PROTEIN EXPRESSION IN C57BL/6J MICE DURING SPATIAL LEARNING IN THE MORRIS WATER MAZE

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DECLARATION

I herein declare that the material in this thesis is my own effort except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged and cited clearly it sources.

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ABSTRACT

PROTEIN EXPRESSION IN C57BL/6J MICE DURING SPATIAL LEARNING IN THE MORRIS WATER MAZE

Learning and memory are two important physiological and biochemical processes that enable a living organism to adapt to the challenging environment. During learning there are changes in behaviour based on experience which consequently can lead to changes in memory. The mechanism of laying down learning and memory involves synaptic plasticity and long-term potentiation (LTP). These processes involve changes in the protein chemistry in different parts of the brain including the hippocampus which plays an important role in spatial cognition and memory. These processes are believed to be dependent on synthesis of new proteins which form the basis of memory. The protein synthesis underlying memory mechanism consolidation takes place in the hippocampus. Memory formation involves a number of signalling pathways associated with activation of many synaptic plasticity-related proteins, such as N-Methyl-D-Aspartic Acid (NMDA) Receptor, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), mitogen-activated protein kinases (MAPKs), glutamate dehydrogenases and many other proteins in the brain. This study was undertaken to investigate the role of the hippocampus in the formation of the spatial learning and memory in C57BL/6J mice undergoing spatial learning in the Morris Water Maze. The mice were sacrificed by cervical dislocation and the total protein in the hippocampus extracted and subjected to 2-dimenssional polyacrylamide gel electrophoresis. The proteins involved in the spatial learning process were identified and comparison was made between proteins expressed in the hippocampus of control and trained mice and also changes in the morphology of hippocampal dendritic arborisation were studid. The proteins were separated according to their isoelectric points and molecular weights. Twenty-six spots were chosen based upon ANOVA statistical analysis. The proteins expressed were analysed using LC/MS Mass Spectrometry. The study indicated high performance of learning in Morris water maze (MWM) and changes in the length and number of dendritic branches in cornu ammonis CA3 pyramidal cells and proteins identified in the mice which is known to be specially involved in, signal transduction and transport. In summary, using a proteomics approach, this study showed that spatial training affects network of proteins involved in energy metabolism and synaptic plasticity in the hippocampus, a brain region central to cognitive function. Further studies can help to identify the proteins and pathways of learning and memory mechanisms and changes of dendrite arborisation during spatial learning.

ABSTRAK

Pembelajaran dan ingatan adalah dua aspek penting dalam proses fisiologi dan biokimia yang membolehkan organisma hidup dan menyesuaikan diri dengan persekitaran yang mencabar. Dalam proses pembelajaran apabila terdapat perubahan dalam tingkah laku berdasarkan pengalaman yang seterusnya boleh membawa kepada perubahan dalam ingatan. Kajian in berkaitan dengan mekanisme pembelajaran dan ingatan yang melibatkan keplastikan sinapsis dan Potensi jangka panjang (LTP). Proses-proses ini melibatkan perubahan kimia di dalam protein di bahagian-bahagian berbeza otak termasuk hipokampus yang memainkan peranan yang penting dalam kognisi ruang dan memori. Proses-proses ini dipercayai adalah bergantung kepada sintesis protein baru yang menjadi asas memori. Sintesis protein dan mekanisme penyatuan memori yang mendasari proses pegikatan berlaku dalam hipokampus. Pembentukan memori melibatkan laluan isyarat spesifik yang berkaitan dengan pengaktifan pelbagai protein-berkaitan keplastikan sinapsis, seperti Asid N-Methyl-D-aspartik (NMDA) Reseptor, Ca^{2+} / calmodulin-bergantung protein kinase II (CaMKII), mitogen-diaktifkan oleh protein kinases (MAPKs), dehidrogenase glutamat dan pelbagai protein yang lain di dalam otak. Kajian ini dilakukan untuk menyiasat peranan hipokampus dalam proses pembelajaran ruang dan memori dalam C57BL/6J, tikus yang diberikan latihan pembelajaran ruangan dalam "Morris Water Maze" (MWM). Tikus tersebut. dikorbankan melalui kaedah kehelan serviks dan jumlah protein dalam hipokampus itu diekstrak dan tertakluk kepada 2-gel elektroforesis dimensi polyacrylamide. Protein yang terlibat dalam proses pembelajaran ruang telah dikenal pasti dan perbandingan dibuat antara protein yang dinyatakan dalam hipokampus kawalan dalam tikus yang terlatih dan juga melihat perubahan dalam morfologi pangoboran dendritik hipokampal. Protein dipisahkan mengikut titik Isoelektrik mereka dan berat molekul. Dua puluh enam tempat telah dipilih berdasarkan analisis statistik ANOVA. Protein yang diungkapkan dianalisis menggunakan liquid chromatography/ mass spectrometry (LC/MS). Kajian ini menunjukkan prestasi pembelajaran dalam (MWM) dan perubahan dalam panjang dan bilangan cawangan dendritik dalam cornu ammonis CA3 sel protein dan piramid yang dikenal pasti dalam tikus yang dikenali terlibat secara khas dalam transduksi isyarat dan transmisi. Secara ringkasnya, dengan menggunakan pendekatan proteomik, kajian ini menunjukkan bahawa latihan ruang menjejaskan rangkaian protein yang berkaitan dengan metabolisme tenaga dan keplastikan sinaps dalam hipokampus itu sekitar rantau otak pusat kepada fungsi kognitif yang berbeza. Kajian-kajian yang lebih lanjut boleh membantu untuk mengenal pasti protein dan laluan pembelajaran dan mekanisma ingatan dan perubahan dendrit arborisat semasa pembelajaran ruang.

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LIST OF ABREVIATION

AC	Adenylcyclase
Ach	Acethylcholine
AD	Alzheimer disease
AMPA	α-amino-3 hydroxy-5-methyl-4-isoxazole propionic acid
Arc	Activity-regulated cytoskeleton associated protein
ARM	Anesthesia-resistance memory
ATF-1	Activating transcription factor 1
BDNF	Brain-derived neurotropic factor
bZIP	Basic leucine zipper
CA3	Cornu ammonis
Ca^{2+}	Ion calcium
Cabin1	Calcineurin-binding protein 1
CaMK	Calodulin-dependent protein kinase II
CaMKIV	Calodulin-dependent protein kinase IV
cAMP	Cyclic adenosine monophosphate
c-fos	proto-oncogene
cGMP	Cyclic guanosine monophosphate
ChAT	Choline acetyltransferase
CNS	Central nervous system
CO	Carbon monoxide
CREB	cAMP response element binding
CREM	cAMP response element modulator
C-S	Conditioned Stimulus

DA	Dopamine
DAG	Diacylglycerol
DHB	Dihydrobenzoic acid
DRP2	Dihydropiramidinase related protein 2
DTT	Dithiolthreitol
E-LTP	Early-long tetrm potential
GABA	ɣ-aminobutyric acid
GAD	Glutamic acid decarboxylase
Glu	Glutamate
Gly	Glycine
GTP	Guanosine triphosphate
GDP	Guanosine diphosphate
HDAC4	Histone deacetylase 4
HPETE	Hydroperoxyeicosatetraenoic acid
Hsp60	Heat shock 60 kDa protein
IEF	Isoelectric focusing
IP_3	Inositol-triphosphate
IPG	Immobilized pH gradient
K^+	potassium Ion
LTM	Long-term memory
LTP	Long-term potentiation
MALDI-TOF	Matrix-assisted Laser Desorption Ionization- Time of Flight
MAPKs	Mitogen-activated protein kinase
MRI	Magnetic resonance imaging

MWM	Morris Water Maze
Na^+	Sodium Ion
NADPH	Nicotinamide adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartic acid
NO	Nitric oxide
pI	Isoionic point
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Protein lipase C
PNS	Peripheral nervous system
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
S-R	Stimulation-Response
S-S	Stimulation-Stmulation
STM	Short-term memory
TEME	N,N,N',N'-Tetramethylethylenediamine
TrKB	tyrosine kinase receptor
UR	Unconditioned response
US	Unconditioned stimuli
VDCC	Voltage-dependent calcium channels
VDAC	Voltage-dependent anion channels

LIST OF SYMBOLES

m	Meter
cm	Centimeter
mm	Millimeter
µm	Micrometer
nm	Nanometer
µl	Microliter
kg	Kilogram
g	Gram
mg	Milligram
µg	Microgram
M	Molar
mM	Milimolar
Min	Minute
s	Second
°C	Degree Celsius
MΩ	MegaOhm
mA	Milliampere
V	Volt
W	Watt
Vhrs	Volt-hours
kDa	KiloDalton
%	Percentage

CHAPTER 1

INTRODUCTION

This chapter is designed to act as a synopsis comprising a brief introduction to the study, objectives and an overview of this thesis.

1.1 Learning and Memory

The brain has been the subject of countless scientific enquiries for the last two centuries. Research has been carried out to find out about brain's special structures and functions; most of the information being obtained from studies performed on patients suffering from brain injuries either through accident or illness, as well as experiments on animals. The findings offer rigorous scientific evidence for new ways of conceptualizing the complex executive control mechanisms in the brain, while many researchers are more focused on understanding the learning and memory mechanisms.

It goes without saying that learning and memory are essential processes for all multi-cellular animals. Memory is essential to recall facts and experiences. Definition of the processes involved consists of encoding and storing information for future retrieval. Observations and experiences leave "engrams" as traces in the brain that can fade with the passage of time, leading to forgetting is strengthened by constant recall recapitulation (lay down memory). An engram is the term given to a hypothetical memory trace. Memory can be reinforced by paying attention and making associations with previous knowledge. This can sometimes lead to permanency of the stored memory.

Learning, unlike memory, is not a process of storing information. Learning is defined as demonstration from a change in behavior as a result of the acquisition of knowledge or new information due to experience (Kandel et al,. 1995). Learning is a process, and memory is the record of that process, stored in the brain.

Both in humans and rodents, the hippocampus is involved in spatial memory consolidation. Memory can be divided into two forms according to its temporal and biochemical properties: short-term memory (STM), which exists for less than a few hours, and long-term memory (LTM), which lasts from several hours to days, or even longer. Learning and memory are not singular processes. They involve a number of distinct processes, each with its own role, that work together. Memory can further be divided into two categories: declarative memory, which is the conscious recall of information about places, people, facts, ideas, etc; and non-declarative, or procedural, memory which is non-conscious recall. These two types of memory seem to involve different neurological structures within the brain (Noback et al., 1996).

Many theories and hypotheses about the mechanisms of memory consolidation and the components that are involved in learning and memory have been proposed. Even though many proteins have been proved to be involved in the process of learning and memory, there are many other proteins yet to be discovered that may be involved in memory consolidation. It is an objective that by using two-dimensional gel electrophoresis, some of these important proteins can be identified, and their roles in the process of learning and memory established.

1.2 Objectives of the Research

The main objective of this research project is the study of spatial learning in Morris Water Maze with 2-dimensional gel electrophoresis technique to identify proteins which are involved in learning consolidation.

I) To develop the 2-dimensional polyacrylamide gel electrophoresis technique for the identification of proteins.

II) To determine the differential proteins expressed in the hippocampus of trained, swim and control.

III) To investigate the proteins identified with the consolidation of spatial learning and memory.

1.3 Hypothesis Formulation

Protein synthesis is an important component of memory formation. The proteins expressed in the hippocampus tissue differ between trained and swim control C57BL/6J mice. Specific hippocampal proteins are involved in memory consolidation. The differences in protein expressed are identified by 2-D polyacrylamide gel electrophoresis and individual proteins may be identified by MALDI-TOF MS/MS.