UNIVERSITI PUTRA MALAYSIA

EVIDENCE OF ASTROCYTIC REACTIVITY DURING VESTIBULAR COMPENSATION

LEE SU ANN

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Chairman : Mohd Roslan Sulaiman, Ph.D.

Faculty : Medicine and Health Sciences

Vestibular compensation is the spontaneous disappearance of postural and oculomotor imbalance exhibited by the vestibular system in response to decreased inputs from the labyrinths. Astrocytes in the vestibular nuclei have been reported to have a role in the plasticity of the central nervous system. The present study was conducted to investigate the behavioural changes of mice and the morphological changes and distribution of astrocytes following unilateral labyrinthectomy.

Thirty-five mice (*Mus musculus*) underwent unilateral labyrinthectomy (UL) and fourteen underwent sham-operation. Their behavioural changes following surgical removal of labyrinths, or sham-operation, were observed. The UL groups displayed behavioural changes including head tilt, circular walking, barrel-rolling and extension and flexion of limbs. These behavioural symptoms disappeared within approximately 3 hours. For the sham-operated animals, these symptoms were absent.

The mice were sacrificed at 4 hours, 6 hours, 24 hours, 4 days, 8 days, 15 days and 25 days post-surgery. Samples of the vestibular nuclei (VN) were processed and studied under light microscope and using anti-GFAP staining, the morphological changes were observed and the immunoreactive astrocytes were quantified. Both biotinylated and FITC-conjugated techniques were used.

Astrocytic immunoreactivity in the UL group was significantly higher ($p<0.05$) than in the sham-operated group. Astrocytic immunoreactivity increased in UL mice as early as 4 hours and reached a peak at 4 days post-UL. At day 4, the astrocytes displayed hypertrophy, which was the most prominent than the other days. The level of immunoreactivity subsequently decreased until day 25. From day 15 to day 25, the astrocytes showed elongation of the processes. Astrocytic immunoreactivity was significantly higher ($p<0.05$) in the ipsilateral side of the vestibular nuclei compared to the contralateral side. There was no significant difference in term of the distribution of the reactive astrocytes among all the ipsilateral vestibular nuclei (superior, medial, lateral and inferior).

Astrocytic hypertrophy may be related to a requirement for increased metabolic activity and the increase in the processes may be related to the formation of the glial scar. Changes in glial cells may also be causally involved in the recovery of the resting activity underlying vestibular compensation.

In conclusion, there were significant changes in the morphology and quantitative aspects of astrocytes during vestibular compensation. Therefore, the objective of the experiment was achieved.

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BUKTI REAKSI ASTROSIT SEMASA PEMULIHAN VESTIBULAR

Oleh

LEE SU ANN

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Pengerusi : **Mohd Roslan Sulaiman, Ph.D.**

Fakulti : **Perubatan dan Sains Kesihatan**

Pemulihan vestibular ialah pemulihan spontan pada postur dan okulomotor yang ditunjukkan oleh sistem vestibular akibat daripada pengurangan input dari labirin. Astrosit dalam nukleus vestibular telah dilaporkan mempunyai peranan dalam keplastikan sistem saraf. Kajian ini dijalankan untuk mengkaji perubahan sifat mencit serta perubahan morfologi dan sebaran astrosit berikutan labirintektomi unilateral.

Tiga puluh lima ekor mencit (*Mus musculus*) menjalani pembedahan labirintektomi unilateral (LU) dan empat belas ekor menjalani pembedahan kawalan. Perubahan tingkahlaku mencit selepas pembedahan labirin dan kawalan telah diperhatikan. Kumpulan LU menunjukkan perubahan tingkahlaku seperti menyengetkan kepala, berjalan berpusing-pusing ke arah lesi, berguling-guling dan fleksi serta ekstensi kaki. Simtom-simtom tersebut hilang dalam lebih kurang 3 jam. Mencit kawalan pula tidak menunjukkan sebarang tanda-tanda selepas pembedahan.

Mencit dibunuh selepas tempoh 4 jam, 6 jam, 24 jam, 4 hari, 8 hari, 15 hari dan 25 hari berikutan pembedahan. Sampel-sampel nukleus vestibular diproses dan dikaji dengan menggunakan mikroskop cahaya dan melalui pewarnaan anti-GFAP, perubahan morfologi diperhatikan dan astrosit imunoreaktif dikira. Kedua-dua teknik yang melibatkan biotin dan konjugasi FITC dijalankan.

Imunoreaksi astrosit dalam kumpulan LU adalah lebih tinggi secara signifikan ($p<0.05$) berbanding dengan kumpulan kawalan. Imunoreaksi astrosit meningkat dalam mencit-mencit LU seawal 4 jam dan mencapai aras tertinggi pada hari ke 4 selepas LU. Pada hari ke 4, astrosit mengalami hipertrofi yang paling jelas berbanding dengan hari-hari lain. Selepas itu, aras menurun sehingga hari ke 25. Pada hari ke 15 hingga 25, astrosit mengalami pemanjangan dalam cabang-cabangnya. Imunoreaksi astrosit adalah lebih tinggi secara signifikan ($p<0.05$) pada nukleus vestibular ipsilateral berbanding dengan sebelah kontralateral. Tiada perbezaan secara signifikan dalam sebaran astrosit yang reaktif di antara semua nukleus-nukleus vestibular ipsilateral (superior, medial, lateral dan inferior).

Hipertrofi astrosit mungkin berkait dengan keperluan untuk aktiviti metabolik yang bertambah manakala pemanjangan cabang-cabang mungkin berkait dengan pembentukan parut glial. Perubahan sel-sel glial mungkin mengakibatkan pemulihan aktiviti rehat semasa pemulihan vestibular.

Kesimpulannya, terdapat perubahan yang signifikan dalam morfologi dan aspek kuantitatif astrosit semasa pemulihan vestibular. Oleh itu, objektif eksperiment ini telah dicapai.

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LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

AEC	: 3-amino-9-ethylcarbazole
ANOVA	: Analysis of variance
BDNF	: Brain-derived neurotrophic factor
CNS	: Central nervous system
CSF	: Cerebrospinal fluid
FITC	: Fluorescein isothiocyanate
GABA	: γ-aminobutyric acid
GFAP	: Glial fibrillary acidic protein
IFN	: Interferon
IgG	: Immunoglobulin G
IP	: Intraperitoneal
LTD	: Long-term depression
LTP	: Long-term potentiation
MHC	: Major histocompatibility complex
MVN	: Medial vestibular nucleus
NaCl	: Sodium chloride
NGS	: Normal goat serum
NMDA	: N-methyl-D-aspartate
NO	: Nitric oxide
PBS	: Phosphate buffered saline
pCREB	: Calcium response element binding protein
PKC	: Protein kinase C
PNS	: Peripheral Nervous System

SPSS	: Statistical Packages for Social Sciences
TNF	: Tumour necrosis factor
UL	: Unilateral labyrinthectomy
UVD	: Unilateral vestibular deafferentation
VIP	: Vasoactive intestinal peptide
VN	: Vestibular nuclei
VNC	: Vestibular nucleus complex
VOR	: Vestibulo-ocular reflex

CHAPTER I

INTRODUCTION

An important type of plasticity is exhibited by the central vestibular system in response to decreased inputs from the labyrinths. This process is called vestibular compensation. Vestibular compensation is the postural and equilibratory adjustments and long-term recovery that follow ablation or damage to the vestibular apparatus.

Glial cells are known as supporting cells. They provide support and form myelin for the neurons, buffer potassium ions in extracellular space, and act as scavengers after injury. They are also involved in other various functions. There are suggestions that glial cells such as astrocytes and microglial cells may be involved in the plasticity of the central nervous system.

Vestibular compensation can be observed after unilateral labyrinthectomy (UL). The present research was conducted to examine astrocytic response during vestibular compensation as a model of lesion-induced plasticity in the central nervous system. The examination of astrocytic reaction was carried out from the morphological and quantitative aspects using immunohistochemistry and computerized microscopy.

Hypothesis 1: There is significant difference in the level of astrocytic reactivity between the unilateral labyrinthectomized mice and the sham-operated mice.

Hypothesis 2: There is significant increase in the level of astrocytic reactivity in the early days following UL and a decrease in the later stages. The astrocytes were expected to show morphological changes.

Hypothesis 3: There is significant difference between the ipsilateral and contralateral sides of the vestibular nuclear complex following UL.

CHAPTER II

LITERATURE REVIEW

Control of Posture

Posture is the relative position of the trunk, head and limbs in space. The body's centre of gravity needs to be maintained in position over its support base to keep a stable posture. Postural reflexes are required to correct changes caused by displacement of the central of gravity by external forces or deliberate movement. Postural change is detected by musculoskeletal propioceptors, the vestibular apparatus, and the visual system (Lasserson *et al.,* 2000)

The Vestibular System

A principal role of the vestibular system is to relay signals from the otoliths regarding linear acceleration, and from the semicircular canals regarding rotation or angular acceleration, to the brain in order to control the motor output of the extrinsic eye muscles and those muscles in the neck (collic) and body (vestibulospinal) concerned with posture and balance (Kelly, 1991). The vestibular nuclei use these signals together with afferent nerves from neck muscles and cervical vertebrae to determine if the head is moving alone or if the head and body are both moving. The nuclei can influence antigravity and axial musculature via a direct projection into the spinal cord.

The Receptor System

The vestibular apparatus consists of a number of interconnected membranous tunnels, collectively the membranous labyrinth, filled with a fluid called endolymph. The labyrinth lies in a fluid-filled space in the temporal bone – the osseous or bony labyrinth and the fluid filling is termed perilymph (Marieb, 1998).

Head movement is detected by movement of the membranous labyrinth relative to the endolymph which because of its inertia, lags behind. Specialized hair cells at certain points in the membranous labyrinths have projections from their surface into jelly-like masses floating in the endolymph. The projections bend as the masses lag behind the movement of the labyrinth. The membrane deformation produced alters the shape of the cation channels and changes the membrane potential – depolarized for stereocilia bending towards the kinocilium, hyperpolarized if bent away (Lasserson *et al.*, 2000).

The otolith organs lie in two areas of the membranous labyrinth: the saccule and utricle, which both contain patches of hair cells called maculae. The projections from the surface of the hair cells lie in a jelly containing calcium salt crystals (the otoliths). The otoliths have a higher specific weight than the endolymph, so the position of the otoliths relative to the maculae is influenced by gravity. This gives information about static head position, coded by slowly adapting receptors. Linear acceleration is detected as the otoliths lag behind movement of the maculae.

The saccular otoliths are oriented vertically, and detect changes in linear acceleration in the vertical plane and changes in head position during lateral tilt. The utricular otoliths are oriented horizontally, and detect changes in linear acceleration in the horizontal plane and changes in head position during flexion and extension of the neck. The semicircular canals are arranged at right angles to each other and together they detect angular acceleration in all three planes of three-dimensional space.

Rotating the head round a horizontal axis stimulates the vertical canals. This can lead to motion sickness. Stimulation of the semicircular canals also causes movements of the eyes to keep them fixed on the same point in the retina for as long as possible. During rotation there is a slow movement of the eyes in the direction opposite to that of rotation, then a quick return to the normal position. This is nystagmus that can also be a pathological sign. It occurs continuously while rotating and continues for a short time after the movement has ceased (Molavi, 1997).

The semicircular canal mechanism predicts ahead of time that mal-equilibrium is going to occur. It allows equilibrium centres to make preventive adjustments. Each canal has a swelling (ampulla) near its attachment to the utricle which contains the hair cells projecting from a ridge (crista) into the cupula in the endolymph.

Hair cells show greatest alteration in the membrane permeability when the stereocilia are moved in one direction. To detect different degrees of tilt and different degrees of flexion, the hair cells in the maculae are oriented differently so that they respond best to a particular head position. The vestibular nuclei can use this information to assess head position precisely (Molavi, 1997).

The brain receives complementary information from the labyrinths as they are located on the opposite sides of the head. For example, as the head turns, one set of hair cells becomes depolarized, whereas the complementary set on the other side becomes hyperpolarized. This organization helps to mediate postural reflexes.

The Vestibular Nuclei

There are four vestibular nuclei within the brain stem (superior, lateral, medial, and inferior). All four cannot be seen in the same cross section, since they are present for a considerable rostrocaudal distance from the rostral medulla to the middle of the pons (Harting, 1997).

The vestibular nuclei lie in the floor of the fourth ventricle and receive information from the hair cells through the vestibular nerve (VIII). The semicircular canals project to the superior and medial nuclei, while the otolith organs project to the lateral nuclei. The medial vestibulospinal tract projects bilaterally, and the lateral vestibulospinal tract projects ipsilaterally. Both tracts influence antigravity, axial, and limb extensor muscles (Molavi, 1997).

The vestibulocochlear nerves (VIII) synapse with the cochlear nuclei (which receive information on auditory inputs), and with numerous vestibular nuclei in both the pons and medulla. The vestibular nuclei, which are collectively called the vestibular nuclear complex, mediate responses that maintain equilibrium (Marieb, 1998).

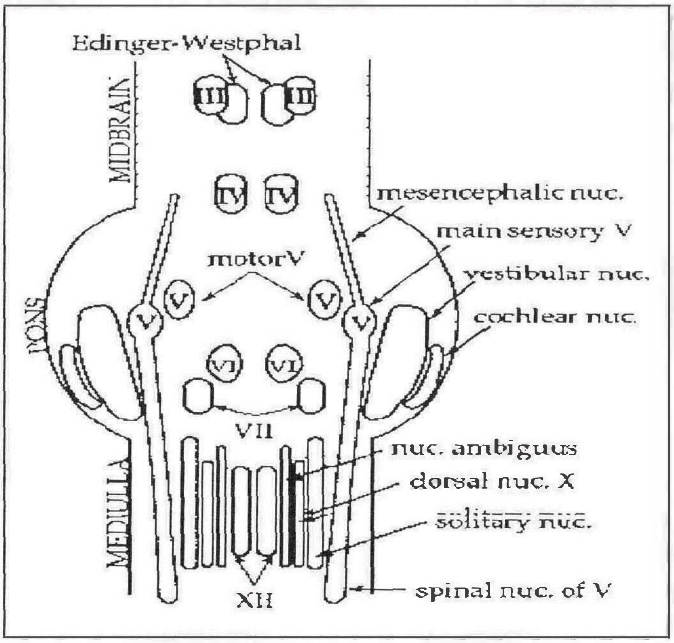

Figure 1: Location of vestibular nuclei in the brain stem.
(Adapted from Molavi, 1997, Neuroscience Tutorial.)

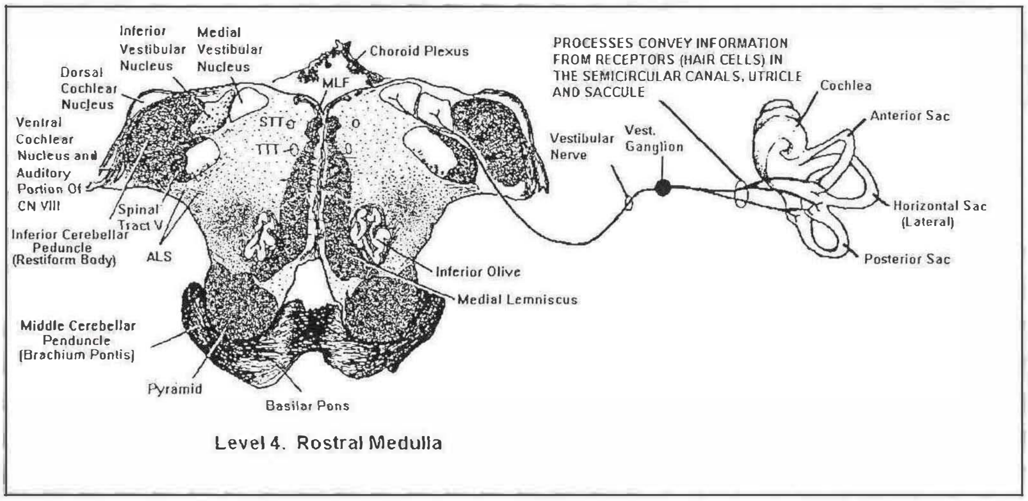

Figure 2: Nerve projections from the vestibular labyrinths to the vestibular nuclei within the rostral medulla. (Adapted from Harting, 1997, Global Brainstem.)