POSTERS

Genetics

P-58

HAPLOTYPES PREDICT HIGH- AND LOW-EXPRESSION OF HUMAN TRYPTOPHAN HYDROXYLASE 2 (*TPH2*) MRNA

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TPH2 is expressed in serotonergic neurons of the raphe nuclei, where it catalyzes the rate-limiting step in the synthesis of serotonin. The goal of this study was to use allele-specific measurements of *TPH2* mRNA expression in raphe neurons in human rostal pons to look for *TPH2* variants that express high- or low-levels of mRNA. A primer extension-based assay was used to measure allele-specific expression of *TPH2* mRNA in total mRNA isolated from the rostral pons of 27 individuals heterozygous for at least one of two "marker" SNPs: rs7305115 (**G**/**A**) and rs4290270 (T/A), located in exon 7 and exon 9, respectively. Differences in mRNA expression between alleles within individual RNA samples were calculated as "allelic expression imbalance" (AEI) ratios. AEI (1.2 to 2.5-fold) was observed in 19 out of 27 pons RNA samples. AEI was observed in 17 out 18 individuals heterozygous for rs7305115. Genotyping 20 additional SNPs within the TPH2 gene revealed four SNPs that were also highly correlated with AEI: rs2171363 (C/T), rs4760815 (T/A), rs6582078 (T/G) and rs9325202 G/A). The alleles of these SNPs define complementary haplotypes, (CT**G**TG) and (TA**A**GA), that correlate with low- and high-levels of TPH2 mRNA, respectively. A recent study from Shantou University (Ke L *et al*, *Brain Research* **1122**, 24–26, 2006) showed that the ***A***-allele of rs7305115 is associated with a significantly lower incidence of suicidal behaviors among a group of depressed patients, suggesting that the high-expression TPH2 allele may be protective against suicide.

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GENETICAL GENOMICS ANALYSIS OF MICROTUBULE-ASSOCIATED PROTEIN TAU GENE, *MAPT*, CONFERS SUSCEPTIBILITY TO PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive, late-onset neurodegenerative disorder. Previous studies have identified microtubule-associated protein tau gene, *MAPT*, as a key gene of PD in human. In this study, we have combined array analysis and QTL mapping approach (genetical genomics) to characterize the genetic variation and regulatory network of *Mapt* in mouse. We identified that *Mapt* shares common patterns of control in two large panels of recombinant inbred strain—the LXS ($n = 77$ strains) and BXD ($n = 67$). Polymorphisms in *Mapt* have a marked effect on steady-state levels of *Mapt* mRNA in hippocampus. Local or *cis* likelihood-ratio statistic (LRS) were >70 in BXD strains and >25 in the LXS. Polymorphism analysis of *Mapt* revealed that eight SNPs between C57BL/6J and DBA/2J strains in the promoter. Whole-brain *in situ* hybridization analysis (Allen Brain Atlas) showed that *Mapt* is well expressed in hippocampus. In addition, network analysis demonstrated that *Mapt* co-varies with many PD-related genes, including *Lrrk2* and *Pink1*. We also characterized numerous novel genes that may be related to PD. However, their biological roles for PD require further investigation. The genetical genomics approach is proved extremely useful for identifying genes and pathways that contribute to complex traits.

P-60

GENOTYPING OF FOUR POLYMORPHISMS POTENTIALLY ASSOCIATED WITH ALZHEIMER'S DISEASE: *A2M*, *APOC1*, *TNF*α-850 AND *TNF*α-308

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Alzheimer's disease (AD) is the most prevalent form of dementia. It is believed to be caused by both environmental and genetic factors. To date, only a few definite genetic risk factors have been determined. We examined the association of three candidate risk-factor genes: *A2M*, *APOC1* and *TNF*α, in AD. We employed PCR-based RFLP analysis on genomic DNA extracted from neuropathologically characterised *post-mortem* brain tissue. The proteinase inhibitor encoded by *A2M*, has been associated with Alzheimer's disease due to its ability to bind Aβ which is found as insoluble plaques in the brains of AD patients. We investigated a SNP in codon 1000 (rs669), close to the functional site of the protein. Our data did not show an association of this locus with AD; however, a nonsignificant trend towards an involvement of the G allele in other neurodegenerative diseases was observed. As apoC1 interacts with the protein encoded by the established risk gene *APOE*, we investigated a 4 bp insertion/deletion in the *APOC1* promoter (rs11568822) and found the allele containing the insertion (H2) to be strongly associated with AD and other neurodegenerative diseases. Another process thought to be

involved in Alzheimer's disease is inflammation. We therefore examined two SNPs in the promoter region of the TNFα gene (position −308: rs1800629; position −850: rs1799724). According to our data, the −308 SNP is not involved in AD, despite a trend towards the G allele as a risk factor. For the −850 position, a significant association of the T allele with AD was observed.

Glia

P-61

TESTOSTERONE MODULATES REACTIVE GLIOSIS AFTER A PENETRATING INJURY IN MALE RATS

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In this study we have assessed the effect of testosterone therapy on gliosis and the proliferation of glial cells after a stab wound injury affecting the hippocampal formation. In addition, to determine whether the effects of testosterone were mediated by its metabolites, some animals were treated with estradiol or dihydrotestosterone (DHT). Wistar male rats were bilaterally orchidectomized at the age of 2 months to reduce circulating levels of testicular secretions. A penetrating injury affecting the hippocampal formation was performed 1 month after orchidectomy. The effects of early and late treatments after injury with testosterone or its metabolites estradiol and DHT on gliosis were assessed in the hippocampus. Both early and delayed administration of testosterone or estradiol resulted in a significant decrease in the number of vimentin-immunoreactive astrocytes in the border of the lesion. DHT administration, either early or delayed, did not affect the number of vimentin immunoreactive astrocytes. Testosterone and estradiol reduced the volume fraction of MHC-II immunoreactive microglia in both early and delayed treatments. Early, but not delayed, administration of DHT significantly reduced the volume fraction of MHC-II immunoreactive cells. Indeed, testosterone is able to decrease significantly the proliferation of glial cells however no significant effects were seen in animals treated either with estradiol or DHT. Our findings suggest that early and late effects of testosterone on reactive astroglia and reactive microglia may be at least in part mediated by estradiol, while DHT may mediate part of the early effects of testosterone on reactive microglia, and this observed effect of testosterone on gliosis is in part due to a decrease in the proliferation of glia.

P-62

INFLUENCE OF GLIAL CELLS ON STRESS-INDUCED NEURITE RETRACTION IN RAT DORSAL ROOT GANGLION CELLS

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We have observed that the neurites of adult rat dorsal root ganglion (DRG) cells retract in a pertussis toxin (PTx)-sensitive manner when cell cultures are removed from their incubator. This observation suggests that the DRG cell culture provides a neurite protective factor acting on Gi-coupled receptors. Because the DRG cell preparation from male Sprague-Dawley rats is primarily a mixture of neuronal and glial cells, the aim of the present study was to explore the role of different subpopulations of cells in the DRG in this neurite retraction response. We therefore prepared a neuron-enriched fraction of cells which was further separated into IB4 + ve (GDNF-responsive) and IB4−ve (NGF-responsive) cells. In the PTx-treated mixed cell preparation, there was a statistically significant decrease (15%, $P<0.05$) in the proportion of neuronal cells expressing neurites one hour following stimulation. In contrast, no neurite retraction response was observed in the more purified neuronal populations. In conclusion, glial cells appear to facilitate the neurite retraction response observed in DRG cell cultures.

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P-63

EPHA4 RECEPTOR TYROSINE KINASE INVOLVEMENT IN CYTOSKELETAL REGULATION AND EXTRACELLULAR MATRIX ADHESION IN ASTROCYTES

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Spinal cord injury in EphA4 null mice revealed functional recovery compared to wildtype animals. This appeared to involve a lack of robust astrocytic gliosis, with only a modest increase in GFAP expression in EphA4 null animals (Goldshmit *et al.* J. Neurosci. 2004). How EphA4 regulates cytoskeletal changes occurring after astrocytic activation and whether these influence adhesion is not yet known. We investigated effects of EphA4 signaling on cytoskeletal rearrangement and adhesion of astrocytes *in vitro*. F-actin cytoskeleton regulation was examined by inducing cytoskeletal collapse with Rho kinase (ROCK) inhibitor HA1077 and recovery of stress fibre formation after HA1077 washout, with and without activation of EphA4 with ephrinA5-Fc. F-actin was stained with phalloidin-FITC. Under basal conditions, no significant differences between genotypes in percentage of cells containing stress fibres were detected. Without EphA4 activation, 15 min after HA1077 removal significantly more wildtype than EphA4 null astrocytes re-established stress fibres. EphA4 activation enhanced recovery of stress fibre formation and was further increased in EphA4 null cells, indicating activation of another Eph receptor. To investigate involvement of EphA4 in astrocyte adhesion, the ability of astrocytes to adhere to laminin, poly-D-lysine or uncoated surfaces was examined. Wildtype astrocytes showed significantly higher adhesion on these substrates suggesting a role for EphA4 in focal adhesion

and integrin regulation. EphA4 therefore plays a role in astrocyte cytoskeletal regulation and adhesion.

P-64

MORPHOLOGICAL POLYMORPHISM OF MITOCHONDRIA IN CULTURED ASTROCYTES BASED ON HIGH VOLTAGE ELECTRON MICROSCOPY

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Mitochondria are dynamic organelles with morphological variations closely linked to many cellular processes, including development, cell cycle progression and death. Morphological characteristics of mitochondria are important to understand the functional status of the cell. We investigated the morphology of the mitochondria in the cultured astrocyte with high voltage electron microscopy (HVEM) without sectioning. Astrocytes were prepared from neonatal rat brain and seeded on the gold grid for HVEM observation. We could observe mitochondria on the whole mount astrocyte with HVEM at KBSI, DaeJeon Korea (JEM-ARM 1300S). The field of observation was randomly selected and the morphology of the mitochondria was classified and we measured the length and area of the each mitochondrion with NIH image. The linear type was most frequent one by 45% and followed by branched type (26%), circular type (22) and ball type (7%). The average length of linear type was 14.3 μm, that of branched was 13.8 μm, that of circular was 4.5 μm, and that of branched of ball type was 0.84 μm. We could put mitochondria into four categories with high penetration image of HVEM in normal cultured condition. The morphological changes of the mitochondria are under evaluation in challenge cellular status. We could get some insight of correlation between morphological shape and function significance.

P-65

PHOSPHORYLATION OF CAMP RESPONSE ELEMENT BINDING PROTEIN INDUCED BY GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED RAT ASTROCYTES

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Glutamate is known as a major excitatory neurotransmitter in the central nervous system. Beside its synaptic transmission properties, glutamate is also involved in intracellular signal transductions via metabotropic glutamate receptors (mGluRs). These signaling cascades might lead to gene regulation by activation of transcription factors. Despite of extensive studies in neurons, effects of glutamatergic signaling remain unclear in glial cells. This study is aimed to investigate the *in vitro* effect of glutamate on activation of transcription factor cAMP response element binding protein (CREB) in astrocytes. Cultures of 1-2 days postnatal rat cortical astrocytes were established, and subjected to potent agonist for group I mGluR, (*S*)-3,5-Dihydroxyphenylglycine ((*S*)-DHPG), at various dosage and time. The level of CREB phosphorylation at serine-133 residue was evaluated by western immunoblotting. Agonist treatment at various time intervals reveals that phosphorylation reaches its peak within 15–20 minute after glutamate treatment, and then declines gradually. For the dose dependency result, the level of phosphorylated CREB is found highest at 1 μM of agonist, and significantly higher than control group. In conclusion, agonist of group I mGluR can elicit rapid, transient phosphorylation of CREB in astrocyte *in vitro*. Proposed further investigations include the use of receptor antagonists, and also inhibition of calcium-responsive signaling molecules will be investigated.

Development

P-66

EXPRESSION OF HYPOTHALAMIC GPR54 IN THE PUBERTAL DEVELOPMENT OF PRECOCIOUS FEMALE MODEL RATS

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To investigate the potential role of G protein-coupled receptor 54 (GPR54) in the advance onset-puberty in precocious puberty, model rats induced by danazol were used in studying the developmental expression of hypothalamic GPR54. The day of vaginal opening (30.97 ± 2.24) and the establishment of two regular estrous cycles (48.75 ± 0.50) showed significant advancement in the model rats compared with those in the intact rats (38.00 ± 0.98, 54.26 ± 2.35) ($P < 0.01$, respectively). Both of the intact and model rats on the day of pre-puberty (the postnatal day 25, 20) were only detected few GPR54 immunoreactive cells in the hypothalamic arcuate nucleus (ARC), periventricular nucleus (PeN) and preoptic area (POA), and there were not significant difference in the two groups. The GPR54 immunoreactive cells expression in the three nuclei were increased in model rats on the day of onset-puberty, and the cell numbers increased in ARC and PeN but decreased in POA on the day of post-puberty. The expression pattern in the intact rats is similar to the model ones but shows a lower level ($P < 0.01$). Furthermore, the hypothalamic GPR54mRNA expression examined by RT-PCR increased obviously in the model rats on the day of pre-puberty, onset-puberty and post-puberty stages compared with the intact rats ($P < 0.01$, respectively), and there appeared a peak level on the day of onset-puberty. Those data suggested that GPR54 might be involved in the advanced onset of puberty in danazol induced female precocious model rats.

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P-67

SYSTEMATIC IDENTIFICATION OF THE GROWTH CONE PROTEINS INVOLVED IN AXONAL GROWTH

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The growth cone is the most important structure for not only the neural wiring but also the axonal regeneration in adult nervous system. The molecular basis of the growth cone in mammalian brain is too poorly understood now. The reason is that we did not understand the proteins of the growth cone explaining the mechanisms and the principles of growth cone motility, guidance and synapse formation. To solve this question, we tried and succeeded in systematically identifying the growth cone proteins that are important for axonal growth, by combining purification of the growth cones from rat brain, proteomic analysis by protein identification technology of the growth cone; immunostaining using antibodies against the proteins identified by the proteomics; and RNAi inducing the inhibition of axonal growth. Among the 900 growth cone proteins identified by proteomics, we selected the 200 proteins for immunostaining, and all of the examined proteins were positively stained in the growth cone of the primary cultured rat cortical neurons. To examine these proteins are functionally important to the growth cone functions, we checked 50 proteins of them using siRNA, and we demonstrated that 15 proteins are involved in axonal growth. We conclude that they are the potential growth cone marker proteins, as well as GAP-43.

P-68

POSTNATAL DEVELOPMENT OF *PER1* AND *AA-NAT* EXPRESSIONS IN THE RAT PINEAL GLAND

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The circadian rhythm of melatonin synthesis in the pineal is controlled by the master clock, suprachiasmatic nucleus (SCN) which its rhythmicity is driven by autoregulated feedback loops consisting of periodical expression of clock genes. Among clock genes, *Per1* is important for maintenance of circadian rhythmicity and entrainment to light cues. *Per1* developed gradually during postnatal ontogenesis in the rat SCN and it is also expressed in pineal with a circadian pattern. In addition, the levels of melatonin change according to the aging process by increased during postnatal life and rapidly drops at pubertal age. It is of interest to study the relationship between the postnatal development of *Per1* and *Aa-nat*, gene of rate limiting enzyme in melatonin synthesis in the rat pineal. Daily profiles of mRNA expressions of genes were analyzed in pineal of pups at postnatal age 4 (P4), P8, P16, and adult rats (at 8 weeks old) by real-time PCR. The results showed that as early as P4, *Per1* and *Aa-nat* mRNA were already expressed and

their expressions were relatively high during the nighttime. The *Per1* expression gradually increased at P8, P16 and adults which paralleled to the increase expression *Aa-nat*. At P16, significant mRNA rhythm of both studied genes were showed with the same pattern of an adult however the level of *Aa-nat* expression was much lower than that of the adult. The present data indicate that there is close relationship between the development of expression of *Per1* and that of melatonin synthesis in the postnatal rat.

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P-69

NESTIN IS ESSENTIAL FOR THE PROLIFERATION OF CORTICAL PROGENITOR CELLS

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Embryonic neuroepithelium is composed of progenitor cells that undergo self-renewal and generate neurons and glia under the control of both extrinsic and intrinsic cues. A precise regulation of the proliferation and differentiation of neural progenitor cells is essential for the morphogenesis of the central nervous system (CNS). Nestin, a type IV intermediate filament (IF) protein, is specifically expressed in mammalian neuroblasts but not in post-mitotic neurons, thereby is widely adopted as a marker for neural progenitors. However, the function of nestin in neurogenesis remains unknown. Through in-utero electroporation in rat embryos, we found that the knockdown of nestin by small interference RNA caused a severe defect in cortical neurogenesis. *In vivo* and *in vitro* analysis showed that cortical progenitors were arrested at G1 phase after the knockdown of nestin expression. In contrast, overexpression of nestin promoted the proliferation of cortical progenitors *in vitro*. Interestingly, we found that of the down-regulation of vimentin, another IF protein which is essential for the assembly of nestin filament in progenitor cells, did not cause defect in neurogenesis, suggesting that the mechanical support of nestin as a component of IF is not necessary for the normal development of cortical progenitors. Further studies will be carried out to explore the molecular mechanisms underlying how nestin regulates the cortical neurogenesis.

P-70

GM-CSF ENHANCES NEURAL DIFFERENTIATION OF BONE MARROW STROMAL CELLS

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Recent reports suggest that bone marrow stromal cells may be induced into neural cells both *in vivo* and in vitro. The factors that regulate the neural differentiation and the mechanism involved, however, remains unclear. Here we demonstrated that granulocyte-macrophage colony-stimulating factor (GM-CSF), a potent hematopoietic factor, was able to enhance the neural differentiation of bone marrow stromal cells. Moreover, we found that GM-CSF receptors are abundantly distributed in the bone marrow stromal cells and GM-CSF significantly upregulated the phosphorylation of cAMP-responsive element binding protein in bone marrow stromal cells. These findings suggest that GM-CSF may activate its receptor and then enhance neural differentiation of bone marrow stromal cells by upregulating phosphorylation of cAMP-responsive element binding protein.

P-71

MATURATION PROFILE OF IONOTROPIC GLUTAMATE RECEPTORS IN CENTRAL VESTIBULAR NEURONS OF RATS

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To investigate the role of ionotropic glutamate receptors (AMPAR and NMDAR) in the development of central vestibular neurons, whole-cell patch-clamp experiments were performed in spinal vestibular neurons of postnatal (P1–16) Sprague Dawley rats. The profiles of AMPAR- and NMDAR-mediated EPSCs evoked by electrical stimulation of the ipsilateral vestibular nerve were also studied. From P1 to P3, glutamatergic transmission is primarily mediated by NMDARs. The relative contribution of AMPARs versus NMDARs at vestibular afferent synapses increased during the first two weeks of life. Using minimal stimulation paradigm, we found that silent NMDAR-only synapses were present only up to P5. From P5 to P7, there was a significant increase in the quantal amplitude of AMPAR-mediated eEPSCs, as confirmed with the use of extracellular strontium instead of calcium, thereby increasing the AMPAR/NMDAR ratio. These age-dependent increase of AMPARs towards the end of the first postnatal week coincides with the disappearance of NMDAR-only synapses. Taken together, our findings provide evidence on the developmental shift in AMPAR and

NMDAR of glutamatergic synapses, contributing to functional maturation of central vestibular neurons in postnatal rats.
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P-72

EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-4 IN THE DEVELOPING BRAIN IN THE RAT

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Introduction: To study temporal expression of Insulin-like growth factor binding protein 4 (IGFBP-4) in the developing brain of rat and explore its function in nervous system development.
Method: IGFBP-4 expression distributing in the developing brain of rat was studied by immunohistochemistry and *in situ* hybridization methods; IGFBP-4 mRNA and protein expression level were detected using Real-time PCR and Western Blot respectively.
Results: Immunohistochemistry and in situ hybridization revealed that IGFBP-4 almost expressed in every brain area-forebrain, mid-brain and hindbrain from E11.5 to E18.5. But Real-time PCR results suggested that IGFBP-4 mRNA level had a regular change from E11.5 to E18.5: on E11.5 IGFBP-4 mRNA could be detected; then IGFBP-4 mRNA level increased gradually and on E13.5 it reached a peak (its level on E13.5 was 3.4 times high of its on E11.5); and from E14.5 its level decreased gradually and on E18.5 its level decreases 8.5 times than its on E13.5. Western Blot results were consistent with Real-time PCR results: on E11.5 IGFBP-4 protein was detected, and reached a peak on E13.5, then from E14.5 its protein expression decreased gradually.
Conclusion: IGFBP-4 maybe take an important role in neural stem cell commitment and differentiation.

P-73

INCREASED OXIDATIVE DAMAGE AND FREE RADICAL GENERATION IN LYMPHOBLASTS FROM AUTISM

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Autism is a severe neurodevelopmental disorder. Currently, there is no biochemical marker to support the behavioral diagnosis of autism. Recent studies have suggested increased oxidative stress in autism. Most of these studies were done with serum, plasma or erythrocytes. The studies with cell cultures are lacking in autism. The aim of this study was to investigate the status of oxidative/nitrosative stress in lymphoblasts from autism by analyzing lipid peroxidation, generation of free radicals (reactive oxygen species -ROS and reactive nitrogen species- RNS) and extent of membrane damage. Cell lysates were prepared from lymphoblasts of autistic and control subjects. Lipid peroxidation was assessed by measuring malonyldialdehyde, an end product of fatty acid oxidation. ROS levels (basal and upon induction by Fenton reaction) were determined by using dichlorofluorescin-diacetate as a fluorescent probe. RNS levels were measured by nitric oxide fluorometric assay kit. Damage of the plasma membrane was evaluated by measuring the amount of intracellular lactate dehydrogenase (LDH) that was released into the conditioned medium. Lipid peroxidation was significantly increased in lymphoblasts from autism as compared with control lymphoblasts, suggesting increased oxidative damage in autism. The levels of ROS and RNS were significantly increased in the lymphoblasts from autism as compared with control lymphoblasts, suggesting increased generation of free radicals in autism. LDH leakage was also increased in lymphoblasts of autism as compared with controls, suggesting that membrane integrity is affected in autism. Our results suggest that autism is associated with increased formation of free radicals (ROS and RNS), which leads to increased oxidative damage and membrane damage. **Sponsors**: This study was supported by OMRDD, Autism speaks, Autism Research Institute, Alexander and Bo MacInnis.

Stress/Biogenic Amines

P-74

MATERNAL RESTRAINT STRESS ALTERS GROWTH-ASSOCIATED PROTEIN-43 (GAP-43) IN POSTNATAL RAT BRAIN

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The brain is very sensitive to prenatal programming and glucocorticoids in particular have powerful brain-programming properties. Exposure to stress during critical periods of an organism's maturation can result in permanent behavioral and metabolic changes and also shows hyper-responsive to novel or aversive stimuli later in adulthood. The hippocampus is a plastic and vulnerable brain structure that is susceptible to damage during aging and repeated stress. The present study examines the effect of maternal restraint stress and corticosteroid (CORT) injection on the level of growth associated protein-43 (GAP-43) by using western blot analysis. Maternal CORT injection during gestation day (GD) 14–21 alters the level of both GAP-43 and its phosphorylated form in the pup's brain. An immunohistochemical staining of GAP-43 was done to localize the effect of prenatal stress on axonal growth in the pup's brain. In the hippocampus, GAP-43 was elevated in the Stratum Lucidum, the Stratum Oriens and the Stratum Radiatum of CA1 and CA3 regions of prenatal stress pups. In prefrontal cortex (PFC), the level of GAP-43 was also elevated in specific cortical layers. These changes indicate the direct effects of elevated maternal stress hormone, since maternal injection of CORT (40 mg/kg) during GD 14–21, also gave the same results. The results suggested that maternal stress may be harmful to the developing brain and the up-regulation of GAP-43 may serve as a protective mechanism against the toxicity effect of maternal stress hormone. Increase of GAP-43 may alter the pattern of axonal growth and the formation of synapses because postnatal day 7–14 has been reported to be correlated with the peak period of synaptogenesis in the rat brain.

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P-75

COMPARISON OF NEUROHYPOPHYSEAL HORMONE OXYTOCINE AND ITS "CARBA ANALOG" CARBETOCIN IN THE OPEN-FIELD; ANTIANXIOUS AND ANTISTRESS EFFECTS

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Neuropeptide oxytoxin becomes intensively studied in the recent neuroscience research because of its newly discovered central effects. Due to these findings there is large interest in behavioral and biochemical effects of oxytocin analogs with agonistic and antagonistic actions on brain oxytocin receptors. From many agonistic analogs synthesized in Prague, we have chosen "carba analog" – carbetocin [deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin]. The replacement of disulfide bridge by thiomethylene group was found to stabilize peptide, and in the case of carbetocin increased the peptide activity. Carbetocin with prolonged action has clinical applications, and it was demonstrated to influence rat behavior. We used male Wistar rats in accordance with the DHEW Publication, NHI 80–23. Open field test was registered in circular arena (150 cm diameter) by video monitoring using AnyMaze (Stoelting, USA). Behavioral parameters following up rats activity, exploration and emotionality were evaluated after application of oxytocin and carbetocin in control animals and rats exposed to restraint stress lasting for 60 min; behavioral testing started 60 min after stress termination and/or drugs application. Our data show differences in behavioral effects of 3–4 doses of oxytocin and carbetocin, given i.p. (0.1–3 mg/kg b.w.). The most remarkable differences in the effects of oxytocin a carbetocin were in total locomotion, rearing, entries in the inner zone and grooming. Carbetocin revealed some anti-anxiety effects, had a prolonged action, and the most significant is its antistress effect in restrained rats.

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NERVE GROWTH FACTOR-INDUCED DIFFERENTIATION OF RAT PHEOCHROMOCYTOMA (PC12) CELLS LEADS TO CHANGES IN ADENYLYL CYCLASE ACTIVITY

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We have previously observed that nerve growth factor (NGF) significantly increases forskolin-stimulated adenylyl cyclase

(AC) activity in PC12 cells. The objective of the current study has been to determine if there is any differentiation-dependent effect on AC expression. PC12 cells were incubated overnight in DMEM plus 1% heat inactivated horse serum, then cultured for up to 6 days ± NGF (50 ng/ml). Cells were harvested on Day 0, 2, 4 and 6 and semi-quantitative RT-PCR analysis was performed on total cellular RNA to determine the mRNA expression patterns of all nine isoforms of transmembrane ACs. We could detect the presence of mRNA for all 9 isoforms of transmembrane ACs in both NGF-treated and non-treated PC12 cells. AC9 mRNA expression was not affected by NGF, whereas there was a small, but statistically insignificant increase in expression of AC3 and AC6 mRNA on Day 2 and Day 4. In conclusion, it is unlikely that changes in AC expression patterns are responsible for the NGF-induced increase in adenylyl cyclase activity observed in PC12 cells.
Acknowledgment: This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region (HKUST3/03C).

P-77
EXPOSURE OF ADULT RATS TO SHORT-TERM ENRICHMENT OR ADMINISTRATION OF BROMOCRIPTINE OR OXOTREMORINE RESTORES CHRONIC RESTRAINT STRESS INDUCED DECREASE IN HIPPOCAMPAL CELL PROLIFERATION AND SURVIVAL

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Adult neurogenesis, particularly in the hippocampus is closely associated with learning and memory. Chronic stress decreases adult hippocampal neurogenesis and impairs learning and memory. Earlier reports from our lab suggest that stress is associated with dendritic atrophy and decreased dopamine and acetylcholinesterase activity in the hippocampus. Further, exposure to enriched environment or administration of the cholinergic muscarinic agonist, oxotremorine or the dopaminergic D_2 receptor agonist, bromocriptine ameliorates the stress-induced behavioral, electrophysiological, biochemical and morphological deficits. Accordingly, in the perspective of exploring the probable mechanisms underlying reversal, we have examined the possible recruitment of neurogenesis in the amelioration of stress-induced cognitive deficits. Adult male Wistar rats were first subjected to 21 days of restraint stress (6 hour/day) followed by administration of bromocriptine (10 mg/kg, i.p.), oxotremorine (0.2 mg/kg, i.p.), or exposure to enrichment (6 hour/day) for 10 days. Hippocampal cell proliferation and survival is analyzed using bromodeoxyuridine (BrdU) immunohistochemistry and stereology. The cell proliferation and survival in the stressed rats was significantly decreased. Both bromocriptine and oxotremorine treatment following stress partially restored the hippocampal cell proliferation. On the other hand, total restoration of hippocampal cell proliferation was observed subsequent to exposure to enriched environment. Similar to the proliferation results, exposure to enriched environment in stressed rats completely restored the cell survival, while bromocriptine or oxotremorine treatment resulted in partial restoration. Our results thus suggest a credible contribution of the newly formed cells in the amelioration of stress-induced cognitive dysfunctions. Thus, stimulating the endogenous precursors to proliferate, differentiate and integrate into the network is a promising approach to treat several neurodegenerative disorders.

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A ROLE FOR PHOSPHORYLATED α-SYNUCLEIN IN THE REGULATATION OF THE TYROSINE HYDROXYLASE

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Several articles have shown that α-synuclein can regulate dopamine biosynthesis and α-synuclein deposited in brain tissue from individuals with synucleinopathy is extensively phosphorylated at Ser-129, our previous experiments have proved that change ser-129 of α-synuclein to aspartic (S129D) can increase the activity of the Tyrosine Hydroxylase (TH) without change its expression level, and if we interference α-synuclein we can examined that the activity of TH is obviously decreased without effects TH expression. In order to know how phosphorylated α-synuclein regulates the activity of TH, we do some experiments to explore whether phosphorylated α-synuclein bound to TH and increase the activity of TH. And PULL-DOWN and immunoprecipitation revealed S129D and WT α-synuclein interacts with TH, but S129A cannot. Also immunocytochemistry confirmed the same results. To explore the consequences of the interaction, we measured the effect of recombinant S129D/S129A/WT-α-synuclein on TH activity in a cell-free system and observed S129D can increase TH activity while WT decreased it and we do not find obvious change of S129A.our results suggest that S129D- α-synuclein interacts with TH and activates some signal transduction pathway protein which can up-regulate the activity of TH.

P-79
RETINOL STIMULATES TYROSINE HYDROXYLASE ACTIVITY BY INCREASING SER40 AND THEN SER 31 PHOSPHORYLATION IN BOVINE ADRENAL CHROMAFFIN CELLS

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Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of the catecholamines. It has been reported that retinol (vitamin A) modulates tyrosine hydroxylase activity by

increasing its expression through the activation of the nuclear retinoid receptors. In this study, we observed that retinol also leads to an acute activation of tyrosine hydroxylase in bovine adrenal chromaffin cells and this was shown to occur via two distinct non-genomic mechanisms. In the first mechanism retinol induced an influx in extracellular calcium, activation of protein kinase C and serine40 phosphorylation, leading to tyrosine hydroxylase activation within 15 min. This effect then declined over the next 2 hour. The retinol-induced rise in intracellular calcium also led to a second slower mechanism; this involved an increase in reactive oxygen species, activation of ERK1/2 and serine31 phosphorylation and the maintenance of tyrosine hydroxylase activation. This occurred for up to 2 hour. No effects were observed with retinoic acid. These mechanisms are likely to operate *in vivo* to facilitate the stress response, especially when vitamin supplements are taken or when retinol is used as a therapeutic agent.

P-80
DIFFERENTIAL EFFECT OF SPECIFIC ANTIDEPRESSANTS ON α_1-ADRENOCEPTORS IN RAT BRAIN

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Alterations in adrenergic neural transmission have been implicated in various behavioral disorders, including Depression and Mania. It is postulated that both α and β adrenergic receptor-mediatedsignal transduction is involved in the mechanism of action of various antidepressants. However, the involvement of subtypes of these receptors is not understood. The present study was undertaken to examine the differential effect of specific antidepressants on the density of α_1-adrenoceptors in rat brain. Density of α_1-adrenoceptors was measured by radioligand binding technique, in cortex and cerebellum of rats treated with Imipramine (IMI), Desipramine (DMI), both TCAs and Floxetine (FLX) a SSRI, for 30 days. The density of cortical α_1-adrenoceptors, measured by using [^{3}H] prazosin, was significantly decreased (25%; $P<0.001$) in DMI treated rats, when compared to control rats, without affecting the affinity of [^{3}H] prazosin. However, in IMI and FLX treated rats the density of cortical α_1-adrenoceptors is not altered. In cerebellum the density of α_1-adrenoceptors was significantly decreased in IMI (37.5%; $P<0.001$), DMI (50%; $P<0.001$) and Floxetine (71%; $P<0.001$) treated rats. The results suggest that chronic AD treatment down regulates α_1-adrenoceptors in rat brain, however the effect is region specific. TCAs like DMI down regulate both cortical and cerebellar α_1-adrenoceptors, whereas, SSRIs like Floxetine, down regulate only cerebellar α_1-adrenoceptors and the extend of down regulation is significantly higher with SSRIs. This could be one of the mechanisms of action of AD and the efficacy of certain ADs.

P-81
THE 5-HT-RELATED MECHANISM UNDERLYING PHYSICAL EXERCISE ATTENUATING STRESS-INDUCED HIPPOCAMPAL DAMAGES

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Physical exercise is benefit for health physiologically and psychologically. Chronic psychological stress is harmful to brain health, especially to the hippocampus. Stress-induced 5-HT system abnormality resulted in depression. Studies revealed exercise and antidepressants have same effects to improve cognitive function. And exercise has been reported to increase 5-HT synthesis and neurotransmission. In the study, exercise was used as preventive intervention to attenuate stress-induced hippocampal damages. Wistar rats were divided into four groups: 4-week voluntary wheel running (exercise, $n=6$), 3-week restraint stress (stress, $n=6$), wheel running 4 weeks then restraint stressed 3 weeks (exercise-stress, $n=6$) and control ($n=6$). Results showed 4-week exercise not only enhanced long-term potentiation (LTP), but also attenuated 3-week stress-induced LTP decrease in hippocampal dentate gyrus (DG). In addition, the hippocampal 5-HT level increased in exercise group, and decreased in stress group. There was not different between exercise-stress and stress groups. To study the related functional proteins involved 5-HT, we found 5-HT1A receptor (5-HT1A-R) mRNA up expressed in exercise group and down expressed in stress group. Additionally, exercise-stress rats' 5-HT1A-R mRNA maintained higher than the stress. As 5-HT1A-R activation regulates the cAMP-response-element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) expression, CREB and BDNF mRNA expressions were detected. Interestingly, they changed as same as the 5-HT1A-R in 4 groups. It is suggested that mechanism underlying exercise attenuating stress-induced hippocampal damages may be close associated with up-activation of 5-HT1A-R -CREB-BDNF pathway, which promotes neuronal survival and activities.

P-82
DECREASED 5-HYDROXYTRYPTAMINE SYNTHESIS AND TRYPTOPHAN HYDROXYLASE EXPRESSION IN SPINAL CORD AFTER EXERCISE-INDUCED FATIGUE

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Objective: The aim of this study was to investigate the changes of 5-hydroxytryptamine (5-HT)and it's synthesis rate-limiting enzyme tryptophanhydroxylase (TPH) in spinal cord after exercise-induced fatigue. To Further study the mechanism of exercise-induced fatigue in spinal level.
Methods: Sixteen healthy adult Wistar rats were randomly divided into 2 groups: exercise-induced fatigue group (E);control group (C). Immunohistochemical staining and quantitative analysis of 5-HT and TPH have been done in spinal cord, the

optic densities of 5-HT and TPH positive fibers or terminals were measured by computerized image analyzer.

Results: Normal rats showed immunoreactive material in nerve cell processes and in a few nerve cell bodies of the ventral horn; After exercise-induced fatigue a marked decrease in 5-HT and TPH immunoreactivity was found in the ventral gray matter of the rats cervical region. Theses immunohistochemical findings were in line with the changes in the contents of 5-HT measured by high pressure liquid chromatography (HPLC) in corresponding spinal segments.

Conclusion: After exercise-induced fatigue, the decreased content of 5-HT and TPH in spinal cord might implicate that biosynthesis of 5-HT in spinal cord might participate in the mechanism and recovery of exercise-induced fatigue.

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INVESTIGATING FUNCTIONAL SELECTIVITY IN RAT 5-HT_{2A} RECEPTOR AGONIST- AND ANTAGONIST-MEDIATED TRAFFICKING

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The serotonin receptor 2A (5-HT_{2A}) has been found to play a pivotal role in a number of disorders including psychiatric diseases and is hence the focus of intense research.The 5-HT_{2A} was one of the first G-protein coupled receptors (GPCRs) where the phenomenon of functional selectivity was described. This refers to a phenomenon whereby different ligands differentially modulate signaling pathways via the same receptor, independently of the intrinsic efficacy and/or potency of the ligand at the receptor. Using the rat 5-HT_{2A} receptor tagged to EGFP, stably expressed in HEK293 cells, we demonstrate another aspect of GPCR functional selectivity in 5-HT_{2A} responses to ligands. The 5-HT_{2A} is differentially internalized and recycled on being exposed to various ligands. We have observed this functional selectivity using a number of receptor agonists such as 5-HT, DOI and dopamine as well as antagonists such as Ketanserin and Clozapine. Furthermore, this differential trafficking is brought about by separate biochemical pathways. Based on the involvement of intracellular molecular players as well as the kinetics of internalization and recycling, we show significant differences between 5-HT_{2A} response to the ligands used.

Plasticity

P-84

STRUCTURAL REQUIREMENTS OF THE SYNAPTOTROPHIN, CBLN1 FOR MULTIMERIC INTERACTIONS AND SECRETION

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Cbln1 is a C1q domain-containing glycoprotein secreted from cerebellar granule neurons and is essential for Purkinje cell synapse structure and function. In cerebellum, Cbln1 exists in complexes containing another family member, Cbln3. Moreover, Cbln1 is essential for Cbln3 to exit the endoplasmic reticulum (ER) and undergo secretion, whereas Cbln1 can be secreted as a homomeric complex in the absence of Cbln3. Here we identify amino acids within Cbln1 that confer these properties. Mutants of Cbln1 containing only the C1q domain were secreted normally, indicating that this region alone specifies assembly and secretion. Next, point mutations were introduced into the C1q domain. Mutations were to a series of phenyalanine residues that are conserved in C1q proteins or they mimicked dominant mutations in the C1q domain of collagen X that cause Schmid metaphyseal chondrodysplasia. Three classes of mutants were identified. The first disrupted protein-protein association resulting in ER retention and proteasomal degradation. A second class of mutant proteins underwent assembly yet were still not secreted and promoted degradation of wild type proteins when they were co-expressed with the mutant. The final group of mutants underwent assembly and secretion and rescued secretion of Cbln3. In conclusion, understanding the structural and functional implications of Cbln1 will provide insights for elucidating the trans-neuronal signaling mechanism of Cbln family members.

P-85

BETA-AMYLOID INDUCED AGE-DEPENDENT CHANGES IN PRESYNAPTIC FUNCTION AND STRUCTURE IN *DROSOPHILA*

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Alzheimer disease (AD) is characterized by a progressive loss in cognitive functions, a process correlated with synapse dysfunction and loss. Beta-amyloid accumulation plays a central role in AD. However, how beta-amyloid accumulation leads to synaptic defect is largely unknown. To address this issue, we have expressed the wild type or arctic beta-amyloid in a subgroup of neurons in the adult *Drosophila* central nervous system, including neurons of the escape circuit that controls flying and jumping. In these beta-amyloid expressing flies (Abeta flies), we found an age-dependent decline of flight ability that correlated with an increased synaptic transmission failure in the escape circuit. Further electrophysiological recording of these Abeta flies showed an age-dependent increase in the susceptibility to activity-induced synaptic fatigue. Electron microscopic examination revealed a significant reduction in the number of presynaptic mitochondria in both young and aged Abeta flies. In addition, we found that synaptic vesicles had a more heterogeneous and generally larger size in the presynaptic terminal of aged Abeta flies. All of the above defects were more severe in flies expressing arctic beta-amyloid than in flies expressing wild type beta-amyloid. More over, the reserve pool (RP) of synaptic vesicles was significantly reduced in aged flies expressing arctic beta-amyloid. We suggest that beta-amyloid accumulation induces presynaptic mitochondria depletion, which in turn causes deficient RP vesicle mobilization and defective synaptic function by depleting the ATP supply. Currently, we are doing a time course analysis of ultra structural changes in presynaptic terminals and their corresponding cell bodies.

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BIDIRECTIONAL CHANGES OF SYNAPTIC TRANSMISSION IN RESPONSE TO SINGLE OR PAIRED PULSE ACTIVATION OF NMDA RECEPTORS IN HIPPOCAMPAL CA1 REGION OF RAT

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The directionality of NMDA-dependent synaptic plasticity is believed to be regulated by the degree of NMDA receptor activation. However, several pieces of work also suggest a role for a temporal factor, implying that the duration of the applied stimulation can influence the direction of the synaptic change. We examined this issue further by an experimental protocol in which paired pulse stimulation (PPS) with a 50 ms interval and basal frequency of 0.1 Hz was temporarily applied to CA1 area of hippocampal slices from 2–3 week old rats, using a low magnesium perfusion solution. In this way, the degree of NMDA receptor activation could be easily switched between two levels. PPS was first tested under conditions of NMDA receptor blockade by AP5. It was found that PPS for 10–60 min led to only a minor depression. In contrast, when PPS was applied in absence of AP5, there was a prominent short-term potentiation (STP), selectively of the AMPA component, followed by a return to baseline. STP was observed with both short and long periods of PPS, peaking at about the same time after 5–10 stimuli but decaying more slowly with the longer periods. Applying AP5 during the STP stabilized the effect, demonstrating that the potentiation was in fact long-term potentiation (LTP) that might be actively reversed in case when

AP5 was not present. We conclude that the duration of NMDA receptor activation is critical in determining the direction of the synaptic change, implying that previous models for LTP/LTD induction are too simplistic.

P-87

TRANSCRIPTIONAL REGULATION OF PSA-NCAM MEDIATED NEURON-GLIAL PLASTICITY IN ADULT HYPOTHALAMUS

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The gonadotropin releasing hormone (GnRH) secreting neurons in the mammal's project principally to the median eminence-arcuate (ME-ARC) region, where they open in the pericapillary space of the primary hypophyseal portal plexus. The present study reports the expression of PSA-NCAM on GnRH neuron cell body and on the glial cells in the medial preoptic area (mPOA) of hypothalamus in the proestrous phase as well as diestrous phase of cycling rats by using dual immunohistofluorescent staining. As regulation of PSA-NCAM in GnRH release is via regulation of PSA biosynthesis by polysialyltransferase enzyme (PST-1), the expression of PST mRNA with GnRH was also studied within GnRH cell bodies by combining fluorescent *in situ* hybridization with immunohistofluorescence and expression of PST mRNA from mPOA using northern blotting. We observed a dynamic temporal upregulation of PSA-NCAM on GnRH cell bodies in proestrous phase, which was accompanied by enhanced PST mRNA expression. The present results may suggest that PSA-NCAM plays permissive role for the structural remodeling of GnRH neuron. Enhanced mRNA expression of PST in proestrous phase suggests that the biosynthesis of PSA on NCAM is regulated at the transcriptional level.

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JUNCTOPHILIN-MEDIATED FUNCTIONAL CROSSTALK BETWEEN RYANODINE RECEPTORS AND SK CHANNELS ESSENTIAL FOR LONG-TERM DEPRESSION IN THE CEREBELLUM

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Functional crosstalk between cell-surface and intracellular ion channels plays important roles in excitable cells and is structurally supported by junctophilins (JPs) in muscle cells. Here we report a novel form of channel crosstalk in cerebellar Purkinje cells (PCs). The generation of slow afterhyperpolarization (sAHP) following complex spikes in PCs required ryanodine receptor (RyR)-mediated Ca^{2+}-induced Ca^{2+} release and the subsequent opening of small-conductance Ca^{2+}-activated K^+ (SK) channels in somatodendritic regions. Despite the expression levels of these channels and overall Ca^{2+}-signaling in dendritic region were normal, sAHP was abolished in PCs from mutant mice lacking JP-3 and JP-4, the neural JP subtypes (JP-DKO), and this defect was restored by exogenously expressing JPs or enhancing SK channel activation by EBIO. The stimulation paradigm for inducing long-term depression at parallel fiber-PC synapses adversely established long-term potentiation in the JP-DKO cerebellum due primarily to the sAHP deficiency. Furthermore, JP-DKO mice exhibited impairments of motor coordination and learning, although normal cerebellar histology was retained. Therefore, JPs support the Ca^{2+}-mediated communication between voltage-gated Ca^{2+} channels, RyRs and SK channels, which modulates the excitability of PCs and is fundamental to cerebellar long-term depression and motor functions.

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CHRONIC ESCITALOPRAM TREATMENT RESTORES IMPAIRED HIPPOCAMPAL LONG-TERM POTENTIATION AND COGNITIVE DEFICITS IN NEONATAL CLOMIPRAMINE MODEL OF DEPRESSION

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Clinical studies show cognitive impairment in depression. Neuroimaging studies in humans revealed that the hippocampus and prefrontal cortex undergo selective volume reduction in major depression. Neonatal clomipramine model is an established animal model of depression that induces behavioral deficits similar to human endogenous depression. Furthermore, neonatal clomipramine administration induces learning and memory impairments, and is associated with cholinergic dysfunction in adult rats [Bhagya *et al.* (2008) Behavioural Brain Research 187:190–194]. The aim of the present study was to evaluate the effect of chronic escitalopram treatment on depression-induced cognitive impairment and hippocampal synaptic plasticity. Male Wistar rat pups were administered with clomipramine (15 mg/kg, twice daily, s.c) from postnatal day 8 to 21 and the induction of depression was evaluated in adulthood. The depressed rats showed impairment in both acquisition and retention in partially baited 8-arm radial arm maze task, decreased hippocampal acetylcholinesterase (AChE) activity, serotonin level and long-term potentiation (LTP) in Schaffer collaterals - CA1 region of the hippocampus. Treatment with escitalopram for 14 days, ameliorated the depression induced cognitive deficits, restored AChE activity, serotonin level and LTP in adulthood. Our results indicate that impaired cognition and hippocampal synaptic plasticity occur as a consequence of depression. These findings demonstrate that early disruption of serotonergic function can have a

deleterious effect on cognition and hippocampal plasticity in the adult life. This work provides a novel perspective into neural basis of depression associated cognitive changes and helps in the development of therapeutic strategies to treat depression related memory dysfunctions.

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DEVELOPMENT OF A NOVEL METHOD FOR THE IDENTIFICATION OF CAMKII BINDING PROTEINS

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Calcium/calmodulin stimulated protein kinase II (CaMKII) is an important regulator of synaptic function. CaMKII is controlled by multi-site phosphorylation and targeting to cellular locations through interactions with specific proteins. CaMKII phosphorylation at Thr286 is well characterised, and recently we identified Thr253 as a new phosphorylation site *in vivo* that enhances CaMKII binding to post-synaptic densities (PSDs) (Migues *et al. J Neurochem* 98:289–99). We hypothesise that phosphorylation at Thr253 or Thr286 differentially regulates CaMKII function *in vivo* by altering the interaction of CaMKII with its specific substrates. To test this, we developed a modified western blot overlay binding assay, using recombinant FLAG-tagged CaMKIIα (wild-type and phospho-mimic mutants Thr253Asp or Thr286Asp), recombinant proteins that are known substrates of CaMKII, and subcellular fractions from rat brain enriched in PSDs, plasma membranes, nuclei or cytosol. CaMKIIα binding to some of the known substrates was demonstrated, with this interaction shown to be dependent on the concentration of substrate and CaMKIIα used. We identified at least twenty distinct proteins in rat subcellular fractions whose ability to bind CaMKII were selectively affected by phospho-mimic mutations. The binding of some proteins were enhanced/blocked by phospho-mimic mutation at 253, but unaffected by 286. Further bands were unaffected by phospho-mimic mutation at 253, but were affected by 286, and still others were affected by both mutations. These results suggest that phosphorylation of CaMKII at Thr253 and Thr286 independently alters binding to specific proteins. This novel modified western blot overlay binding assay is therefore a new tool that is semi-quantitative and can be used to examine the interactions between CaMKIIα and its binding proteins, thereby enabling the characterisation of a new regulatory mechanism of CaMKIIα function *in vivo*.

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NUCLEAR FACTOR OF ACTIVATED T CELLS (NFAT) MEDIATES ACTIVATED-DEPENDENT EXPRESSION OF PROTOCADHERIN8 IN NEURONS

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Transcriptional activation is a key link between neural activity and long-term synaptic plasticity. Little is known about genes responding to this activation whose products effect functional and structural changes at the synapses. Protocadherin8 (PCDH8) is an activity-regulated gene encoding a transmembrance adhesive molecule that is implicate in the induction of long-term potentiation (LTP). In this study, we identified that the neuronal depolatization up-regulates the expression of PCDH8 mRNA via voltage-sensitive L-type calcium channels. Because it requires *de nevo* protein synthesis, it appears that the immediate-early gene (IEG) class transcriptation factors mediate the depolarization-induced PCDH8 expression. *In silico* analyses and reporter assay with serial deletion of PCDH8 promoter region demonstrated that the-380/-137 region is essential for the transcription of PCDH8. These regions contain putative binding sites for nuclear factor of activated T cells (NFAT) and AP-1. Especially, site-directed mutagenesis of NFAT binding site completely abolished PCDH8 promoter activity. In consistent, expression of dominant-negative or NFAT activation inhibitor abolished the depolarization-induced PCDH8 expression. Taken together, these results suggest that NFAT plays an essential role in the regulation of PCDH8 expression, and accordingly NFAT-PCDH8 cascades may be involved in the regulation of activity-dependent long-term changes in the synaptic connections.

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SPATIOTEMPORAL SPECIFICITY IN ACTIVITY-INDUCED PLASTICITY OF DENDRITIC INTEGRATION

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Most neurons in the mammalian brain receive thousands of excitatory synaptic inputs that are widely distributed along the dendritic arbor and activated with varying degrees of synchrony. Summation of unitary synaptic excitatory post-synaptic potentials (EPSPs) at the dendrite is crucial for initiation of the action potential. Patterned neuronal activity, which is known to modify synaptic transmission, has been shown to modulate dendritic structure and ion channel properties. However, whether and how neuronal activity can modify dendritic integrative function is largely unknown. In this study, we examined the efficacy of EPSP summation in hippocampal CA1 pyramidal neuron before and after the induction of long-term synaptic potentiation (LTP) induced by a paired theta burst stimulation (pTBS). We found that the linearity of both spatial summation (summation of synchronous EPSPs from two independent synaptic inputs) and

temporal summation (consecutive EPSPs from the same input) of EPSPs increased following LTP induction, in an input-specific manner. Such enhancement of summation efficacy was critically dependent on the spatial location and arrival timing between synaptic inputs at the dendrite, and was attributed to a local modulation of dendritic channels following LTP induction. Furthermore, these spatiotemporally specific modifications in the EPSP summation linearity differentially enhanced the coincidence detection and temporal integration functions at the distal and proximal dendrites, respectively. Thus, our findings underscore a notion that the activity-induced plasticity of dendritic functions, together with synaptic plasticity, constitutes an integral part of activity-dependent information processing and storage in neural circuits.

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EFFECTS OF 24 HOURS OF SLEEP DEPRIVATION ON LTP AND NEUROGRANIN MRNA EXPRESSION IN HIPPOCAMPUS OF RATS

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Recent evidence indicates that Sleep deprivation (SD) causes impairments in cognitive functions, but the mechanisms are not clear. Our objective is to study the effects of 24 hours SD on long-term potentiation (LTP) and Neurogranin (Ng) mRNA expression of hippocampus in rats. Ng is a postsynaptic brain-specific protein, and is involved in synaptic plasticity through the regulation of calmodulin (CaM)-mediated signalling. Male Wistar rats were divided into two groups: sleep deprivation group (SD group) and control group (C group). After 24 hours of SD by sleep deprivation box, LTP was induced in hippocampal dentate gyrus (DG) by high-frequency stimulation (HFS). One step methods by Trizol was used to extract hippocampus neuronal total RNA. The changes of Ng mRNA expression were detected by SYBRA green - RT-PCR. We found that compared with C group, the changes of amplitude of population spike (PS) were lower in SD group, especially at 25 min, 30 min, 35 min, 40 min, 45 min and 50 min after HFS ($P<0.05$). The expression of Ng mRNA of SD group was significantly lower than that of C group ($P<0.05$). These results indicate that the down regulation of Ng mRNA by 24 h of SD might be relevant to the impairment of LTP in hippocampus, suggesting that Ng plays an important role in cognitive processing during SD and maybe a novel target for intervention studies in future.

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BOTH EVOKED AND SPONTANEOUS NMDA RECEPTOR RESPONSES ARE TUNED BY THE ION PASSAGE THROUGH NMDA CHANNELS DURING SYNAPTIC ACTIVATION

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We have shown that the interaction between the excitatory neurotransmitter glutamate and AMPA receptors is influenced by the ion passage through the receptor channels during synaptic activation. Does this electrodiffusion phenomenon of glutamate influence the activation of other glutamate receptors? Here we studied both evoked unitary and spontaneous NMDA receptor mediated excitatory postsynaptic currents (NMDAR EPSCs), using visualized patch-clamp and imaging technique. We carried out experiment in cultured hippocampal neurons that are not surrounded by dense neuropil. We showed that the decay time ratio of (τ_{+50}/τ_{-50}) are 1.37 ± 0.08 for evoked EPSCs (mean $\pm$ SEM, $n = 15$, $P<0.001$) and 1.38 ± 0.11 for spontaneous EPSCs ($n = 7$, $P<0.01$). This asymmetry does not depend on glutamate uptake. In contrast, a relatively low concentration of glutamate applied diffusely by pressure-puff to the dendrites evoked responses that showed voltage-independent kinetics. Furthermore, reduction of NMDAR EPSCs by a fast-dissociating competitive antagonist was greater at negative than at positive voltages. Thus, the novel mechanism of glutamate electrodiffusion applies not only for AMPARs at some central synapses but also for the NMDARs. It would allow the local postsynaptic depolarization to enhance activation of NMDARs by extending the dwell time of intra-cleft glutamate. It is likely to interact synergistically with the depolarization-dependent attenuation of postsynaptic glutamate transport to facilitate induction of NMDAR-dependent synaptic plasticity. This phenomenon can also play a significant role in the process of neuronal injury/protection.

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THE EXCITATORY NEUROTRANSMITTER GLUTAMATE TUNES ITS INTERACTION WITH AMPA RECEPTORS VIA A NOVEL MECHANISM DURING SYNAPTIC ACTIVATION

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Although ion currents through postsynaptic receptors are small, they can exert a large lateral voltage gradient, i.e. electric field inside the synaptic cleft, raising the possibility that they can affect the dwell time of electrically charged neurotransmitters. The excitatory neurotransmitter glutamate is negatively charged at physiological pH, suggesting that postsynaptic depolarization should in principle retard its escape from the synaptic cleft during synaptic activation. We evoked dendritic AMPA receptor mediated excitatory postsynaptic currents (AMPAR EPSCs) in CA1 pyramidal cells by stimulating Schaffer collaterals in rat hippocampal slices using visualized patch-clamp and imaging technique. We found that the EPSC decay time τ (defined as the area/peak ratio) increased monotonically with depolarization. The ratio between τ recorded at $+40\,mV$ and at $-70\,mV$ (τ_{+40}/τ_{-70}) was consistently above unity (average $\pm$ SEM: 2.17 ± 0.09, $n = 49$, $P < 0.001$). This asymmetry did not depend on the EPSC amplitude, glutamate transport, recording temperature, or on unknown voltage-dependence influences of receptor antagonists. Furthermore, the voltage-dependent asymmetry of the EPSC decay is prominent at distal, but not proximal, synapses in CA1 pyramidal cells. In contrast, the kinetics of receptor-mediated currents evoked by direct application of glutamate is voltage-independent, as are synaptic currents mediated by the electrically neutral neurotransmitter GABA. A novel mechanism of electrodiffusion of glutamate thus may explain, at least in part, why AMPAR EPSCs at some central synapses are retarded by depolarization and why AMPAR EPSCs recorded locally at distal dendrites of CA1 pyramidal cells have faster decays than those at proximal dendrites. The electrodiffusion mechanism can play significant physiological roles by shortening the coincidence detection window of synaptic signaling or by enhancing receptor activation to facilitate synaptic plasticity.

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ROLES AND SIGNALING MECHANISMS OF THE NEURAL-SPECIFIC SHC PHOSPHO-TYROSINE ADAPTOR IN DENDRITIC SPINE MORPHOGENESIS OF HIPPOCAMPAL NEURONS

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Accumulative evidence suggests that various signal transduction molecules are involved in the neuronal activity-dependent remodeling of actin networks in post-synaptic spines. We previously isolated a neuron-specific homologue of phospho-tyrosine adaptor molecule Shc, i.e., N-Shc. In an attempt to explore the possible functions of N-Shc, we generated a line of N-Shc deficient mice, and examined the phenotype. We found that the hippocampal cognitive function such as learning and memory was elevated in the N-Shc gene deficient mice. Consistent with this finding, the hippocampal long-term potentiation (LTP) was elevated in the mutant mouse. As a basis of this functional change, we further found that the N-Shc protein interacted with the NMDA-receptor, possibly depending upon tyrosine phosphorylation by Fyn or Src-related kinases. Thus, N-Shc is a novel neural specific modulator of NMDA receptor function. There is growing evidence that the hippocampal synaptic stimulation modulate actin dynamics at the post-synaptic spines. Therefore, we further wondered whether N-Shc might also be involved in the process of actin structural modifications in the post-synaptic spines. When we overexpressed N-Shc in primary cultured hippocampal neurons, numbers and lengths of dendritic spines significantly diminished. When certain tyrosine residues were replaced by phenylalanine, the mutant N-Shc was unable to modulate the spine size and structure. These results indicate that N-Shc is certainly involved in the activity-dependent synaptic modifications in the hippocampal neurons. We will discuss further additional involvement of p250-Rho GAP/Grit/RICS, and Homer/Vessl/Cupidin in the Shc-dependent modulation of actin dynamics.

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THE KV4.2 MEDIATES EXCITATORY ACTIVITY-DEPENDENT REGULATION OF NEURONAL EXCITABILITY IN RAT CORTICAL NEURONS

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Neuronal excitability can cooperate with synaptic transmission to control the information storage. This regulation of neuronal plasticity can be affected by alterations in neuronal inputs and accomplished by modulation of voltage-dependent ion channels. Here, we report that enhanced excitatory input negatively regulated neuronal excitability. Enhanced excitatory input by glutamate, electric field stimulation or high K^+ increased

transient outward K^+ current, whereas did not affect the delayed rectifier K^+ current in rat cultured cortical neurons. Both the voltage-dependent K^+ channel 4.2 and 4.3 subunits contributed to the increase. The increase in the K^+ current density by Kv4.2 was ascribed to its cytoplasmic membrane translocation, which was mediated by NMDA type of glutamate receptor. Furthermore, enhanced excitatory input inhibited neuronal excitability. Taken together, our results suggest that excitatory neurotransmission affects neuronal excitability via the regulation of the K^+ channel membrane translocation.

Metabolism

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NMDA, KAINATE AND BICUCULLINE ELICIT EFFLUX OF GLUTATHIONE AND OTHER AMINO ACIDS IN RAT HIPPOCAMPUS *IN VIVO*

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In a previous study we have shown that *in vitro* application of kainate and NMDA caused a selective increase in exctracellular concentrations of glutathione, phosphoethanolamine and taurine. Here, we investigated here changes in extracellular amino acids and glutathione levels in the hippocampus of urethane-anesthetized rats by combining *in vivo* microdialysis techniques with local administration of NMDA or bicuculline, or systemic injection of kainate. During intrahippocampal perfusion with NMDA or bicuculline, glutathione increased immediately, and reached a maximum efflux rate after wash-out of the drug. Systemic administration of kainate, a powerful neuroexcitant and structural analogue of glutamate, caused a similar immediate increase of glutathione, reaching maximum at 80 min after the injection. Both NMDA and kainate, but not bicuculline, also led to increases in the concentrations of phosphoethanolamine and taurine. Interestingly, kainate administration caused also a delayed and stable increase of glutamate, not observed with either NMDA or bicuculline. No change was observed in glutamine, aspartate or glycine following any of the applied drugs, demonstrating selectivity of effects. It has previously been shown that the here tested substances have strong neurotoxic and/or convulsant effects. Our finding of an induced glutathione efflux points to a possible mechanism for the neurotoxic effects. The implied release of glutathione from intracellular to extracellular space might contribute to a lack of glutathione intracellularly. The consequential loss of scavenging mechanisms for eliminating reactive oxygen species may then induce cell damage. Our results may also be helpful in understanding the underlying mechanisms of status epilepticus and probably of epileptogenesis.

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LACTATE GENERATED IN BRAIN DURING ACTIVATION IS UNLIKELY TO BE A MAJOR NEURONAL FUEL IN NORMAL CONSCIOUS SUBJECTS

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The possibility of astrocyte-to-neuron lactate shuttling during brain activation is a controversial idea that is very difficult to assess *in vivo*. Astrocytes and neurons may increase glycolytic metabolism during excitatory conditions and both cell types can oxidize lactate. Local lactate oxidation requires proportionate changes in oxygen and glucose consumption and quantitative trapping of labeled products of glucose in amino acids derived from the oxidative pathway. However, these requirements are not satisfied; the oxygen/glucose ratio falls during most studies of brain activation and autoradiographic studies reveal poor trapping of metabolites of glucose during activation. Brain images obtained with [1- or 6-^{14}C] glucose greatly underestimated focal metabolic activation compared to [^{14}C] deoxyglucose (DG) during acoustic stimulation of the conscious rat. Extracellular lactate transiently doubled during stimulation and labeled lactate levels are much higher than labeled CO_2 in interstitial fluid, indicating that oxidation of glucose-derived lactate does not match the rate of lactate generation. During microinfusion, spreading of labeled glucose metabolites within activated brain tissue was 3 times greater than for DG, and glucose label dispersal could be reduced by gap junction and lactate transporter inhibitors. Because spreading of lactate and glutamine increased during sensory stimulation, whereas that of glucose fell, there must be rapid loss of metabolites, consistent with low autoradiographic registration of labeled metabolites of glucose. Low retention of labeled metabolites of glucose, low $^{14}CO_2$ formation, lactate spreading, and metabolite release from tissue are consistent with rapid lactate release from activated tissue, not shuttling to and oxidation by nearby cells.

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THE CHANGES OF LACTIC ACID AND CORTEX MCT GENE EXPRESSION OF RATS DURING CENTRAL FATIGUE INDUCED BY EXERCISE

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To probe the changes of cortex lactic acid and MCT1 (monocarboxylate transporter (MCT), MCT2 and MCT4 gene expression during central fatigue induced by exercise. It was found that lactic acid was the major substrate in brain energy metabolism, produced by astrocytes and released in the extracellular space via MCT1 or MCT4. During prolonged and intense exercises, both the uptake and concentration of lactic acid in brain increased, but it is still unclear that how lactic acid changes during central fatigue and how to affect MCT gene expression to regulate its uptake and transport. In our study, the changes of weight, Hb, BUN and blood lactic acid were determined to estimate rats exercise abilities; SEP, 5-HT and DA/5-HT of cortex were measured to evaluate central nervous system function. According to the comprehensive analysis of above indexes, the central fatigue model was established. Lactic acid content was detected by chemical method and MCT mRNA was measured by real time RT-PCR. During central fatigue, cortex lactic acid contents became elevated to 1.46 ± 0.67 mg/100 g while 0.92 ± 0.08 mg/100 g in the control group ($P < 0.05$); the expression of MCT2 mRNA obviously increased 3.19 ± 1.24 times, but MCT1 and MCT2 present a decrease and a increase tendency respectively with no significant difference. It is indicated that accumulation of lactic acid occurred in central fatigue; The MCT2 mRNA expression was up regulated with the lactic acid increase, which may involve in the mechanisms of lactic acid on central fatigue, While MCT1 and MCT4 may partly participate in the regulation.

Methods

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A NOVEL METHOD OF RNAI APPLIED TO THE NEURON FOR THE GENOME-WIDE RESEARCH

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RNA interference (RNAi) is widely accepted as the simplest way of inhibiting the gene expression to examine its function for the present. However due to the low rate of introducing short interfering RNA (siRNA) into the neuron, it is difficult to measure the RNAi-dependent effect clearly by discriminating the siRNA-untransfected from the siRNA-transfected one. To apply the RNAi widely to neuroscience, this problem should be overcome. Here, we used the cultured cortical neurons derived from the transgenic rat expressing enhanced green fluorescent protein (EFGP) and applied the siRNAs against EGFP and a targeting gene simultaneously to the EGFP-expressing neurons. We also confirmed by quantitative fluorescent immunocytochemistry that the loss of EGFP is closely correlated to that of the target protein in a given neuron. The method we take was much easier to measure the effect of siRNA, in this case, inhibition of axonal growth. In the present study, we demonstrated that fatty acid-binding protein-7 (FABP-7), concentrated in the growth cone, was involved in axonal growth by this method. These results show that this method is simple and useful in wider range of the neuroscience using RNAi, particularly to the genome-wide research.

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A STUDY ON FABRICATION OF NEURAL NETWORK WITH MICRO PATTERNING PEI *IN VITRO*

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In vitro micro-electrode arrays provide a means by planting neural cells on it to record electrical activities. The key point is how the neurons adhere and grow as the MEA design. It is convinced PEI can fabricate satisfied neural network. But it is unknown so far whether can be thought as a high Signal/Noise Ration interfacial material to direct neuron cell bodies to electrodes. To address this problem, three-dimensional microfluidic systems in poly (dimethylsiloxane) (PDMS) were fabricated. And different kinds of substrate patterns were pre-defined on the microelectrode arrays (MEAs) with the help of PDMS. Primary neuronal cell cultures from striatum and substantia nigra regions were completed on MEAs. As a result, the comparisons of these three different macromolecule patterns, i.e., polyethyleneimine, poly-l-lysine and laminin-patterns were done for observing neuronal adhesion and living status. The immunohistochemical results showed many dopaminergic neurons and GABAergic neuron run parallel on the PEI patterned grids. Furthermore, a synaptic structure was found near the cell body. Finally, extracellular electrical signals will be expected to successfully record from both cells placed inside the patterns and present for several weeks. The advantage of this approach is that it can be integrated with microfluidic devices and MEAs to investigate the relationship between these two kinds of coherent neurons and construct an artificial neural network *in vitro*.

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CULTURING ADULT RAT HIPPOCAMPAL NEURONS: THE EFFECT OF LONG-INTERVAL CHANGING MEDIA ON NEURONAL SURVIVAL

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Cultures of embryonic neurons have been used to introduce a model of neurons in physiological situations and investigate pathological conditions in neurodegenerative diseases. However, age-related cellular events would limit this method in adult neurodegenerative diseases studies. Besides, changing medium in short intervals could remove the effects of some released factors from neurons and the environment, which are important in natural condition. Meanwhile, such short-interval changing media which was done in previous cultures model, decreases the effectiveness of this model in the situations that the study on some neuronal secreted factors are important or in those which the influence of some experimental materials on neurons which use manually is investigated. In this study, the method for isolation and culturing adult rat hippocampal neurons with long intervals medium changing has been described. By using Neurobasal ™/B27 culture medium and Papain for neuronal isolation, survival of neurons was increased. Neuronal sprouting and viability were elevated by using Optiprep density gradient separation of neurons from other cell types and debris. The adult neuronal culturing and their regeneration were nearly impossible without FGF2 which has been demonstrated as an important factor for adult neuronal viability. Adding new fresh medium every 4 days and exchanging half of the medium every 8 days, instead of every 4 days, had no detrimental effect on neuronal viability, and also resulted in neuronal survival till 42 days. This investigation shows the possibility of culturing adult neuronal cells and their maintaining in a nearly natural environment for a long period for physiological and pathological studies.