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Poster Presentations

P01: Poster Presentation 1

P01-1

Inhibition of Rac1-derived reactive oxygen species by AMPK decreases blood pressure in a fructose-induced rat model of hypertension

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Nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase and its regulatory subunit, Rac1 (a small GTPase), play important roles in ROS generation in the brain. Recent studies have reported that the activation of AMP-activated protein kinase (AMPK) suppressed oxidative stress. The aim of this study was to examine whether the activation of AMPK in the brain decreased Rac1-induced ROS generation, thereby reducing blood pressure in rats with fructose-induced hypertension. Our results demonstrated that the ROS levels in the nucleus tractus solitarii (NTS) were higher in fructose-fed rats than in control Wistar-Kyoto (WKY) rats. The inhibition of ROS by treatment with an AMPK activator (oral resveratrol) for 1 week decreased the blood pressure and increased the NO production in fructose-fed rats but not control rats. In addition, resveratrol treatment abolished the Rac1-induced increases in the activity of the NADPH oxidase subunits (p67 and p22-phox) and reduced the activity of SOD2, while treatment with an AMPK inhibitor (compound C) had the opposite effect, in the fructose-fed rats. Interestingly, the overexpression of AMPK abolished Rac1 activation and decreased blood pressure by inducing the activities of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and ribosomal protein S6 kinase (RSK) and nNOS phosphorylation in the fructose-fed rats. We conclude that the activation of AMPK decreased blood pressure, abolished ROS generation, and enhanced ERK1/2-RSK-nNOS pathway activity by negatively regulating Racl-induced NADPH oxidase levels in the NTS during oxidative stress–associated hypertension.

P01-2

Fyn tyrosine kinase activates Cdk5 by stabilizing Cdk5 activator p35 but not phosphorylation of Cdk5 at Tyr15

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Cdk5 is a member of cyclin-dependent kinase family. Cdk5 play a role in various neuronal activities such as neuronal migration, synaptic activity and neuron death. Cdk5 is activated by binding to p35 or p39. In contrast to cycling Cdks such as Cdk1 and Cdk2, which are inhibited by phosphorylation at Tyr15, Cdk5 is reported to be activated by phosphorylation. However, it is not known how cycling Cdks and neuronal Cdk5 are oppositely regulated by their Tyr15 phosphorylation. In this study, we have reinvestigated the effect of Tyr15 phosphorylation of Cdk5 on its activation using the COS-7 cell overexpression system and cultured neurons. Tyr 15 phosphorylation was observed only when Cdk5 alone was coexpressed with Fyn. However, The phosphorylation was decreased coexpressed with p35. Further, we found by immunoprecipitation experiments that binding ability of phosphorylated Cdk5 to p35 is lower than unphosphorylated Cdk5. These results indicate that Cdk5 is phosphorylated at Tyr15 by Fyn only when it is a monomeric free form, and is not activated by Tyr15 phosphorylation. Further, phosphorylated Cdk5 at Tyr15 was not in the immunoprecipitates with anti-p35 in neurons. If so, how is Cdk5-p35 activated by Tyrosine Kinases? When Cdk5-p35 was coexpressed with Fyn, the total activity of Cdk5-p35 was increased along with increase in p35, suggesting that, extracellular signals, which activate Tyrosine Kinases, would stimulate the Cdk5-p35 activity by stabilizing p35.

P01-3

New aspects of tau phosphorylation obtained by Phos-tag SDS–PAGE

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Aggregates of hyperphosphorylated tau are commonly seen in brains of tauopathies such as Alzheimer disease (AD), and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Mutations in tau gene are a cause of FTDP-17. Therefore, an understanding of the pathological environment that induces hyperphosphorylation of wild-type and mutant taus is required. Most previous studies have employed phospho-specific antibodies to examine tau phosphorylation, which, while useful, have weakness in quantitative and combinatorial phosphorylation analysis of multiple sites. Here, we applied Phos-tag SDS–PAGE to characterize tau phosphorylation *in vitro*, in cultured COS-7 cells, and tau from AD or FTDP-17 model mice and human brains. *In vitro* and in the COS-7 cell expression system, we focused on tau phosphorylation by Cyclin-dependent kinase 5 (Cdk5). p35-Cdk5 and p25-Cdk5 phosphorylated tau to a similar extent. When P301L or R406W FTDP-17 mutant tau was expressed in COS-7 cells with p35-Cdk5, R406W mutation produced a phosphorylation pattern different from that of wild-type and P301L tau. The P301L mutant tau in JNPL3 P301L tau transgenic mice was hyperphosphorylated only in tau aggregates. Individual Alzheimer patients had different tau phosphorylation levels, and the phosphorylation profiles of Alzheimer and corticobasal dementia patients differed. All data indicate that Phos-tag SDS–PAGE is useful for the characterization of hyperphosphorylated tau in various experimental systems including the brains of tauopathy patients.

P01-4

Aurora-A, a cell cycle regulator, is activated in advanced stage of head and neck cancer and promotes head and neck cancer cell invasion by a neuroprotective factor, osteopontin stimulation

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Head and neck cancer (HNC) is one of the tumors of poor prognosis despite the therapeutic advances in the past few years. It has been reported that amplification and overexpression of Aurora-A takes place in many human carcinomas. However, the clinical significances, cellular effects, and molecular mechanisms by which Aurora-A mediate its invasive effects in HNC still unclear. Through a meta-analysis on 14 microarray studies of HNC in Oncomine databases, we found that Aurora-A expression is significantly higher in tumor tissues. In the present study, we demonstrated that Aurora-A was not only overexpressed in HNC specimens, but also significantly correlated with advanced-T-classification, positive-N-classification, TNM-stage and the poor 5-year survival rate. Stimulation of HNC cells with osteopontin results in an increase in Aurora-A expressions and translocates in centrosome. Functionally, Aurora-A had the abilities to stimulate cell invasion in HNC cells through increase ERK1/2 activity in the presence of osteopontin stimulation. Conversely, depletion of Aurora-A expression by siRNAs suppressed ERK1/2 activity as well as inhibition of cell invasiveness. In addition, treatment with anti-CD44 antibodies in HNC cells not only resulted in a decrease of mRNA and protein levels of Aurora-A and ERK1/2 activity upon osteopontin stimulation, but also affected the abilities of Aurora-A-elicited cell motility. Finally, immunohistochemical and western blotting analysis of human aggressive HNC specimens showed a significant positively correlation among osteopontin, Aurora-A and ERK1/2 activity. These findings suggest that Aurora-A is not only an important prognostic factor but also a new therapeutic target in the osteopontin/CD44/Aurora-A/ERK pathway for HNC treatment.

P01-5

The potential of indole and synthetic derivatives for polyQ aggregation reduction by the enhancement of chaperone and autophagy systems

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In polyQ-mediated disorders, the expansions of translated CAG repeats in the disease genes result in long polyQ tracts in the respective proteins, leading to intracellular accumulation of aggregated polyQ proteins, production of reactive oxygen species and cell death. The molecular chaperones act in preventing protein misfolding and aggregation to inhibit a wide range of harmful downstream events. In the circumstance of accumulation of aggregated polyQ proteins, the autophagic pathway is induced to degrade the misfolded or aggregated proteins. In this study, we used Flp-In 293/SH-SY5Y cells with inducible SCA3 ATXN3/Q_{75}-GFP expression to test indole and synthetic derivatives for neuroprotection. We found the ATXN3/Q_{75} aggregation can be significantly prohibited in Flp-In 293 cells by indole and derivative NC001-8. Meanwhile, indole and NC001-8 up-regulated chaperones and autophagy in the same cell models. Both of them further promote neurite outgrowth in neuronal differentiated SH-SY5Y ATXN3/Q_{75}-GFP cells. Our results demonstrate how indole and derivative NC001-8 are likely to work in polyQ-aggregation reduction, and provide insight into the possible working mechanism of indole compounds in polyQ SCA patients. These findings may have therapeutic applications in a broad range of clinical situations.

P01-6

The role of PIAS1-mediated neuroprotection through HDAC1 sumoylation upon beta amyloid stimulation

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Alzheimer's disease (AD) is a common neurodegenerative disease. The amyloid plaque is a significant feature and biological hallmark in the brain of AD patients. Beta amyloid 1-42 is generated by beta- and gamma-secretase cleavage of the amyloid precursor protein (APP) and beta amyloid aggregates to form the plaques to result in neuronal death eventually. Protein inhibitor of activated STAT1 (PIAS1) is considered as an E3 ligase implicated in protein sumolyation. Previous study has shown that PIAS1 promotes the sumoylation of STAT1 and inhibits the DNA binding of STAT1 to enhance spatial learning and memory in rats. But other roles of PIAS1 implicated in pathogenesis of Alzheimer's disease in the nervous system are barely known. We found that acute beta amyloid 1-42 treatment up-regulates PIAS1 expression and increases ERK1/2 phosphorylation in the hippocampus CA1. Up-regulation of PIAS1 by beta amyloid 1-42 could be eliminated via de-phosphorylation of ERK1/2 by U0126 treatment. Beside, acute beta amyloid 1-42 treatment in CA1 also increased the expression of Mcl-1, an anti-apoptotic gene. This up-regulation of Mcl-1 could be reduced via PIAS1 siRNA micro-injection. Furthermore, acute beta amyloid 1-42 treatment in CA1 increased the sumoylation of histone deacetylase 1 (HDAC1), whereas knockdown of PIAS1 reversed this effect of beta amyloid 1-42. Our results demonstrate that PIAS1-mediated HDAC1 sumoylation regulates Mcl-1 expression through beta amyloid stimulation.

P01-7

Effects of SKF 83959 on rat's operant performance and CREB expression in the mesocorticolimbic dopamine system

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SKF 83959 is a benzazepine compound previously recognized as an atypical dopamine D1 receptor partial agonist. More recently, SKF 83959 is suggested for acting as an agonist on D1-D2 receptor heteromers, whose activation leads to the intracellular calcium release through phospholipase C-mediated phosphoinositide hydrolysis which was independent of the traditionally discovered adenylyl cyclase-mediated signaling cascade. The present study tested the effects of SKF 83959 on rat's operant behaviors maintained by two schedules of reinforcement: the fixed-interval (FI-30 sec) and differential reinforcement of low rate (DRL-10 sec). The two tasks are assumed to comprise different behavioral components and may be differentially affected by psychoactive drug manipulation. SKF 83959 significantly disrupted both types of operant behavior by reducing the responses in a dose-dependent fashion. Rats were re-tested by an effective dose (1.0 mg/kg) on both behavioral tasks that was followed by western blot analysis on brain tissues to compare the levels of cyclic AMP response element binding protein (CREB) expression in the prefrontal cortex, dorsal striatum, nucleus accumbens, and hippocampus. The drug-treated rats of DRL-10 sec exhibited a significant increase in the levels of phosphorylated CREB only in the nucleus accumbens. In contrast, those of FI-30 sec group exhibited significant increases in the levels of phosphorylated CREB in the nucleus accumbens and hippocampus. From the present study, SKF 83959 was found to exhibit dopamine antagonist-like effects for producing operant behavioral impairment. These data also demonstrated the differential signaling regulations of SKF 83959 on rats trained under different schedules of reinforcement.

P01-8

Characterization of hypothalamic adenylyl cyclase type VIII in response to stress

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Adenylyl cyclases (AC) are associated with neuronal transmission and AC8 is the only AC isoform found in the hypothalamus. AC8 knock-out mice showed a decreased anxiety response to stress, suggesting AC8 is related to emotional regulation to stress responses. In the present studies, we tested the hypothesis that AC8 is anatomically and functionally related to stress response. Male Sprague–Dawley rats subjected to electrical footshock were sacrificed at different time courses, and the brain sections were processed for in situ hybridization and/or immunohistochemistry to examine the PVH expression of AC8 transcripts. Footshock upregulated AC8 mRNA expression and cAMP evels in a time-dependent pattern in the paraventricular nucleus of the hypothalamus (PVH), indicating that AC8 is involved in the cellular signaling under stress challenge. Moreover, we found that the AC8 neurons in the PVH express corticotrophin-releasing factor receptor (CRFR) and/or serotonin receptor type 7 (5-HT7), implicating CRF and 5-HT is associated with AC8 signaling via receptor mediated mechanisms. Our findings provide the evidence that AC8 play a role in neuroendocrine cellular responses to neurogenic stress.

P01-9

Precocious development of visual cortical networks by the removal of molecular restraints leads to dysfunctional vision

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Much of our adult behavior is shaped by experiences during the critical period (CP) in early postnatal development. Since its discovery by Hubel and Wiesel, the visual system has been the classical model for activity dependent neuronal plasticity. We hypothesize that external environment influences the dynamic epigenetic state of the brain and in turn impacts CP plasticity. One epigenetic modification is protein methylation. Protein methylation is catalyzed by enzymes called protein arginine methyltransferases (*Prmt*). Interestingly, *Prmt8* is selectively expressed in the central nervous system (CNS), possibly indicating a special role. *Prmt8* is up-regulated in the murine visual cortex, both during developmental CP and experience-dependent when dark-reared. Using a proteome-wide approach (iTRAQ) to compare the synaptic proteins between *Prmt8* mutants and their wildtype counterparts, we identified a number of structural proteins to be significantly upregulated in *Prmt8* knockout mice. One protein, Tenascin-R (TNR), is a main component of perineuronal nets (PNNs). These nets are implicated in maintaining structural integrity of neuronal networks during the CP. Upon closer inspection of dendritic morphology, *Prmt8* knockout mutants display increased spine number and density. This suggests that PRMT8 may act as a molecular brake on structural brakes on plasticity in the visual cortex. Consistent with this hypothesis, removal of PRMT8 causes a drop in strength of V1b neuronal responses and visual acuity in these mice, both functionally and behaviorally respectively. Our findings, at this juncture, suggest that PRMT8 plays a role in synaptic plasticity by regulating structural brakes in the brain.

P01-10

Analysis of postsynaptic densities (PSDs) and postsynaptic membrane rafts (PSRs) in rat forebrain

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Both postsynaptic density (PSDs) and postsynaptic membrane raft (PSR) are isolated from detergent-treated synaptic plasma membrane (SPM). However, PSD and PSR form a complex under certain conditions (Suzuki et al., J. Neurochem. 2011, Liu et al., J. Neurogenetics, 2013). To elucidate the more detailed structural relationship between PSD and PSR, we investigated purification process of PSD (both type I and type II) and PSR from rat forebrain SPM using three different detergents, Triton X-100, *n*-octyl-beta-D-glucoside and CHAPSO at varied concentrations. The synaptic subdomains are separated on sucrose density gradient and analyzed by western blotting and electron microscopy. This type of systematic examination has not been carried out before. Three types of detergents used showed distinct separation profiles of the subdomains. Type I and type II PSD protein markers showed completely different distributional profiles: most of type I and type II PSD proteins were found in insoluble and soluble fractions, respectively. Type I PSDs were recovered in two fractions: pellets and lighter fractions, although distribution was dependent on the detergent concentrations. Different types of association of PSDs and PSRs were observed between type I and type II PSD proteins. These results are valuable in further characterizing and purifying the type I and type II PSDs and PSRs.

P01-11

Nerve guidance channel constructed by Schwann cell seeded aligned chitosan nanofiber

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Schwann cell-seeded guidance channels have been exploited to bridge and guide axonal re-growth across gaps in lesioned nerves. By orienting the Schwann cell growth on aligned nanofibers, we hypothesized that axonal growth can be guided along the designated direction towards the target. Chitosan was the choice scaffold material given its biocompatibility and the tunable susceptibility to biodegradation. Chitosan was dissolved in trifluoroacetic acid/methylene chloride solution and was electrospun onto a high speed rotating drum. Stability of the chitosan fibers in aqueous, physiological environment was achieved with the use of sodium carbonate to neutralize residual acidity in the chitosan fiber preparation. Schwann cells seeded onto these stabilized aligned chitosan nanofibers aligned uniaxially with the chitosan nanofibers. In addition, by seeding dissociated cells of dorsal root ganglia (DRG, E14/15 rats) onto the uniaxially aligned nanofibers, both neurons and Schwann cells were aligned with the uniaxial arrangement of nanofibers. The Schwann cells could be induced to myelinate neurites extending from neuronal cell bodies. The Schwann cell-seeded nanofiber was rolled into a model of nerve conduit. The Schwann cell aligned along the longitudinal axis of the conduit. These *in vitro* results provide proof of principle for pursuing improvement in post-traumatic recovery from nerve injury with use of Schwann cell-seeded uniaxially aligned chitosan nanofibers as a nerve guidance channel. (Supported by ITS/100/10 of the Innovation and Technology Commission, HK Government).

P01-12

Active participation of vasculature in immune-to-brain communication in the sensory circumventricular organs

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Withdrawn by author.

P01-13

Astrocytes in the adult globus pallidus change morphologies in response to motor activities

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The basal ganglia control activities of motor cortex. Recently we found Olig2-expressing astrocytes in the basal ganglionic nuclei including the globus pallidus (GP). Given the distribution pattern and so-called 'tripartite synapse theory', these Olig2+ astrocytes may modulate neuronal activities under exercise stimuli. To test the hypothesis, we first focused on the relationship between astrocytic morphologies and voluntary exercise, using olig2-CreER/ ROSA-EGFP-GAP43 double transgenic mice that express membrane-targeted EGFP in the olig2-positive cells. Mice were divided into three groups; first group of mice were kept in a cage with a locked running wheel to limit the exercise (A-group) and second group of mice were given a free running wheel for voluntary exercise (B-group) for three weeks. Some mice in the B group were kept additional three weeks with locked running wheel (C-group). As expected, the more complex the astrocytic process arborization became after voluntary running, the more EGFP fluorescence per area increased. Taking advantage of this phenomenon, we measured average fluorescence intensity of an astrocyte and extrapolated the complexity of the astrocytic morphology from the intensity. The EGFP-fluorescence intensities of astrocytes in the GP are significantly greater in the B-group (runners) than those of A-group (sedentary mice). Interestingly, fluorescence intensities of C-group tended to decrease, suggesting that the morphological changes may be plastic in response to motor activities. Furthermore, we observed fine processes of olig2+ astrocytes frequently surrounded synapses and construct 'tripartite synapse' in immuno-electron microscopic analysis.

P01-14

Learning induces sonic hedgehog signaling in the amygdala which promotes neurogenesis and long-term memory formation

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It is known that neurogenesis occurs throughout the life mostly in the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricle. Here we investigated whether neurogenesis occurred in the amygdala and its function in fear memory formation. Mice were injected intraperitoneally with 5-bromo-2′-deoxyuridine (BrdU) 2 h before receiving 15 tone–footshock pairings. The number of $BrdU^+/DCX^+$ and $BrdU^+/NeuN^+$ cells was significantly higher in the conditioned mice suggesting that association of tone with footshock induced neurogenesis. To determine the relationship between neurogenesis and memory formation, mice were given cell proliferation inhibitor methylazoxymethanol acetate (MAM). MAM markedly reduced neurogenesis and impaired fear memory formation. Similarly, intra-amygdala infusion of cytosine arabinoside (Ara-C) which interferes with DNA synthesis decreased freezing responses. Sonic hedgehog (Shh), its receptor patched1 (Ptc1) and transcription factor Gli1 protein levels increased at 1 day and returned to baseline at 7 days after fear conditioning. Immunohistochemistry confirmed that Shh^+ cells increased after conditioning. Chronic infusion of cyclopamine (Shh antagonist) through an osmotic pump into amygdala reduced the number $BrdU^+/DCX^+$ cells and decreased freezing responses. Silencing *Shh* gene expression with small hairpin interfering RNA (shRNA) by means of a lentivirus expression system or with a retrovirus vector encoding *Shh* shRNA (Retro-*Shh*-shRNA) which allowed us to knockdown Shh specifically in the mitotic neurons reduced the number of $BrdU^+/NeuN^+$ cells and decreased freezing responses. Taken together, these results suggest that fear learning induces Shh signaling activation in the amygdala which promotes neurogenesis and long-term memory formation.

P01-15

TRIP6 regulates the maintenance of postnatal mouse neural stem cells

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Postnatal neurogenesis persists throughout life in the subventricular zone (SVZ)-olfactory bulb pathway in mammals. Extrinsic or intrinsic factors have been revealed to regulate properties of neural stem cells (NSCs). Thyroid hormone receptor interacting protein 6 (TRIP6) belongs to zyxin family of LIM proteins, which interact with various proteins to mediate cellular functions. However, the role of TRIP6 in NSCs is still unknown. By performing double immunofluorescence staining, we found that TRIP6 was expressed by Sox2-positive NSCs in postnatal mouse SVZ. To study the function of TRIP6 in NSCs, we performed overexpression experiments with neurospheres derived from postnatal day 7 SVZ. We found that TRIP6 increased the sphere size and proliferation of NSCs. To test whether TRIP6 regulates multi-potency in NSCs, we overexpressed and knocked down TRIP6 in NSCs cultured in differentiation condition and found that TRIP6 inhibited NSC differentiation. To further investigate the mechanism of TRIP6 in NSCs, we performed luciferase assay and found that TRIP6 activated Notch signalling, a pathway required for NSC self-renewal. In conclusion, our data suggest that TRIP6 regulates postnatal NSC maintenance. Taken together, our results suggest that TRIP6 regulates NSC maintenance in the postnatal mammalian SVZ.

P01-16

The circadian rhythm of sirtuin mRNA expression in the rat brain

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Sirtuins belong to the third class of deacetylase enzymes, which are dependent on NAD+ for their activityt that is associated with variety of mechanism in mammals. Sirtuins expressed in several tissues and organs involved in systemic metabolism have been clearly reported. However, the studies of sirtuins in the brain, where is the central of nervous system are remain unknown. The aim of this study was to examine the pattern of Sirtuin mRNA expression in the rat hippocampus and striatum which are related to many of neurodegenerative diseases. Rat brains were disected and daily profile of *Sirt1* and *Sirt2* mRNA levels were analyzed using semi-quantitative RT-PCR. Results showed that *Sirt1* and *Sirt2* were expressed in a different pattern of each specific brain areas. *Sirt1*mRNA level displayed a circadian rhythm of expression only in the striatum but not in hippocampus. The highest level of *Sirt1* expression was occurred during night time. The diurnal rhythm of *Sirt2* mRNA expression was found only in hippocampus which peaked at ZT3. These results indicate that the rhythm of Sirtuin may play an important role in circadian rhythms of physiological processes in the specific brain area. [Acknowledgement: TRF(MRG5680016) & Mahidol University]

P01-17

In utero analysis of *miR-3099* role in the development and function of the mouse brain

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MicroRNAs (miRNAs) are small non-coding RNAs about 18–24 nucleotides long that are emerging as key regulator of post-

transcriptional protein synthesis through translational repression or cleavage of target mRNA. A novel miRNA, *miR-3099* was discovered in a small RNA sequencing analysis of a developing (E15.5) mouse brain. The miR-3099 was found expressed throughout the embryo in early development. At E13.5, *miR-3099* expression was restricted to specific regions of central nervous system suggesting a potential role in the development and function of neuronal cells. To confirm the roles of *miR-3099* in the development of the mouse brain, an expression vector carrying precursor sequence of *miR-3099* was constructed for a gain-of-function study *in utero*. The expression vector, pEGP-miR-3099, was transfected into HEK293 cell lines for 24 h. Observation of GFP expression in the transfected cells indicated successful transfection and total RNA was extracted from the cells. Stem-loop RT-qPCR was then performed on the extracted RNA to validate the expression of miR-3099 by the vector. Expression of miR-3099 in pEGP-miR-3099 transfected cells showed significant difference ($p < 0.0001$) compared to expression in null plasmid control and mock control. This validated the expression of miR-3099 from the expression vector pEGP-miR-3099. Subsequently, *in utero* electroporation was performed using pEGP-miR-3099 into ventricles of developing E13.5 mouse embryonic brain. The electroporated brains were then harvested after 2 days and expression of miR-3099 was confirmed by GFP expression.

P01-18

Spatiotemporal expression profiling and molecular characterisation of *miR-344b* and *miR-344c* in the developing mouse brain

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MicroRNAs are small non-coding RNAs of about 22 nucleotides that regulate gene expression through inhibition or repression processes during post-transcriptional or translational stages. Studies have shown that miRNAs play a crucial role in spatiotemporal regulation of the brain development. A recent study suggested that *miR-344* family is globally expressed in a developing mouse brain. In this study, we focused to characterise the expression of *miR-344b* and *miR-344c* during the development of mouse brain. Results have shown that both *miR-344b* and *miR-344c* are strongly expressed in the germinal layer during the early stages of mouse brain development via *in situ* hybridisation. Later at P1, both miRNAs are highly expressed in the cerebral cortical layer, cerebellum and olfactory bulb of the brain. Interestingly, in mature adult brain, *miR-344b* was not expressed whereas *miR-344c* was highly expressed, particularly in the olfactory bulb. We then further profiled the expression level of these miRNAs in the brain and other multiple organs via RT-qPCR. The expressions of these miRNAs in the brain and in contrast to other tissues were performed via RT-qPCR. Bioinformatics analysis was employed to identify the downstream target genes of *miR344b* and *miR-344c*. Initially, *miR-344b* and *miR-344c* were found to target a total of 1540 and 863 genes respectively. Only genes known to be identified by 3 independent bioinformatics tools and also associated with transcription regulation and nervous system development were screened further. We found that the gene that fulfilled these criteria and targeted by *miR344b* and *miR-344c* was *Olig2* and *Otx2* respectively. These targeted genes will be validated via Luciferase assay. In conclusion, *miR-344b* and *mir-344c* are expressed in the developing mouse brain and may play a crucial role during brain development and function.

P01-19

The effects of *kctd 12.1* in brain lateralization by using zebrafish model

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Recent studies suggested the mutant of KCTD family is related with emotion and memory of human. The telencephlic region in zebrafish has been proposed as homologous to the mammalian hippocampus and amygdala. *Kctd12.1(lefty1,lov)* has been proven asymmetry distribution on habenula of zebrafish in early developmental stage. We use morpholino(MO) to block the mRNA translating to protein, then observe the behaviours of larvae. The results show that the 6 day post fertilization (dpf) and 8 dpf of MO groups have different performance in mirror viewing task compare with control groups. But there is no significant difference in the locomotor activity. And there are no differences on 14 dpf between both groups. The study pointed out that we confirm that *kctd12.1* may play an important role on the development of zebrafish brain lateralization in early developmental stage.

P01-20

Keratan sulfate regulates mouse spinal cord development by modulating a sonic hedgehog signaling

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In the embryonic spinal cord, Wnt and Shh act as morphogen. They are involved in the patterning of the spinal cord. Wnts and Shh bind to acidic sugar chains. We hypothesized that the interaction between morphogens and acidic sugar chains play essential roles in the pattern formation of embryonic spinal cord. In this study, we analyzed involvement of keratan sulfate (KS). First, we analyzed localization of KS in the embryonic spinal cord. Highly sulfated KS was expressed in the floor plate and the notochord. This expression pattern colocalized with Shh expression. Next, to estimate roles of KS, we analyzed the KS null mouse. At E12.5, the domain structure, including the pMN domain, shifted ventrally in the KS null mice, whose formation of is controlled by Shh signaling. Patched1, Shh

signaling reporter gene, expression pattern in the pMN domain was different between WT and KS null mice at E12.5. We analyzed cell types generated from the pMN domain. The pMN domain generates motorneurons and subsequently oligodendrocyte. Oligodendrocyte precursor cells were hardly generated in the E12.5 spinal cord. Moreover, motor neuron production detected by Islet1/2 expression was increased. It is likely that the switch from motor neuron production to oligodendrocyte production is delayed in KS null mice. Taken together, our study suggests that KS plays important role in the pattern formation and oligodendrocyte development in the embryonic spinal cord.

P01-21

Chemical exposure and nutritional deficiency induced pregnancy outcomes and neuropsychiatry development of children in India

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Developing fetuses and infants are exquisitely sensitive to environmental chemicals which may disrupt the specific developmental processes and cause neurodevelopmental disabilities. At any stage during brain development alteration or disruption in the process imposed by environmental toxins & deficiency of iodine may affect its functioning leading to behavioral/functional abnormalities. Present study considers the environmental as well as nutritional factors to associate the changes in the developing children and fetus. Detailed history about any antecedent medical facts was collected from all women, and the necessary clinical examination was done to understand the interdependence of neuropsychiatric development and life style factors. Determination of maternal thyroid function at the end of each trimester by estimation of total T3 (TT3), total T4 (TT4), free T3 (FT3), FT4, and TSH levels. Affect of socialization on the development of children. Exposure to a number of chemicals may adversely affect child development through altered endocrine function. Variations in the urinary iodine excretion during pregnancy were recorded demonstrating physiological adaptation allowing energy conservation. Iodine is an important requirement during pregnancy as it effects the formation of thyroid & thus affects neurodevelopment of fetus directly. Environmental factors & life style plays a very significant role in the neuropsychiatric behavior of children. The studies are is in progress to understand the details mechanism of neurobehavioral development in pesticide exposed children.

P01-22

Effect of amla extract on cerebral ischemia reperfusion injury in rats

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Reactive oxygen species (ROS) contribute to brain damage after ischemia reperfusion (IR) which is responsible for neuronal death in cerebral stroke. ROS generation cause oxidative damage to essential proteins, DNA, and other mitochondrial components. Lipid peroxidation during brief period of ischemia plays a pivotal role in triggering the ischemic neuronal damages. Lipid peroxidation diminish the anti-oxidant system and also disrupt the blood brain barrier. The aim of this study was to investigate the neuroprotective effects of amla extract on ischemia reperfusion injury in rats. Rats were treated with amla extract and then subjected to cerebral ischemia induced by a middle cerebral artery occlusion (MCAO). The infarct volume and neurological deficit were determined by 2,3,5 triphenyltetrazolium chloride (TTC) staining and Longa's score. Blood brain barrier integrity and Brain water content was measured to assess the neuroprotection. Lipid peroxidation levels were evaluated by malondialdehyde assay and Glutathione (GSH) level was evaluated by 5,5-dithiobis-2-nitrobenzoic acid (DTNB) which is readily reduced by sulfhydryls forming a yellow substance which was measured at 412 nm. Amla extract significantly reduced infarct volume, ameliorated the neurological deficit and blood brain barrier dysfunction. The biomarker of oxidative stress malondialdehyde (MDA) was also found to be significantly reduced following amla extract administration and increased the level of the antioxidant enzyme glutathione (GSH). These results show that amla extract has a preventive effect against cerebral stroke in animal model.

P01-23

Sulfuretin suppresses inflammatory responses by blocking the NF-κB and AP-1 translocation signaling pathways in BV-2 microglial cells

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Sulfuretin, a flavonoid from the stem bark of Albizzia julibrissin and heartwood of Rhus verniciflua, has been shown to possess antioxidative, anti-inflammatory, and anti-cancer properties. In this study, we studied the signaling pathways involved in the anti-inflammatory effects of sulfuretin as well as their influence on the expression of several genes known to be involved in inflammation using BV-2 microglial cells. We found that sulfuretin inhibited LPS-stimulated pro-inflammatory responses by suppressing the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2). Also, sulfuretin inhibited inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) at both the protein and mRNA levels. In addition, in LPS-stimulated BV-2 microglial cells, sulfuretin inhibited pro-inflammatory cytokines and chemokines, as well as reactive oxygen species (ROS) production. Subsequent mechanistic studies revealed that sulfuretin inhibited LPS-induced activation of nuclear factor-kappa B (NF-κB), activator protein-1 (AP-1) translocation, phosphorylation of mitogen-activated protein kinases (MAPKs), and phosphatidylinositol 3-kinases (PI3K)/Akt, as well as the Janus kinase1 (JAK1)/ signal transducer and activator of transcription (STAT)1, which are upstream molecules responsible for controlling inflammatory reactions. These results suggest that sulfuretin may exert anti-neuroinflammatory responses by suppressing the LPS-induced expression of pro-inflammatory mediators via a blockade of the activation of NF-κB, AP-1, MAPKs, and PI3K/Akt, as well as JAK1/STAT1 signaling pathways in BV-2 microglial cells. Therefore, sulfuretin may provide a novel therapeutic approach for the prevention and treatment of neurodegenerative diseases.

P01-24

Synergistic effects of two old medicines on neurodegeneration and cognitive dysfunction in MPTP-induced Parkinson's disease rat model

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Parkinson's disease (PD), a neurodegenerative disorder, shows cognitive impairment in the early phase of the disease. Neuroproection of CFT and EPX has been reported in recent studies. Wistar rats were induced with PD by stererotaxically perfused with MPTP in the substanatia nigra pars campacta (SNc). CFT (5 and 10 mg/kg), EPX (100 and 250 IU/kg), and combination of CFT (5 mg/kg) and EPX (100 IU/day) were daily administered, i.p., after MPTP lesioning. A battery of behavioral tests, bar test, T-maze test, and object recognition test was taken after MPTP lesioning, for measuring motor function, working memory, and object recognition, respectively. The brain was then taken for histological assay. Motor dysfunction was observed on the next day after MPTP lesioning and was spontaneously recovered to control level in a week. CFT and EPX treatment prevented MPTP-induced working memory deficits. CFT treatment prevented MPTP-induced object recognition dysfunction. The combination of CFT and EPX showed higher therapeutic effect. This phenomenon was observed in neuroprotection in the hippocampal CA1 area and dopaminergic system. This study demonstrated that combination of CFT and EPX reveals synergistic effects on preventing cognitive dysfunction and neurodegeneration in MPTP-induced PD rat model.

P01-25

Alteration in adenylate cyclase activity mediated by coexpressed mu-, kappa-opioid and nociceptin receptors in HEK 293 cells following buprenorphine exposure

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Buprenorphine is used in maintenance therapy for heroin addicts. It is a mu-opioid (MOP) receptor partial agonist and a potent kappa-opioid (KOP) receptor antagonist as well as a nociceptin/opioid receptor-like 1 (NOP) receptor agonist. In this study, we established an *in vitro* cell model overexpressing human MOP, KOP, and NOP receptors individually or simultaneously in human embryonic kidney (HEK) 293 cells, and compared the effects of U-69593, DAMGO, nociceptin, and buprenorphine on adenylate cyclase (AC) activity in these cells (KOP, KOP+MOP, KOP+NOP, and KOP+MOP+NOP). Saturation radioligand binding assay using [^{3}H]-diprenorphine was performed to verify surface expression of KOP receptor. After acute exposure, U-69593 inhibited AC activity in all four stable clones, showing that KOP receptor was successfully expressed. Acute application of DAMGO and nociceptin could elicit AC activity inhibition in cells expressing MOP and NOP receptors, respectively. Buprenorphine, when applied acutely, was able to inhibit AC activity to about 90% of the E_{max} in cell expressing MOP, NOP and KOP receptors simultaneously, which is more efficacious than the effects obtained in the other three stable clones. Chronic exposure to buprenorphine induced AC superactivation in cells coexpressing KOP and NOP receptors, and the level of AC superactivation was further elevated in KOP+MOP+NOP-expressing cells. The study demonstrated that MOP receptor might act as an enhancer in AC superactivation in HEK 293 cells coexpressing KOP, MOP and NOP receptors during long-term exposure to buprenorphine.

P01-26

The effect of soluble epoxide hydrolase inhibition on seizure generation in two mouse models of temporal lobe epilepsy

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Some inflammatory signaling has been upregulated following an epileptogenic injury, and they may persist during the latent phase that precedes spontaneous recurrent seizures (SRS). It has been proposed that cerebral inflammation, besides contribution to ictogenesis, may play a role in the process of epileptogenesis. Epoxyeicosatrienoic acids (EETs) are the metabolites of arachidonic acid via cytochrome P450 epoxygenases, which regulate cerebral blood flow and offer anti-inflammatory and anti-apoptotic effects. Soluble epoxide hydrolase (sEH) is a key enzyme in the metabolic conversion of EETs into their less active form, dihydroxyeicosatrienoic acids. Our preliminary data showed that inhibition of sEH hydrolase activity by 12-(3-adamantan-1-yl-ureido) dodecanoic acid butyl ester (AUDA) attenuated neuroinflammation and the frequency and duration of SRS *in vivo*, suggesting that sEH may play a crucial role in the ictogenesis. We hypothesized that sEH inhibition might affect ictogenesis via modulating neuroinflammatory responses. Pretreatment of a single dose of AUDA (80 mg/kg) was performed in two mice ictogenesis models: pilocarpine induction and electrical kindling. Their electroencephalography and behavior responses were recorded and analyzed. We found the seizure severity of epileptic mice was attenuated. The onset time to Racine scale stage 1 were prolonged in both models when the mice were pre-treated with AUDA. Our results demonstrated that inhibition of sEH hydrolase activity reduced the process of ictogenesis in both mice models. Therefore, sEH may play an important role in the generation of epilepsy, suggesting that further studies are warranted to explore its potential for clinical use in the future.

P01-27

The effects of melatonin on dexamethasone exposure-induced changes in melatonin receptor of adult mouse hippocampus

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Dexamethasone (DEX), a potent glucocorticoid receptor (GR) agonist, may mimic the effects of GR possession. The chronic administration of high doses of DEX impairs long-term memory, reduces body weight, decreases neurogenesis and induces onset of depressive-like behavior in mice. Melatonin, a hormone mainly synthesized in the pineal gland, is a potent free radical scavenger, antioxidant and antidepressant. Melatonin plays various physiological functions via the melatonin receptor (MT1). It may be interesting to explore the mechanism of melatonin especially MT1 that is associated with the role of stress as a key factor to precipitate depression and memory impairment. In the present study, we investigated whether melatonin could counteract the effects of chronic DEX treatment for 21 days on body weight and MT1. Our results showed that the body weight and MT1 were significantly reduced in the DEX-treated mice. Pretreatment with melatonin was found to restore body weight. Moreover, melatonin pretreatment prevented the reduction of MT1 protein expression. This finding indicates that MT1 may underlie mechanism of chronic stress. However, the precise mechanism by which melatonin possesses anti-stress requires further investigation.

P01-28

Angiotensin II type 1 receptor blocker attenuates cell growth *in vitro* and *in vivo*

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Angiotensin II type 1 receptor blocker (ARB) are not only extensively used for the treatment of cardiovascular disease, but also play a role of neuroprotection in microglia cell. However, there are few reports demonstrated that how ARB effects the survival of cancer cells. In the present study, we found that irbesartan, an ARB, suppressed cell growth of oral cancer cells in a dose- and time-dependent manner by MTT and soft agar assay. Flow cytometry was used to determine whether the irbesartan induced apoptosis. By time-lapse confocal microscopy we observe the viability of cancer cells under irbesartan treatment. Furthermore, we also administered irbesartan to nude mice with experimental tumors to determine its *in vivo* effects and toxicity. Functionally, irbesartan inhibited angiotensin II-elicited FLJ10540 expression, a cell growth related protein, in oral cancer cell lines by Q-RT-PCR, western blotting and immunofluorescence approaches. The relationship between irbesartan and FLJ10540 was also confirmed in nude mice model by immunohistochemical staining. Our results suggest that irbesartan might be a new therapeutic option for the treatment of human cancer cells.

P01-29

Gender differences in serum adipokines, appetite-related neuropeptides, and anthropometric indicators of the risk of metabolic syndrome

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Although increased body weight is associated with the metabolic syndrome (MetS), MetS does not occur in all overweight or obese individuals, and prevalence rates of MetS differ between genders. We aimed to investigate the association of serum adipokines, appetite-related neuropeptides (ARNPs), and anthropometric indicators with the risk for MetS in Taiwanese adults of both genders. Subjects in this cross-sectional study were recruited from the health examination centers and metabolic clinics located in 2 medical centers in northern Taiwan. Overall, 171 subjects (51 men and 120 women), of whom 81 were of normal weight and 90, overweight as per the Taiwanese BMI index, participated. One hundred and fifteen subjects did not have MetS according to the Department of Health Taiwan definition, while 56 did. Levels of serum adipokines (leptin, adiponectin, visfatin, and resistin) and ARNPs (ghrelin and obestatin), anthropometric indicators (body mass index, bioelectrical impedance analysis, and waist-to-hip ratio), and metabolic serum parameters were analyzed. Positive associations were identified between anthropometric indicators and the risk of MetS. Serum triglyceride, high density lipoprotein-cholesterol (HDL-C), total cholesterol (T-CHOL)/HDL ratio, aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), high-sensitivity C-reactive protein (HS-CRP), and adiponectin levels were associated with MetS in women, whereas triglyceride, HDL-C, and T-CHOL/HDL ratio showed significant effects on MetS in men. This study demonstrates that the effects of serum adipokines and ARNPs on MetS are different between men and women after controlling for gender, body weight and BMI.

P01-30

Mangosteen pericarp improves spatial memory retrieval in triple transgenic Alzheimer's disease mice through pleiotropic intervention

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Mangosteen (Garcinia mangostana) is a tropical fruit native in Southeast Asia and reported to contain multiple health promoting properties. In this study we investigated the effects and molecular mechanisms of Mangosteen pericarp powder (MP) in AD mice. First, the pre-treatment of MP in the mouse hippocampal slice culture induced neuroprotective effect against the neurotoxicity of oligomeric Aβ42 via increasing BDNF level. We further applied MP to the 3 × Tg-AD mice from 5 to 13 months of age. We found dietary MP supplement improved mouse spatial memory retrieval associated with protected hippocampal pyramidal neurons. Further pathological and molecular characterization revealed that MP

increased the molecules associate with anti-oxidative stress, cognitive-related pathway, neurotrophic, and calcium binding protein. MP also reduced inflammatory response (IL-6, pp38, and COX2) and astrogliosis and the levels of Aβ42, APP, BACE, active form of GSK3β, and p-tau (262/202) in mouse hippocampus. These results reveal that the multifunctional properties of MP supplement might have the potential to delay the progression of AD.

P01-31

Adrenomedullin activates baroreflex response via Akt-dependent mechanism in the nucleus tractus solitarii of rats

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Adrenomedullin (ADM), a 52-amino acid vasoactive peptide, is critical for the central regulation of cardiovascular functions. We have previously demonstrated that ADM in the nucleus tractus solitarii (NTS) enhances baroreflex response (BRR) through the mechanism mediated by protein kinase A (PKA)-dependent activation of neuronal nitric oxide synthase (nNOS). In NTS, phosphatidylinositol 3-kinase (PI3K)/Akt/nNOS pathway is reported to play an important role in central cardiovascular regulation. This study was thus undertaken to examine the hypothesis that Akt may mediate the PKA-dependent nNOS activation by ADM in the NTS. Male, adult Sprague–Dawley rats used in this study were anesthetized with pentobarbital sodium. We found that BRR enhancement by bilateral microinjections of ADM into NTS was significantly reduced after pharmacological inhibition of PI3K/Akt signaling. Furthermore, ADM-elicited increase of phospho-nNOS (Ser1416) level was attenuated by co-administration of PI3K, Akt, or PKA inhibitors. These results suggested that a PKA-dependent activation of PI3K/Akt signaling participates in the activation of nNOS, which in turn mediates ADM-induced BRR enhancement. This novel signaling pathway activated by ADM was substantiated by the co-localization of Akt and nNOS proteins in NTS neurons. In conclusion, these results suggested that ADM enhances baroreflex response though PKA-dependent PI3K/Akt/nNOS signaling pathway in the NTS of rats. (Supported by the grants NSC 99-2314-B-010-012-MY3 and NSC 102-2314-B-010-020).

P01-32

Role of alpha4- and alpha6-containing nicotinic receptors in the acquisition & maintenance of nicotine self-administration

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Tobacco smoking is a major cause of death and disease and as such there is a critical need for the development of new therapeutic approaches to treat nicotine addiction. Here we utilise genetic and pharmacological tools to further investigate the nicotinic acetylcholine receptor (nAChR) subtypes that support intravenous self-administration of nicotine. α4-S248F mice contain a point mutation within the α4 nAChR subunit which confers increased sensitivity to nicotine and resistance to mecamylamine. Here we show that acute administration of mecamylamine (2 mg/kg, i.p.) reduces established nicotine self-administration (0.05 mg/kg/infusion) in WT, but not α4-S248F heterozygous mice, demonstrating a role for α4* nAChRs in the modulation of ongoing nicotine self-administration. Administration of *N,N*-decane-1,10-diyl-*bis*-3-picolinium diiodide (bPiDI), a selective α6β2* nAChR antagonist, dose-dependently (5 and 10 mg/kg, i.p.) impairs the acquisition of nicotine self-administration, and reduces established nicotine self-administration in WT mice when administered acutely (10 mg/kg, i.p.). This was not due to a general reduction in locomotor activity and the same dose of bPiDI did not affect operant responding for sucrose. bPiDI treatment (10 mg/kg, i.p.) also impaired both the acquisition and maintenance of nicotine self-administration in α4-S248F heterozygous mice. This provides further evidence for the involvement of α6β2* nAChRs in the reinforcing effects of nicotine that underlies its ability to support ongoing self-administration. Taken together, selective targeting of α6β2* or α4α6β2* nAChRs may prove to be an effective strategy for the development of smoking cessation therapies.

P01-33

Melatonin attenuates methamphetamine toxicity-induced increase in mitochondrial fission proteins, Bax and cytosolic calcium levels and cell death in neuroblastoma SH-SY5Y cells

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Methamphetamine (METH) is an addictive drug that can cause toxicity and degeneration in the brain. Several pieces of evidence have demonstrated that METH-toxicity exhibit increase in oxidative stress, which regulates intracellular signaling cascade leading to cell death. METH is a lipophilic substance that can cross plasma membrane and sub-cellular membrane to several intracellular compartments including the mitochondria. Recently, several studies have been emphasized that mitochondrial fission into a small mitochondrial structure and overload of cytosolic calcium levels play some roles in cell death processes. In this study, we aimed to investigate the effects of METH toxicity on cell viability, mitochondrial fission and cytosolic calcium overload in neuroblastoma SH-SY5Y cells. In addition, the protective effect of melatonin against METH-induced toxicity was also investigated. The results of the present study demonstrated that METH significantly decreases cell viability and increases the levels of mitochondrial fission (Fis1 and Drp1) proteins and Bax in isolated mitochondria. METH also significantly increased the cytosolic calcium levels. Melatonin can reverse the toxic effects of METH on reduction in cell viability, increase in mitochondrial fission proteins and Bax levels in isolated mitochondria. In addition, melatonin was able to decrease METH-induced increase in cytosolic calcium levels in SH-SY5Y cells. The results of the present study demonstrate the potential effects of melatonin to maintain the homeostasis of mitochondrial dynamics and cytosolic calcium levels in METH-induced toxicity in neuronal cells. (Supported by a research grant from Mahidol University and the Thailand Research Fund-Royal Golden Jubilee Ph.D. Program).

P02: Poster Presentation 2

P02-1

Sigma-1 receptor stimulation by fluvoxamine rescues depression-like behaviors in CaMKIV null mice

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Lack of activity-dependent promoter IV-driven expression of BDNF leads depression-like behavior in mice (Sakata K et al., Genes, Brain and Behavior 2010;9:712-721). Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and IV (CaMKIV) are involved in the activity-dependent BDNF, especially in the promoter IV-driven BDNF expression in the hippocampus. As reported, CaMKIV null male mice exhibited depression-like behaviors with concomitant decreases in the promoter I- and -IV driven BDNF expression. The depression-like behavior in CaMKIV null mice was closely associated with impaired neurogenesis in the hippocampal dentate gyrus. We found that fluvoxamine but not paroxetine improves the depression-like behavior in mice concomitant with improvement of BDNF expression and neurogenesis in the hippocampus. Importantly, the fluvoxamine-induced improvements of neurogenesis and depression-like behaviors are totally inhibited by sigma-1 receptor antagonist, NE-100. Consistent with the crucial role of sigma-1 receptor, SA4503, a selective agonist improved the depression-like behaviors in CaMKIV null mice. The improvement of depression-like behaviors by fluvoxamine and SA4503 was associated with BDNF expression following activation of CaMKII and protein kinase B (Akt) in the dentate gyrus. Taken together, CaMKIV exhibits SSRI-resistant depression-like behaviors, which is improved by sigma-1 receptor agonists.

P02-2

Nitric oxide synthase activation is involved in midkine-mediated cardiovascular effect in the nucleus tractus solitarii

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The renin-angiotensin system plays a central role in the regulation of blood pressure. Neuronal nitric oxide synthases (nNOS) is distributed throughout the central nervous system and has been proposed to modulate neuronal activity in the nucleus tractus solitarii (NTS). Previous studies demonstrated that Ang II may modulate central blood pressure effects via reactive oxygen species to downregulate ERK1/2, RSK and nNOS in the NTS. Recent evidence has suggested that midkine is produced in the lung and in turn upregulates angiotensin-converting enzyme (ACE) expression. However, the signaling mechanisms involved in midkine-mediated depressor effects in the NTS remained uncertain. Hence, the aim of this study was to investigate these signaling mechanisms. Male Wistar–Kyoto rats (WKY) were anesthetized with urethane, and blood pressure was monitored intra-arterially. Microinjection of midkine into the NTS produced a dose-dependent decrease in blood pressure and heart rate, and increased nitrate levels in anesthetized WKY. We also evaluated the cardiovascular effects of midkine before and after microinjection of the *N*-nitro-L-arginine methyl ester (L-NAME), NOS non-selective inhibitor in the NTS. The depressor and bradycardic effects of midkine were significantly attenuated by L-NAME. In addition, the depressor and bradycardic effects of midkine were significantly attenuated by MK801, non-competitive *N*-methyl-D-aspartate (NMDA) antagonist. In contrast, ACE inhibitor, Lisinopril, did not diminish these midkine-mediated effects. We further investigated what kinds of the NOS regulated central cardiovascular effects through midkine in the NTS. Taken together, these results suggest that the midkine regulated central cardiovascular effects through NOS and NMDA receptor mediation in the NTS.

P02-3

Prenatal stress alters hippocampal synaptic plasticity in young rat offspring through preventing the proteolytic conversion of pro-brain-derived neurotrophic factor (BDNF) to mature BDNF

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Prenatal stress (PS) has been associated with a higher risk of development of various neurological and psychiatric disorders later in life, but the underlying mechanisms are not yet fully understood. Here, using a chronic prenatal restraint stress model where the rat dams were immobilized for 45 min three times per day during the last week of pregnancy, we explored the long-lasting effects of PS on hippocampal synaptic plasticity in the offspring of both sexes. We found that PS switched the direction of synaptic plasticity in hippocampal CA1 region, favouring low-frequency stimulation-induced long-term depression (LTD) and opposing the induction of long-term potentiation (LTP) by high-frequency stimulation in young (5-week-old) rat offspring, but these changes disappeared at adult age (8 weeks old). Fostering of PS offspring to control dams did not alter the effects of PS on LTP and LTD. In addition, PS-induced changes in LTP and LTD induction were correlated with increasing endogenous pro-brain-derived neurotrophic factor (pro-BDNF) and decreasing of the mature form of BDNF (mBDNF) levels. Furthermore, PS resulted in a significant decrease in the activity and expression of tissue plasminogen activator (tPA), a key serine protease involved in the extracellular conversion of pro-BDNF to mBDNF. No significant differences were observed between the sexes for the effects of PS on hippocampal synaptic plasticity, the levels of pro-BDNF and mBDNF, and tPA expression. These results suggest that PS downregulates tPA levels within the hippocampus, inhibiting the proteolytic conversion of pro-BDNF to mBDNF, thereby leading to long-lasting alterations of the properties of synaptic plasticity.

P02-4

Studies on a novel sialylated N-glycan expressed in the mouse brain

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Glycosylation of proteins is one of the major posttranslational modifications. N-glycans harbored on glycoproteins profoundly affect the character of proteins by altering their structure or capacity to bind to other molecules. Sialic acid is an acidic monosaccharide present at the non-reducing terminal of sugar residues attached through α2,3-, α2,6- or α2,8-linkage. Some Sialic acid binding Ig-like lectin (Siglecs) are crucial for exerting brain function. We refined a method to purify and detect trace amounts of N-glycansand analyzed sialylated N-glycans from the mouse brain. In this study, we identified 6-sialyl-LewisC ([Galβ1,3 (NeuAcα2,6)GlcNAc-]), a novel sialylated N-glycan structure, in the mouse brain. Glycoproteins harboring 6-sialyl-LewisC and Siglec that recognize 6-sialyl-LewisC have not been identified. Thus to identify a glycoprotein that has 6-sialyl-LewisC, membrane proteins of mouse brain were separated by 2D-PAGE. After CBB staining, N-glycans were extracted from protein spots and analyzed by HPLC. Finally, we detected Calreticulin (CRT) as a candidate for a glycoprotein that harbors 6-sialyl-LewisC. CRT mRNA and protein were mainly detected in neuron using in situ hybridization and immunohistochemistry. We also try to identify Siglec that recognize 6-sialyl-LewisC by using Surface Plasmon Resonance. Result of this experiment shows one Siglec might interacts with 6-sialyl-LewisC. Cell surface CRT and this Siglec are known to involve in the immune system in the brain. The roles of CRT harboring 6-sialyl-LewisC and its Siglec in the brain will be discussed.

P02-5

Phosphorylation of drebrin by cyclin-dependent kinase 5 and its role in neuronal migration

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Cyclin-dependent kinase 5 (Cdk5)-p35 is a Ser/Thr kinase which plays a key role in brain development. It is not fully understood how Cdk5-p35 regulates cytoskeletal reorganization. Since actin filaments are critical for the neuronal movement and process formation, we aimed to find Cdk5 substrates among actin-binding proteins. We isolated actin gels from mouse brain extracts and phosphorylated them by Cdk5-p35. Drebrin, a side binding protein of actin filaments, was identified as a phosphorylated protein. Drebrin has two isoforms, an embryonic form drebrin E and an adult isoform drebrin A. Ser142 was identified as a common phosphorylation site to drebrin E and A and Ser342 as a drebrin A-specific site. Phosphorylated drebrin is localized at the distal area of total drebrin in the growth cone of primary neurons. By expressing nonphosphorylatable or phosphorylation mimicking mutants in developing neurons *in utero*, the phosphorylation/dephosphorylation reaction of drebrin was shown to be involved in radial migration of cortical neurons.

P02-6

The role of type VI adenylyl cyclase in the regulation of synaptic activity

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Type VI adenylyl cyclase (AC6) is a membrane bound, calcium-regulated adenylyl cyclase which mediates the synthesis of cAMP from ATP during extracellular stimulation. Unlike other ACs, AC6 is of particular interest in the brain as it is expressed on neuronal cells and distributed in various brain regions. Furthermore, it is also negatively regulated by multiple signals (such as Giα, Ca^{2+}, PKA, PKC, and NO). Therefore, AC6 might be a key integrator in maintaining proper neuronal functions. In addition to the production of cAMP, we previously showed that N-terminus of AC6 could modulates neurite outgrowth by interacting with Snapin in primary hippocampal neurons and Neuro2A cells, which revealed that AC6 is an important modulator in neuritogenesis. Nonetheless, the physiological functions of AC6 in the central nervous system remain elusive. By using AC6 KO mice, we surprisingly found that the absence of AC6 increased dendritic spine density of hippocampal CA1 pyramidal neurons without affecting the gross anatomy of the brain. Besides, electrophysiological analyses showed increased membrane time constant, lower rheobase, reduced action potential threshold, and an increase in the ratio of NMDA to AMPA receptor-mediated EPSCs in CA1 pyramidal neurons of AC6 KO mice, indicating that AC6 negatively modulates neuronal excitability. Furthermore, the glutamate-induced cAMP/calcium responses were enhanced in primary hippocampal neurons of AC6 KO mice. Our results demonstrated that AC6 plays pivotal roles in regulating in the glutamate-dependent neuronal activity in the hippocampus.

P02-7

The hypoxia-recovering responsive cells in the zebrafish spinal cord during regeneration after hypoxia stress

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After hypoxia, some neural stem/progenitor cells (NSPCs) are able to proliferate and differentiate into neuron, and the quiescent astrocytes (ASTs) become reactive and proliferate in brain, indicating that multiple cell types might be involved in neural regeneration. However, *in vivo* model system that can identify what cell types and fates are involved in neural regeneration is not available. To solve this issue, we employed a zebrafish transgenic line *huORFZ,* which harbors an upstream reading frame of human *chop* fused with GFP reporter. We verified that GFP signals were exclusively apparent in some cells located at CNS when hypoxia was treated *huORFZ* embryos for 2 h, followed by 10 h of oxygen recovery. This result suggested that multiple cell types which are sensitive response during recovery after hypoxia do exist, termed hypoxia-responsive recovering cells (HrRCs). We further found that HrRCs were mostly NSPCs, along with some ASTs and a small portion of oligodendrocytes (OLs). These HrRCs did not undergo apoptosis, and some of them proceeded migration. Some GFP^+ NSPCs could proliferate, and then proceeded differentiation into ASTs, while GFP^- NSPCs could not. Moreover, some GFP^+ reactive ASTs could proliferate, and then proceeded differentiation into neurons. Taken together, we demonstrated that (i) *huORFZ* embryos serve to label and trace HrRCs; (ii) Some NSPCs, ASTs and OLs in spinal cord consist of cells which are sensitive response during hypoxia-recovery; (iii) Some HrRCs can migrate; and (iv) Some GFP^+ NSPCs and reactive ASTs are able to proliferate and differentiate into ASTs and neurons, respectively.

P02-8

Role of Cathepsin C and Cystatin F in demyelinating diseases

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Multiple sclerosis (MS) is the most common demyelinating disease in CNS. The studies on MS have been significantly increased and improved therapeutic outcome. Animal models have been critically important for addressing and establishing MS treatment. We previously found CathepsinC (CatC) is upregulated in chronic demyelinated lesions in one of demyelinating model, $plp^{4e/-}$ mouse. Additionally, expression of its inhibitor Cystatin F (CysF) was also induced during early phase of demyelination and ceased expression in chronic demyelinated lesions. To study the role of CatC and CysF involvement in demyelinating disease, we generated mouse lines to manipulate CatC and CysF expression. When remyelination is active in $plp^{4e/-}$ mouse at 4 months, CysF Knockdown (CysFKD) mouse significantly worsened demyelination. Conversely, at the chronic demyelination phase (8 months) CatC knockdown (CatCKD) mouse diminished demyelination. CatC overexpression (CatCOE) mouse also gave a similar result with CysFKD mouse showing early demyelination. In MOG-EAE model, CatC was expressed in the chronic demyelinating region but not CysF. CatCKD showed delayed demyelination in EAE. Conversely, CatCOE in microglia significantly enhanced demyelination. This result is similar with chronic demyelination model, $plp^{4e/-}$ mouse. We have discovered CatC and CysF are strongly related with demyelinating diseases in both acute and chronic phase, even in different cause and state of pathology.

P02-9

Pre- and post-synaptic identities dictate the type of synapse in an amygdala inhibitory network

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The amygdala complex is a key brain structure for fear learning and memory. The central amygdala (CeA) is the main output station and controls the expression of fear responses via projections to the hypothalamus and brainstem. The CeA contains γ-aminobutyric acid-releasing (GABAergic) neurons and is divided into the lateral (CeL) and medial (CeM) subdivisions. The CeL controls fear expression by gating activity of the CeM, the main output center of the CeA. Although local inhibition in the CeL impacts information transfer across CeL-to-CeM connections, the functional properties of inhibitory synapses between spatially intermingled CeL neurons have not been fully examined. Using unbiased cluster analysis, we first identified two major physiologically and pharmacologically distinct CeL neuron classes, early-spiking (ES) and late-spiking (LS) neurons, in acute mouse amygdala slices. We next recorded from CeL neuron pairs or triples and found that CeL neurons were chemically but not electrically coupled. Analysis of individual connections revealed that efficacy of synaptic transmission at ES→LS or LS→ES neuron connection was approximately 5-fold stronger than that at LS→LS or ES→ES neuron connection. The temporal dynamics of GABAergic transmission when tested at 20 Hz showed that synapses between electrically heterogeneous neurons were strongly depressing. By contrast, synapses between electrically homogeneous neurons displayed slight depression following initial weak facilitation. Our results indicate that phenotypic nature of both presynaptic and postsynaptic neurons is involved in dictating the type of synapse. Such combinatorial interactions between two neurons could maximize synaptic diversity and may be relevant for synaptic computations in the CeL.

P02-10

Inhibition of STAT3 activation suppresses maintenance of glioma stem-like cells and implication for combination therapy in glioblastoma

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Glioma stem cells (GSCs) are thought to contribute to tumorigenesis and recurrence. In this study, we isolated glioma stem cells

(GSCs) from human glioablastoma cell line, U87 and rat glioblastoma cell line, C6 by using fluorescence-activated cell sorting for CD133 as a cancer stem cell marker. These $CD133^+$ glioma cells exhibit self-renew property and stem cell markers. The $CD133^+$ glioma cells also perform significant drug resistant compare to parental and $CD133^-$ glioma cells. Signal transducer and activator of transcription 3 (STAT3) regulates cell growth, differentiation and apoptosis and correlates with glioma malignancy and poor prognosis. We found that STAT3 was constitutively active in $CD133^+$ glioma cells and inhibition of STAT3 activation repressed the proliferation and neurosphere formation of $CD133^+$ glioma cells. Besides, inhibition of STAT3 activation decreased the stem cell markers expression of $CD133^+$ glioma cells. These results indicate that STAT3 plays an important role in glioma tumorigenesis. Further, STAT3 inhibitor increased the sensitivity to chemo-drugs in $CD133^+$ glioma cells. It suggests that additional targeting STAT3 may provide an effective combination therapy for patients with malignant glioma.

P02-11

The role of modulator of apoptosis (MOAP-1) in acute ischemic stroke

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Cerebral ischemia triggers two general pathways of apotosis: the intrinsic pathway and extrinsic pathway. Modulator of apoptosis 1 (MOAP-1) has been identified to be a Bax-associating protein. While the role of MOAP-1 in promoting caspase-dependent cell death has been studied in cell lines, the *in vivo* physiological role of MOAP-1 is not understood. In the present study, we investigated the influence of MOAP-1 in ischemic injury using MOAP-$1^{-/-}$ mice. Results showed that MOAP-$1^{-/-}$ primary neurons aremore resistant against oxygen glucose deprivation (OGD) than MOAP-$1^{+/+}$ primary neurons with significantly higher cell viability at 4 h after OGD. Consistently, SH-SY5Y cells overexpressing MOAP-1 has lower cell survivability than vector control during OGD. Immunocytochemistry demonstrated enhanced co-localization of MOAP-1 with RASSF-1A during OGD indicating that MOAP-1 recruited RASSF-1A and stimulated death receptor dependent apoptosis. After transient middle cerebral artery occlusion (tMCAO, 24 h), MOAP-$1^{-/-}$ mice showed smaller infarct volume (57.8 mm^3 vs. 128.9 mm^3, $p < 0.001$) and better performance on the rotarod test (117.6 s vs. 32.75 s, $p < 0.05$) than MOAP-$1^{+/+}$ mice. At 1 h tMCAO, MOAP-1, Trim39, BAX, and TNF-alpha were significantly upregulated in MOAP-$1^{+/+}$ mice showing that the MOAP-1-dependent apoptotic pathway is activated. In addition, MOAP-$1^{-/-}$ mice subjected to tMCAO has lower Bax/Bcl-2 ratio and higher NeuN expression than MOAP-$1^{+/+}$ supporting the idea that MOAP-$1^{-/-}$ neurons are more resistant to ischemic injury. In conclusion, both our *in vitro* and *in vivo* data demonstrates that MOAP-1 is an important modulator in cell death in ischemic injury.

P02-12

Oxidative damage to lipids in white matter lesions induced by chronic hypoperfusion in mice

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White matter lesions(WMLs), appearing as hypodensities on computed tomography or as hyperintensities in T2-weighted and proton density MRI, is one of the major pathological aspects in vascular cognitive impairment (VCI). However, the molecular mechanism in white matter changes is still not clearly understood. Due to the unique high lipid constituent of myelin in white matters, changes in lipids such as oxidative damage may play an essential role in white matter injury. Therefore, we used a mouse chronic hypoperfusion model induced by bilateral common carotid artery stenosis (BCAS) to study the molecular changes in WM during chronic hypoperfusion. Myelin loss and a decrease in myelin basic protein (MBP) as well as an elevation of HIF1-α were observed in the corpus callosum (CC) from 15 days after BCAS, indicating white matter injuries due to chronic hypoperfusion. At 30 days, the MBP level increased when compared to that at 15 days, but still significant lower than the sham control group. CD31 expression increased at 30 days after BCAS both in the cortex and corpus callosum and elevation of NG2-positive oligodendrocytes precursor cells may suggest spontaneous angiogenesis in a recovery phase after prolonged hypoxia. Lipids changes under hypoperfusion were profiled by lipidomics analysis. A trend of sulfatide content loss was demonstrated in CC but not in cortex. Besides, percentage of sulfatides with very long acyl chain (C22-C24) decreased while those with C16-C18 elevated slightly though both not statistically significant. We are currently investigating other sphingolipids.

P02-13

Overexpressed interferon alpha or beta receptors in the brain of adult Ts1Cje mouse model of Down syndrome

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Down syndrome (DS) is a genetic disorder with trisomy of human chromosome 21 (HSA21) and all DS patients exhibited intellectual

disability. Ts1Cje mouse model of DS has partial triplication of mouse chromosome 16 (MMU16) which is homologous to HSA21. The JAK (Janus kinase) and STAT (signal transducer and activator of transcription) signalling pathway was firstly described in immune system however its role has extended to central nervous system (CNS) as it is involved in neurogenesis and gliogenesis regulation. Cytokines especially interferons family is the major activator of JAK-STAT signalling pathway. Furthermore, interferon receptor genes (*Ifnar*1, *Ifnar2* and *Ifngr2)* are located at the triplicated region in MMU16 and also in HSA21. Thus, we investigated the gene expression of *Ifnar1, Ifnar2* and *Ifngr2* and associated genes in JAK-STAT signalling pathway (*Jak1, Jak2, Stat1, Stat3* and *Stat6)* in Ts1Cje mouse brain. We further validated and compared these genes in cerebral cortex and cerebellum at four time-points; post natal day (P)1, P15, P30 and P84 by using qRT-PCR technique. Our data showed *Ifnar1, Ifnar2, Ifngr2* and *Stat1* are significantly over-expressed in cerebral cortex and cerebellum at various time points whereas the rest of the genes showed no significant differences in expression as compared to control littermates. Protein expression analysis further confirmed the over-expression of Ifnar1, Ifnar2 and Stat1 in Ts1Cje mouse cerebral cortex and cerebellum at P84 as compared to wild type. Thus, we suggest that over-expression of interferon receptors will increase sensitivity towards interferon levels in Ts1Cje mouse brain. Consequently, the over-stimulated JAK-STAT signalling pathway may contribute to the neuropathology in the Down syndrome mouse brain.

P02-14

Oxidative stress at rostral ventrolateral medulla underlies long-term binge methamphetamine-induced cardiovascular dysfunction and fatality in rats

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Abuse of methamphetamine (METH; *N*-methyl-1-phenylpropan-2-amine) is a serious health concern worldwide. Although cardiovascular collapse is a common cause of death within the abuse population, much less studies are devoted to the brain stem mechanisms contributing to METH-induced cardiovascular collapse. The rostral ventrolateral medulla (RVLM) is the key neural substrate at brain stem, which is responsible for the mediation of baroreflex responses and homeostasis of blood pressure (BP). The present study investigated the molecular mechanism in the RVLM contributing to METH-induced dysfunctional baroreflex and circulatory responses and fatality via an animal model of long-term binge METH consisting of four-injections of METH (5 or 10 mg/kg, 2-h intervals, i.p.) to conscious Sprague–Dawley male rats, whose BP and heart rate (HR) were recorded by telemetry. We found that METH dose- and repeat-dependently elicited an increase of superoxide in the RVLM followed by a decrease of BP, HR, survival rate, accompanied by a decrease of the power density of the low frequency (LF; 0.25–0.8 Hz) component in the BP spectrum (an index for baroreflex-mediated sympathetic vasomotor tone) and a less decrease of the baroreflex sensitivity (BRS; an index for cardiac vagal baroreflex). Moreover, intracerebroventricular application of superoxide scavenger FeTMPyP prevented METH-induced oxidative stress in the RVLM and a decrease of BP, HR, LF, BRS or survival rate. These results indicated that chronic binge METH, the typical regimen used by METH abusers, induced oxidative stress in the RVLM and disrupted baroreflex responses, contributing to subsequent cardiovascular dysfunction and fatality. (Supported by NHRI-EX102-10252NI and NHRI-EX103-10252NI).

P02-15

Identification of potential HDAC inhibitor compounds for polyQ diseases with SCA17 mouse system

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Spinocerebellar ataxia (SCA) is a group of heterogeneous autosomal dominant neurodegenerative diseases. Clinical symptoms include ataxia, dementia, dystonia, seizures, and significant cerebellum atrophy in pathology. Spinocerebellar ataxia type 17 (SCA17) is one type of SCAs. It is caused by CAA/CAG repeat expension of the *TATA-binding protein (TBP)* gene. TBP is a crucial transcription factors for all the three RNA polymerase in transcription initiation. The polyglutamine (polyQ)-expanded mutant TBP accumulates as aggregates in the cells and leads to cell degeneration, especially the cerebellar Purkinje neurons. Histone deacetylases (HDAC) make the histones more tightly bind to DNA by removing the acetyl groups from histones, which in turn suppresses the gene transcription and causes many diseases. HDAC inhibitors could alleviate transcription suppression and show neuroprotecive effect in several neurodegenerative diseases models. We found two HDAC inhibitors, NC105 and NC109, can increase Purkinje cell total neurite outgrowth on primary culture and decrease TBP aggregation on slice culture. These two HDAC inhibitors were applied to SCA17 mice to verify their efficacy *in vivo*. Our results show these two inhibitors alleviate TBP aggregation, Purkinje cell degeneration and increase histone acetylation. In addition, mouse motor coordination was improved from thebehavior evaluation. These results suggest that NC105 and NC109 could be potential HDAC inhibitors for SCA17 treatment.

P02-16

Therapeutic effects of add-on low-dose dextromethorphan plus valproic acid in bipolar disorder

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The change of inflammatory cytokine and dysfunction of the neurotrophic factors is thought to be involved in the pathology of bipolar disorder (BP). Whether the inflammatory or neurotrophic factors or both were changed in BP was evaluated. We further investigated whether the low dose dextromethorphan (DM) with

mood stabilizer valproic acid (VPA) is more effective than treating BP with VPA only, and whether DM affects plasma cytokines and brain-derived neurotrophic factor (BDNF) levels. In 12-week, randomized, double-blind study, patients were randomly assigned to groups: VPA plus either placebo, DM30 or DM60 mg/day. The Young Mania Rating Scale (YMRS) and Hamilton Depression Rating Scale (HDRS) were used to evaluate symptom severity, and ELISA to analyze plasma cytokine and BDNF levels. One hundred twenty three healthy control and 309 patients with BP were recruited in this study. Before the treatment began, patients with BP had significantly higher plasma cytokines and lower plasma BDNF levels than did healthy controls. After 12 weeks treatment, each groups showed significant HDRS and YMRS score improvement. In plasma molecular, VPA+DM groups showed the tendency of plasma cytokines improvement. Moreover, in the VPA+DM60 group showed more change of plasma BDNF levels compared with the VPA+placebo group. Patients with BP have a certain degree of systemic inflammation and BDNF dysfunction. VPA plus DM treatment provided patients with BP more anti-inflammation and neurotrophic benefit than did VPA treatment alone.

P02-17

Investigation of the role of dopamine D4 receptor in methamphetamine-induced psychosis

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Chronic methamphetamine (METH) not only leads to addiction, but also gives rise to the development of psychotic symptoms. Previous studies addressed the significance of dopamine system in METH psychosis; however, the role of dopamine D4 receptor (D4R) signalling in METH psychosis remains unclear. The purpose of this study is to investigate the role of D4R in METH psychosis using pharmacological manipulations and behavioral evaluation. Male ICR mice from 6 to 8 weeks were used in this study. Selective D4R antagonist (also an atypical antipsychotic) clozapine (1, 2.5, 5 mg/kg/day, i.p.) was pre-treated before daily METH (2 mg/kg/day, i.p.) administration for 7 consecutive days. Horizontal locomotor activity and stereotypy were evaluated on the first and last day 1 h post METH treatment to evaluate if D4R is involved in METH-induced behavioral sensitization development. Results showed that clozapine (1, 2.5, 5 mg/kg/day, i.p.) pre-treatment dose-dependently inhibited METH-induced behavioral sensitization (by 25%, 50%, 80%, respectively), suggesting that D4R is critically involved during the sensitization process. In future study, the molecular mechanism underlying D4R-mediated METH sensitization antagonism will be further assessed to investigate the role of D4R in METH psychosis.

P02-18

NMDA receptor antagonism in the hippocampus ameliorates acute stress potentiation of aggressive behaviors in the post-weaning isolation-reared mice

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Despite epidemiological evidence showing that early life events have long-term effects on the susceptibility to subsequent stress exposure during adulthood, there has been very little work examining the underlying cellular mechanisms. We used post-weaning social isolation mice as an animal model of early life adversities to test the action of NMDA receptor antagonists on acute stress-induced exaggeration of aggressive behaviors in the social isolated (SI) mice. Synaptic protein levels of NMDA receptors were measured using synaptosomal preparation. Acute stress giving before test markedly exacerbated offensive behaviors and attack number in the SI mice. Post-weaning social isolation increased hippocampal surface expression of NR2A and NR2B without affecting NR1 subunit of NMDA receptors, PSD-95 and α2 subunit of $GABA_A$ receptors. Bilateral hippocampal injection of NMDA antagonists reversed acute stress-induced exaggeration of aggressive behaviors in the SI mice. Acute stress induced phosphorylation of eukaryotic elongation factor-2 (eEF2) which was abrogated by NMDA antagonist. Furthermore, eEF2 kinase inhibitors reversed acute stress-induced exaggeration of aggressive behaviors and exhibited anti-depressant effect in the SI mice. These results suggest the involvement of NMDA receptors and eEF2 kinase in the isolation-induced alterations of behavioral phenotypes and NMDA receptor antagonists may be useful to ameliorate child neglect-induced exacerbation of aggressive behaviors.

P02-19

AMPA receptor endocytosis in the amygdala is involved in the disrupted reconsolidation of methamphetamine-associated contextual memory

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Repetitive drug taking induces neural long-lasting changes and results in compulsive drug use behavior known as addiction. Here, we used a conditioned place preference (CPP) procedure in mice to examine the role of AMPA receptor endocytosis in the basolateral amygdale (BLA) in the disrupted reconsolidation of Methamphetamine (MeA M) memory. Conditioning MeAM (2 mg/kg, i.p.) for 3 days in mice markedly increased the time spent in the MeAM-paired compartment tested 24 h after the last injection (CPP test), indicating that MeAM induced a significant rewarding effect. Mice then received anisomycin or saline 1 h after CPP test and CPP was assessed 24 h after CPP test. Mice injected with saline exhibited CPP for the previously MeAM-paired chamber whereas mice injected with anisomycin did not. Anisomycin had no effect on the CPP when CPP test was omitted. In addition, anisomycin treatment prevented MeAM priming-induced reinstatement of CPP suggesting the disruption of MeAM memory reconsolidation. MeAM CPP increased surface expression of GluR1 and GluR2 subunits of

AMPA receptor in the BLA. Bilateral injection of Tat-GluR2$_{3Y}$, a synthetic peptide that blocked AMPA receptor endocytosis, into the BLA prevented anisomycin-induced disruption of MeAM memory reconsolidation. These results suggest that AMPA receptor endocytosis in the BLA is critical for the anisomycin-mediated disruption of reconsolidation of MeAM reward memory.

P02-20

Depression/anxiety phenotyping of neuropeptide FF receptor type 2 transgenic mice

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Neuropeptide FF (NPFF) belongs to a family of FMRF-NH2 peptide and was known as an opioid modulation peptide regulating both pronociceptive and anti-nociceptive responses. Two receptor subtypes were cloned, namely the NPFF1 and NPFF2. According to radioligand receptor competition assay, NPFF2 was suggested to be a physiological receptor of NPFF (Liu et al., 2001). Other than pain involvement, it was reported that NPFF receptor antagonist would modulate ethanol and amphetamine withdrawal induced-anxiety behaviors. Few reports also indicated NPFF system could regulate serotonin levels in different brain area, though how NPFF system links with depression and anxiety behaviors and its underlying mechanism remain largely unknown. The aim of this study was to explore the functional significance of brain NPFF in limbic system, taking advantage of in-house made NPFF2 over-expressed transgenic mice. We observed that NPFF2 Tg mice exhibit depressive- and anxiety-like behaviors (force-swimming test, tail suspension test, novelty suppressed feeding and elevated plus maze) along with hypothalamus-pituitary-adrenal (HPA) axis overactivation as compared to WT mice. In addition, reduced serotonin levels in different brain area were also found in Tg mice, quantified by HPLC-ECD. The cellular mechanisms of NPFF-NPFF2 system on expression of depressive- and anxiety-like behaviors are currently under investigation.

P02-21

Pentedrone induces conditioned place preference and self-administration through dopaminergic system

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A new group of recreational drugs, known as "bath salts" or "plant food" has recently emerged in many countries. The active compounds in "bath salts" are cathinone derivatives continuously developed and modified by drug designers to avoid detection, yet little information is available regarding the mechanism of action for these drugs. Here, we investigated rewarding effects of pentedrone, a kind of cathinone derivatives, in conditioned place preference (CPP) in mice and self-administration in rats. Intraperitoneal injections of pentedrone 3 and 10 mg/kg showed significant increases of CPP. In addition, the number of infusion per session (2 h) is significantly increased at 0.3 mg/kg/infusion in rats. In order to confirm the involvement of dopaminergic system in rewarding effects of pentedrone, we performed RT-PCR and western blot. Pentedrone decreased tyrosine hydroxylase (TH) mRNA level and increased dopamine transporter (DAT), dopamine 1 receptor (D1), dopamine 2 receptor (D2) mRNA levels and phosphorylation of CREB in PC-12 cells. Furthermore, to assess dopaminergic activation of pentedrone in mice, we also carried out apomorphine-induced climbing behavior test. Pentedrone 10 mg/kg showed significant increase of apormorphine-induced climbing behavior in mice. Additionally, pentedrone 10 mg/kg decreased VMAT2 (vesicular monoamine transporter 2) and did not affect DAT in mouse striatum. These results suggest that pentedrone has a dependence-liability and it may be due to the activation of dopaminergic system.

P02-22

Social interaction with a helper rescues memory deficit in an animal model of Alzheimer's disease by increasing BDNF-dependent hippocampal neurogenesis

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It has been recognized that the risk of cognitive decline during aging can be reduced if one maintains strong social connections, yet the neural events underlying this beneficial effect have not been vigorously studied. Here, we show that Amyloid precursor protein (APP) and presenilin 1 (PS1) double-transgenic (APP/PS1) mice improved memory after co-housing with wide-type (WT) mice. The improvement was associated with increased protein and mRNA levels of BDNF in the hippocampus. Concomitantly, the number of $BrdU^+/NeuN^+$ cells in the hippocampal dentate gyrus was significantly elevated after co-housing. Methylazoxymethanol acetate (MAM), a cell proliferation blocker, markedly reduced BrdU- and BrdU/NeuN-positive cells and abolished the companion effect. Selective ablation of mitotic neurons using diphtheria toxin (DT) and retrovirus vector encoding DT receptor (DTR) system abolished the beneficial effect of company. Knockdown of BDNF by shRNA transfection blocked while overexpression of BDNF mimicked the memory improving effect. A tropomyosin-related kinase B (TrkB) agonist, 7,8-dihydroxyflavone (7,8-DHF), occluded the companion effect. These results provide the first evidence that increased BDNF expression and neurogenesis in the hippocampus underlie the reversal of memory deficit by co-housing in APPPS1 mice.

P02-23

The role of posterior insular cortex in spontaneous pain and mechanical allodynia of neuropathic pain rats

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In human functional imaging studies, posterior insular cortex (PIC) consistently activates by noxious stimulation. Activation and

inactivation of this region produce altered pain sensation. In animal studies, PIC has been shown to have somatotopic organization and to be important for mechanical allodynia caused by chronic constriction injury of the sciatic nerve. Our previous PET studies in the spared nerve injury (SNI) model also revealed that PIC was activated both under spontaneous pain condition and mechanical stimulation. It would be of interest to test whether PIC is important for different pain modalities, including spontaneous pain and evoked pain under neuropathic condition. Permanent bilateral PIC lesion was produced by 5% NMDA solution microinjected 14 days before (pre-lesion) or after (post-lesion) SNI surgery. In the post-lesion group, behavioral tests showed that spontaneous paw lifting gradually and significantly recovered, while evoked pain showed no improvement. In the pre-lesion group, rats exhibited less paw lifting and milder mechanical allodynia, while cold allodynia and heat hyperalgesia remained unchanged. PIC lesion without SNI (the lesion-only group) caused no significant change in all four behaviors tested. These data indicated that PIC is critically important in the initiation of spontaneous pain and mechanical allodynia, and the maintenance of spontaneous pain in neuropathic rat. Moreover, thermal hypersensitivity of neuropathic pain might be differentially processed in the forebrain. (Supported by grants from NSC100-2311-b002-002-MY3 and NHRI-EX101-10104NI).

P02-24

Betaine facilitates extinction of conditioned place preference by methamphetamine and morphine in rats

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Betaine (trimethylglycine) is a neutral chemical compound found naturally in many plants. Recently, we have found that betaine potentially acts on glycine binding site of NMDA receptor as a partial agonist. NMDA receptor glycine binding site agonist and partial agonists have been reported to accelerate extinction of drug seeking behavior. The present study aimed to determine whether betaine facilitates extinction of methamphetamine (MA) and morphine (MOR) conditioned place preference (CPP). After MA/MOR CPP, rats received betaine (100 mg/kg) or saline immediately after confined extinction, with saline injection in previous MA-paired compartment for 3 days. MA CPP was significantly reduced during retest after extinction in rats treated with saline and betaine, but MA priming induced reinstatement in the saline-treated, but not betaine-treated rats. MOR CPP was effectively extinct in betaine- , but not saline-treated rats. The MOR priming did not induce reinstatement in the betaine-treated rats. In addition, after complete extinction, acute betaine treatment 30 min prior to MA/MOR priming injection significantly reduced the reinstatement. These results demonstrated that betaine facilitates memory consolidation for extinction of approach behavior to environmental stimuli previously paired with MA/MOR and suppresses MA/MOR-induced reinstatement of CPP behavior, suggesting that betaine is a potential therapeutic agent for MA/MOR addiction.

P02-25

Mice model of central post stroke pain

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Chronic pain is a serious health challenge in aged society. Patients' life quality decreased and the cost of medical care increased massively. Chronic pain could be induced by many factors such as peripheral limb damage, spinal cord injury, chronic inflammation and stoke. Hemorrhage stroke is an important neurological disorder that causes chronic pain. About 7~15% patients with stroke would develop chronic pain. This type of chronic pain is called central post stroke pain (CPSP). But mechanism of CPSP is not clear. Although a hemorrhage rat model was well established for CPSP research. Due to the efficiency to test gene function in transgenic mice, it is valuable for establishing a mouse hemorrhage CPSP model that could help to distinguish more detail mechanisms of CPSP. Followed the same experimental protocol of rat hemorrhage CPSP model, mice were injected with 0.01 U/0.2 μL type4-collagenase into ventrobasal complex of thalamus. After 28 days of lesion, the mechanical and thermal thresholds were decreased in lesion mice. Multiunit activities in medial dorsal nucleus of thalamus were enhanced and lengthened after noxious stimuli in CPSP mice. Those data fit to some basic features, allodynia and hyperalgesia, of chronic pain. In contrast with GABA inhibitory effect in control mice, spontaneous unit activity in medial dorsal nucleus could be enhanced by muscimol (20 μM, 0.2 μL) application. Plasma chloride concentration was measured with 6-methoxy-*N*-ethylquinolinium iodide method. The preliminary result showed abnormal chloride accumulation in neuron cytoplasma after CPSP. Those data indicated that a mouse CPSP model could be established and change of cytoplasmic chloride concentration plays an important role in CPSP.

P02-26

The nociceptin receptor involved in the prenatally and postnatally buprenorphine-induced withdrawal syndromes

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Buprenorphine is a partial mu-opioid receptor agonist, full/partial nociceptin receptor (NOPR) agonist and kappa-opioid receptor antagonist. It has been used as a selectively new therapeutic medication for heroin maintenance, recently. Buprenorphine has benefits than methadone as a maintenance medication due to it has less addictive liability and respiratory depression. Because the patent for buprenorphine as prescription medication had been expired. It can be predicted that buprenorphine will be widely used after the generic formulae are available. Recently, buprenorphine formulations for sale by online pharmacies (from internet) without a prescription were increased. This indicates that illicitly obtained buprenorphine formulations appear to be easily obtained in the future. Unfortunately, there are still lacking detail information to know whether buprenorphine intrauterus exposure would not exhibit toxicological effects, behavioural and cellular changes in the offspring. Our previous studies showed that prenatally higher dose, 3 mg/kg/day (~ 32 mg for 70 kg human), of buprenorphine-

exposed rats exhibited a marked change in the postnatally morphine-induced tolerance and dopaminergic system in the nucleus accumbens (NAc). In the current study, we provided NOPR antagonist could precipitate the withdrawal syndromes in both of prenatally and postnatally buprenorphine-exposed rats. The expression of NOPR in locus coeruleus was also changed after prenatal or postnatal exposure to buprenorphine. The changes of receptors presented as a region-dependent manner in previous and current studies. The study reveals that prenatal exposure to buprenorphine caused long-term effects on the offspring. [Supported by China Medical University Hospital (DMR-103-023) and National Health Research Institutes (NHRI-EX103-10224NC and NHRI-103A1-PDCO-1312141)].

P02-27

A novel mechanism for restraint stress-induced cocaine relapse: orexin-induced endocannabinoid retrograde disinhibition in the ventral tegmental area

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Orexin A and B are hypothalamic neuropeptide ligands of orexin receptors, OX1R and OX2R. Orexin-containing neurons are localized in the lateral hypothalamus (LH) and perifornical area (PFA), but send projections widely throughout the brain, including the ventral tegmental area (VTA), an important reward area. Activation of hypothalamic orexin neurons has been reported to be associated with stress-induced reward seeking while its mechanism remains unclear. Here, we reveal a novel mechanism for stress-induced cocaine relapse involving orexins and endocannabinoids acting in the ventral tegmental area (VTA). Acute restraint stress significantly activated lateral hypothalamic orexin neurons, increased orexin A levels in the VTA, and reinstated extinguished cocaine place preference. This stress-induced cocaine reinstatement was prevented by OX1 receptor (SB 334867), diacylglycerol lipase (DAGL) inhibitors (THL) CB1 receptor (AM 251) antagonists, respectively, and abolished in CB1 receptor-knockout mice. In VTA slices, orexin A presynaptically inhibited GABAergic transmission onto dopaminergic neurons. This effect was antagonized by SB 334867 and AM 251, prevented by internal GDP-beta-S and by phospholipase C (PLC) and DAGL inhibitors, and potentiated by inhibiting 2-arachidonoylglycerol (2AG) degradation. These results suggest that stress-released orexins activate postsynaptic OX1 receptors on VTA dopaminergic neurons, and through a Gq protein-PLC-DAGL cascade, generate 2AG. This endocannabinoid then retrogradely inhibits GABA release through presynaptic CB1 receptors, leading to disinhibition of VTA dopaminergic neurons and drug relapse. (Supported by NHRI-EX103-10251NI, NSC 102-2321-B-002-066 and NSC 102-2325-B-002-047)

P02-28

Fragile X mental retardation-1 knockout zebrafish showed precocious development in social behavior

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Fragile X syndrome (FXS) is most prevalent hereditary form of mental retardation in human. It had been proven that FXS was resulted from triplet repeat expansion (CGG) mutation in the fragile X mental retardation 1 (*fmr1*) gene, induced the loss-of-function in *fmr1* gene. The most commonly symptoms of FXS patients included learning disabilities, inattention, hyperactivity and anxiety. Even though the gene responsible for the pathogenesis of FXS had been targeted. Its' detail mechanism remains unclear. The *fmr1* mediated behavioral function deserves further investigation. The present study was aimed to study the possible role of *fmr1* on social behavior by using *fmr1* KO transgenic zebrafish model. We focused on three different types of behavior including social behavior, locomotor activity and anxiety-like behavior. Results obtained from this study will not only expanded our knowledge regard to the function of *fmr1*, but also benefit to the development of novel therapeutic strategy for the patient suffered by FXS. Results showed precocious development of social behavior in *fmr1* KO fishes. We also found anxiety- like behavior elevated in *fmr1* KO fishes compared with the control animals, Sequent control experiment showed the locomotor activity declined in *fmr1* KO animals. In conclusion, our results showed consistency with the previous observation that *fmr1* KO zebrafish model displayed abnormal behaviors. Moreover we proven that *fmr1* participated in the development of social behavior and the regulation of emotional behavior in zebrafish. We suggested that *fmr1* KO zebrafish is an idea model for pre-clinical drug screening.

P02-29

The effects of maternal deprivation on the development of adrenal glands

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Social and physical stresses in our environment that we experience during childhood can have powerful and lasting effects on our physiology and behavior. Among the most important early life influences is the interaction between the primary caregiver and the offspring. Adrenal glands are important for stress response and daily physiological regulation. There are 2 types of chromaffin cells (derived from neural crest), epinephrine secreting cells and norepinephrine secreting cells, in adrenal glands. The enzymes responsible for converting norepinephrine to epinephrine, PNMT (phenylethanolamine-*N*-methyl transferase), is abundant in epinephrine secreting cells but not norepinephrine cells. Thus, it was used as a marker to identify epinephrine secreting cells. After long term maternal deprivation, there is no major morphological difference in adrenal glands between control and maternal deprived animals. The content of epinephrine in adrenal glands of the maternal deprived juveniles is significantly lower than in regular juveniles. However, there is no

change in the number of PNMT containing chromaffin cells between the maternal deprived juveniles and regular juvenile. The content of norepinephrine in adrenal glands was no difference between two groups of animals. It is likely that early postnatal stress induced by maternal deprivation increase the response of sympathetic system by increase the output of epinephrine, but not affecting the population establishment of epinephrine secreting cells.

P02-30

The effect of cyclin-dependent kinase 5 on voltage-dependent calcium channels in PC12 cells varies according to channel type and cell differentiation state

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Cyclin-dependent kinase 5 (Cdk5) is a proline-directed Ser/Thr protein kinase that is activated by binding a p35 regulatory subunit. Cdk5-p35 plays an important role in a variety of neuronal activities including neuronal migration during brain development, survival and neuron death. Cdk5 also plays an important role in synaptic transmission. The synaptic transmission involves neurotransmitter release from presynaptic terminal and the activation of postsynaptic signaling cascades via neurotransmitter receptors. The neurotransmitter release is triggered by Ca^{2+} influx into the presynaptic cytoplasm through voltage-dependent Ca^{2+} channels (VDCCs). It is reported that Cdk5 regulates L-, P/Q-, or N-type VDCC, but there is conflicting data as to the effect of Cdk5 on VDCC activity. To clarify the mechanisms involved, we examined the role of Cdk5 in regulating the Ca^{2+}-channel property of VDCCs, using PC12 cells expressing endogenous, functional L-, P/Q-, and N-type VDCCs. The Ca^{2+} influx, induced by membrane depolarization with high K^+, was monitored with a fluorescent Ca^{2+} indicator protein in both undifferentiated and nerve growth factor (NGF)-differentiated PC12 cells. Overall, Ca^{2+} influx was increased by expression of Cdk5-p35 in undifferentiated PC12 cells but suppressed in differentiated PC12 cells. Moreover, we found different VDCCs are distinctly regulated by Cdk5-p35 depending on the differentiation states of PC12 cells. These results indicate that Cdk5-p35 regulates L-, P/Q-, or N-type VDCCs in a cellular context-dependent manner.

P02-31

Developmental modulation of long-term plasticity at vestibular GABAergic synapses

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Determining the plastic properties of neurotransmission within the neonatal vestibular nucleus (VN) is essential to our understanding of how the central vestibular system undergoes maturation during development. Using whole-cell patch-clamp recording in brainstem slices, we found that most VN neurons of postnatal day (P) 3-5 rats exhibited long-term depression (LTD) of $GABA_A$ receptor-mediated evoked-postsynaptic currents. At this stage, these currents were excitatory in nature. By P14, LTD of these currents, which became inhibitory, was observed in only a small proportion of VN neurons. In contrast, long-term potentiation (LTP) of these currents was observed in about half of VN neurons. By P28, however, a sharp rebound of LTD in conjunction with a significant decrease in LTP occurred, such that the proportion of VN neurons showing LTD became higher than that showing LTP. These results indicate a postnatal period during which biphasic plastic feature of GABAergic VN synapses is observed. To further study the role of GABAergic transmission in the VN on developmental acquisition of vestibular behavior, we implanted above the VN of P1 rats with Elvax slice loaded with $GABA_A$ receptor antagonist bicuculline or agonist muscimol. These rats, when tested at adult stage, showed deficit in vestibular function, as indicated by impairment of performance in the rota-rod task, a vestibular-dependent behavior. Taken together, we have delineated the developmental profile of central vestibular plasticity in GABAergic transmission, which contributes to the establishment of mature vestibular function. (Supported by RGC 761812M)

P02-32

Somatostatin-positive inhibitory interneurons mediate inter-dentate gyrus inhibition

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Local-circuit GABAergic interneurons provide local inhibition and thereby control normal network function. Recent studies reported that GABAergic interneurons also innervate remote targets in the cortex by long-range projections. However, the function of the long-range GABAergic projections remains largely unknown. Using retrograde dye tracing, optogenetics, mouse genetics, and electrophysiology, we investigated somatostatin-positive (SOM^+) GABAergic interneuron-mediated long-range projections in the dentate gyrus (DG). In addition to local innervation, SOM^+ interneurons located in the hilus of the DG send their axons to the contralateral DG. Our preliminary results revealed that SOM^+ neurons directly target onto granule cells, fast-spiking interneurons, and non-fast-spiking interneurons in the DG. The functional role of contralateral inhibition by SOM^+ neurons warrants further investigation.

P02-33

The potentiate effect of CGRP on TRPV1 activity in rat trigeminal neurons

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Growing evidence has been emphasized that calcitonin gene related peptide (CGRP) participate in trigeminal nociceptive responses including primary headache nociceptive pathway. Our previous study demonstrated that CGRP can induce increase in TRPV1 levels in trigeminal neurons and activate the central neurons in TNC.

Therefore, we hypothesized that up-regulation in TRPV1 levels in trigeminal ganglion (TG) by CGRP may impact on pain mediator-activated TRPV1 responses in trigeminal neurons. In this study, we sought to investigate the consequent role of CGRP-induced up-regulate in TRPV1 levels in response to nociceptive stimuli in trigeminal neurons. The increase in TRPV1 levels was also observed in CGRP-incubated TG organ cultures. TRPV1-binding substance, capsaicin significantly increased pCaMKII levels in CGRP-incubated organ cultures compared to capsaicin- and CGRP-incubated organ cultures. The potential role of CGRP-induced increase in TRPV1 levels and activity was further investigated in the primary cultured trigeminal neurons. CGRP incubation with 1 μM for 4 h significantly increased the current density of capsaicin-stimulated inward current and this effect was inhibited by the CGRP receptor antagonist (BIBN4068BS) and PKA inhibitor (H-89). These finding reveal that CGRP may play a key role to facilitate cellular events which contribute to peripheral sensitization of TG in nociceptive transmission.

P03: Poster Presentation 3

P03-1

Mitochondrial defects in ASD and ADHD: evidence from cybrid neurons

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Autism spectrum disorders (ASD) and attention-deficit-hyperactivity disorder (ADHD) are childhood-onset neurodevelopmental disorders. Brain areas that govern behaviours are found to be affected in these disorders. Although the basis of neuropathology is unknown, literature available support a hypothesis that ASD and ADHD are mitochondria mediated diseases. We proposed to create mitochondrial transgenic where Rho0 cells (neuronal cells without mitochondrial genome) inherit platelet mitochondrial DNA from the patients, and could be used for investigations of mitochondrial dysfunctions with a single blood sampling from the affected young patients. In our lab we prepared two control cybrids, three ADHD and four ASD cybrids. Characterization of Rho0 cells and cybrids was done by following long-template PCR, and PicoGreen® and Mitotracker Green® staining. PicoGreen® binds to dsDNA and stained only nuclei in Rho0 cells, but SH-SY5Y as well as cybrids stained mitochondria and nuclei. Mitotracker Green® binds to mitochondrial proteins to produce green fluorescence that allows visualizing mitochondria within the cells, and Rho0 cells depicted uneven pattern of staining, and SH-SY5Y and cybrids showed tubular pattern of mitochondria. Cellular respiration was found to be significantly lower in ASD and ADHD cybrids as compared to control cybrids as measured by a Clark's type electrode. 5-Hydroxytryptamine level, as measured employing HPLC-electrochemical detection procedure, was significantly higher in ASD and ADHD cybrids as compared to the control cybrids. Protein levels of monoamine oxidase (MAO) A and B was not different between the groups. Transcriptome levels of ND1, ND3, ND5, ND6, COIII and ATP6 revealed loss of the latter protein subunit in disease cybrids. We conclude here that ASD and ADHD have inherent mitochondrial defects, which could be one of the underlying reasons for the disease phenotype.

P03-2

Neonatal lipopolysaccharide-induced long-lasting learning impairment and hippocampal injury can be attenuated by IL-1 receptor antagonist

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The present study was to further determine whether the co-administration of lipopolysaccharide (LPS) with IL-1 receptor antagonists (IL-1ra) can protect against neonatal LPS exposure-induced hippocampal injury and learning deficits in later life of rats. Intracerebral injection of LPS (1 mg/kg) or co-administration LPS with IL-1ra (0.1 mg/kg) was performed in rat pups on postnatal day 5 (P5) Sprague–Dawley male rat pups. Neurobehavioral tests were carried out on P21, P49 or P70 and brain injury was examined on P71. Neonatal LPS exposure resulted in learning deficits in the passive avoidance task (P21, P49 and P70), and reductions in the hippocampal volume and the number of NeuN+ cells in the CA1 region of the middle dorsal hippocampus in P71 rats. Neonatal LPS exposure also resulted in sustained inflammatory responses in the P71 rat hippocampus, as indicated by an increased number of activated microglia and elevation of interleukin-1β (IL-1β) contents in the rat hippocampus, as well as increased number of cyclooxygenase-2+ cells which were double labeled with GFAP+ (astrocyte) or NeuN+ (neuron) cells in the CA1 region of P71 rats. Neonatal administration of IL-1ra significantly attenuated the LPS-induced long-lasting learning deficits, hippocampal injury, and sustained inflammatory responses in the P71 rats. Our study demonstrates that neonatal LPS exposure causes persistent injuries to the hippocampus and results in long-lasting learning disabilities which are related to the chronic inflammation in the rat hippocampus, and these effects can be attenuated with an IL-1 receptor antagonist.

P03-3

Is cystathionine beta synthase a viable therapeutic target for acute ischemic stroke?

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Hydrogen sulfide (H_2S) produced via cystathionine beta synthase (CBS) catalytic reaction, was reported to exacerbate the stroke outcome in an experimental animal model. Our present study aims to investigate the effect of H_2S in the aggravation of stroke outcome and by selectively inhibiting CBS could ameliorate stroke outcome. By using immunofluorescence staining and western blot approach, CBS protein expression was found increased in the ipsilateral cortex and striatum at 8 h and 24 h after pMCAO respectively. In order to endogenous increased H_2S level, we used supplemented the CBS overexpressed cells lines with cysteine (cys) and homocysteine (hcy) with both physiological and high concentration. Our results suggested that following OGD, cell viability was significantly reduced only in CBS overexpressed cells under high substrates condition and the effect was reversed by a non-selectively CBS inhibitor. A novel cystathionine analogue (CBSI) which was designed to selectively inhibit CBS was showed to restore cell death in both *in vitro* (0.5 mM, $p < 0.005$) and *in vivo* stroke model (1.6 μmol/kg ICV, $p < 0.005$). Results showed the novel CBSI dose dependently reduced H_2S level with minimal inhibition of GABA

transaminase activity (IC50 0.36 μM). Taken together, our study suggested that (i) high endogenous production of H_2S under ischemic conditions could exacerbate cell death; (ii). the novel CBSI is a selective CBS inhibitor; and (iii) by inhibiting of H_2S production could be a viable approach for the treatment of acute ischemic stroke.

P03-4

Therapeutic potentials of human adipose-derived stem cells in mouse model of Parkinson's disease

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Human adipose-derived stem cells (hASCs), a kind of mesenchymal stem cells (MSCs) isolated from adipose tissue, are well known for their pluripotent ability to differentiate into various cell types including adipocytes, osteocytes, cartilage cells, and muscle cells. Furthermore, the differentiation potential of hASCs into neuron-like cells was reported in the recent studies. Autologous hASCs have significant advantages, such as the lack of immune rejection responses, tumorigenesis, or ethical problems. However, the consistency and high reliability of the experimental results verified by the animal models of diseases have to be considered greatly important factors for the stability of hASCs transplantation. Therefore, the aim of this study is to investigate preventive and therapeutic potential of ASCs for Parkinson's disease (PD). In our previous study, we found that intravenously transplanted hASCs passed through the blood brain barrier (BBB) and migrated into the injuries of the brain. hASCs was intravenously injected into tail vein of Parkinson's disease mouse model induced by 6-hydroxydopamine (6-OHDA). Consequently, the behavioral performances were significantly improved at 3 weeks after the intravenous injection of hASCs in PD mouse model. It suggests that intravenously transplanted hASCs may have a therapeutic potential for PD.

P03-5

Targeting the p53 pathway to protect against traumatic brain injury

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Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Programmed death of neuronal cells plays a crucial role in acute and chronic neurodegeneration following TBI. The tumor suppressor p53 has been recognized as an important regulator of apoptotic neuronal death. The aim of our study is to investigate whether inhibition of p53 using pifithrin-α (PFT-α) or PFT-α oxygen analogue (PFT-α(O)) would be a potential neuroprotective strategy for TBI. To investigate whether these drugs protect excitotoxicity *in vitro*, primary rat cortical cultures were treated with glutamate in the presence or absence of various concentration of p53 inhibitor PFT-α or PFT-α(O). Cell death was estimated by LDH assays. *In vivo*, adult SD rats were subjected to controlled cortical impact (CCI). Five hours after injury, PFT-α or PFT-α(O) (2 mg/kg, i.v.) was administered to rats. Brain contusion volume, sensorimotor and cognitive functions were be evaluated. Apoptotic cells were identified by doubled staining with cell -specific markers. Levels of mRNA encoding for proinflammatory/p53 related genes were measured by real time-PCR. We found that PFT-α(O) (10uM) enhanced neuronal survival against glutamate-induced cytotoxicity more effectively than PFT-α (10uM). PFT-α (O) treatment enhanced functional recovery and decreased contusion volume at 24 h post-injury. Neuroprotection by PFT-α (O) treatment also reduced cleaved-caspase 3 and p53 binding protein 1 (53BP1) positive cells in the cortical contusion region. Our data suggests that the inhibition of p53-induced apoptosis by PFT-α (O) may develop into a novel neuroprotective strategy for TBI.

P03-6

DJ-1 deficiency augments expression and release of INF-gamma and I-TAC in microglia: a cytokine-chemokine circuit causing death of dopaminergic neurons

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Loss-of-function mutations of DJ-1 (PARK7) has been linked to the development of early-onset Parkinson's disease (PD), but the underlying molecular mechanism is still unclear. Our preliminary tests showed that knockout (KO) of DJ-1 can sensitize substantial nigra to lipopolysaccharide (LPS), causing loss of dopaminergic neurons. This study was therefore aimed to explore the role of microglia-derived cytokines in the LPS-induced neuronal loss in DJ-1 deficient conditions. Firstly, protein-microarray and bioinformatics analyses were performed to investigate the expressional profiles of cytokines in substantia nigra in DJ-1 KO mice for identifying a microglia-derived cytokine that can activate an autocrine/paracrine network. Secondly, quantitative RT-PCR and various immunostaining assays were done to compare levels of INF-gamma (the hub cytokine) and I-TAC (a downstream mediator) in microglia cells with absence or deficiency of DJ-1. Thirdly, western blot and luciferase reporter assays were used to check the basal and LPS-evoked IKK signaling/NF-kappa B activation in microglia cells with DJ-1 deficiency. Finally, mixed cell culture, transwell assay and neutralized antibodies were applied to study the glial-neuronal crosstalk. Our results showed that DJ-1 deficiency increased the basal activity of NF-kB that sensitized microglia to LPS stimulation, and demonstrated that the sensitized microglia released higher INF-gamma to build an autocrine-paracrine circuit to affect survival of nearby neurons. These data indicate that INF-gamma derived from DJ-1 deficient microglia is responsible for the LPS-induced neuronal loss, and suggest that interaction between genetic (i.e. DJ-1) and inflammatory (eg. LPS) factors contribute to the development of PD.

P03-7

Dysfunction of mitochondrial respiration capacity underlies methamphetamine-induced neuronal death

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Methamphetamine (METH; *N*-methyl-1-phenylpropan-2-amine) is a cationic lipophilic molecule with potent stimulant actions on the central nervous system. Previous results from our laboratory demonstrated that mitochondrial dysfunction is responsible for acute necrotic cell death in the rostral ventrolateral medulla (RVLM) within the brain stem and subsequent cardiovascular collapse and fatality in rats received lethal doses of METH administration. The present study aimed to specifically investigate the fate of neurons receiving METH treatment and underlying cellular and molecular mechanism of mitochondria. The neuronal PC12 cells were treated with METH (0.1–20 mM) for 1–6 h and XTT or WST-1 assay was carried out to determine the cell viability. The apoptotic and necrotic cell death were examined by activated caspase-3 or LDH assay. The affected cellular respiration of mitochondria and ATP turnover rate were determined by the outcome of oxygen consumption rates (OCR) in mitochondria by using the Seahorse system. Our results showed that METH elicited a dose-dependently decrease of cell viability in neuron cell, represented by both necrotic and apoptotic cell death. Furthermore, METH elicited a significant decrease of basal respiration, maximal respiration or ATP turnover rate and an increase of proton leak in neuronal mitochondria. More importantly, the METH-induced detrimental responses in neuronal cells and neuronal mitochondria were significantly antagonized by pretreatment of coenzyme Q10, which is a highly mobile electron carrier in the mitochondrial respiratory chain and also acts as an antioxidant. The present results suggested that disturbance of mitochondrial respiration capacity underlies METH-induced neuronal cell death.

P03-8

Participation of inflammation in organophosphate-elicited mitochondrial dysfunction and necrotic cell death in glia

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Serving as an acetylcholinesterase inhibitor, the poisoning mechanisms for organophosphate compounds are generally believed to entail overstimulation by the accumulated acetylcholine on muscarinic receptors in peripheral and central synapse. However, our previous studies demonstrated that organophosphate pesticide mevinphos-induced muscarinic receptor-independent mechanism in rat brain stem contributed to the cardiovascular responses during mevinphos intoxication and we further found that mevinphos-induced cholinergic receptor-independent mechanism was responsible for necrotic cell death of neuronal PC12 cells via an induction of mitochondrial dysfunction. Concerning the linkage of inflammation to organophosphate intoxication is recently suggested and mitochondria is a vulnerable target affected by inflammation, the present study aimed to investigate whether inflammation is an alternative cholinergic-independent mechanism underlies the mitochondrial dysfunction and subsequent cell death in glia, the other key players in the nervous system, by using human glioma LN18 cells. We found that organophosphate mevinphos (Mev) or diisopropylfluorophosphate (DFP) elicited dose-dependent (1–20 mM) and time-dependent (6–12 h) decrease of cell viability, determined by XTT or WST-1 assay, accompanied by an upregulation of LDH but not that of activated caspase-3. Mev or DFP also elicited an increase of IL-1beta, accompanied by a decrease of basal respiration, maximal respiration or ATP turnover rate in glia cells. The present results suggested that inflammation might be an alternative cholinergic-independent mechanism underlies mitochondrial dysfunction and subsequent necrosis in glia cells during organophosphate intoxication.

P03-9

Differential neurotoxicity by arsenics in primary cultured cortical neurons

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Arsenic exposure via contaminated water and food as well as insecticides and chemotherapy, reportedly induces neurotoxicity in the central and peripheral nervous systems. In the present study, the neurotoxic effects of arsenics, including arsenite, monomethylarsonous acid (MMA^{III}), arsenate and dimethylarsinic acid (DMA^{V}) were studied using primary cultured cortical neurons. Incubation with equal molar arsenics (5 μM) for 24 h caused cell death of primary cultured cortical neurons and the cell viabilities of MMA^{III}, arsenite, DMA^{V} and arsenate were 22 ± 6%, 60 ± 2%, 94 ± 2% and 99 ± 2% of control, respectively. Furthermore, profound increases in heme oxygenase (HO)-1 and HSP-60 levels were observed in arsenite and MMA^{III}-treated cells. The apoptotic mechanism underlying arsenics-induced neurotoxicity was demonstrated by inducing caspase 3 activation and decreasing procaspases 12 level. The potency in caspase 3 activation and reduction in procaspase 12 levels was as follows: MMA^{III} >arsenite>>arsenate and DMA^{V}. Moreover, MMA^{III} was more potent than arsenite in autophagy activation and decreasing α-synuclein levels while DMA^{V} and arsenate scarcely altered these events. In addition, co-incubation with glutathione attenuated arsenics-induced HO-1elevation and HSP-60 elevation as well as apoptosis, suggesting that oxidative stress is involved in the arsenics-induced neurotoxicity. Furthermore, autophagy appears to play a pro-death role in arsenite-induced neurotoxicity.

P03-10

Investigation of the impact of ERK pathway in SCA17 transgenic mice

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Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant neurodegenerative disease caused by the CAG expansion in the TATA binding protein (TBP) gene. Clinical symptoms of SCA17 include ataxia, spasticity, chorea and cognitive decline. The neurological feature of SCA17 is Purkinje cell loss and gliosis. ERK pathway has been implicated in neurogenesis and behavioral function, but the role of activated ERK is unclear in SCA17 disease. Our laboratory have established the SCA17 transgenic mice that express human TBP with 109 CAG repeats (hTBP-109Q). These SCA17 mice showed ataxia and Purkinje cell loss, which recapitulate the patients' phenotypes and are suitable for study of SCA17 pathomechanism. Our results showed a significant reduction of calbindin expression at SCA17 transgenic mice since 4 weeks old. The overexpression of GFAP and TBP aggregation were also observed since then. We also found the pERK level in SCA17 transgenic mice was higher than wild type mice. The G-CSF treatment at 3 weeks old of age could ameliorate the behavioral and the pathological phenotypes of SCA17 transgenic mice. Furthermore, immunofluorescence staining analysis showed a significant increase of pERK expression in mouse cerebellum after 2 months old, and the activation of pERK was observed in the Bergmann glia cells. We applied the MEK activator and inhibitor in the SCA17 mouse cerebellar primary cultures to uncover the relationship between the ERK pathway and Purkinje cells degeneration. The modulation of ERK pathway could be considered as therapeutic strategy in SCA17 treatment.

P03-11

Study of SOD-like compounds on SCA17 transgenic mice

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Spinocerebellar ataxia 17 (SCA17) is an autosomal dominant neurodegenerative disease caused by trinucleotide CAA/CAG repeat excessive expansion in TATA box binding protein (*TBP*) gene. Expanded CAA/CAG repeat encodes polyglutamine (polyQ) in TBP, which causes mutant polyQ proteins aggregate and the cerebellar Purkinje cell degeneration. Some effective treatments for neurodegenerative disease using anti-oxidants have been reported in cellular and animal studies. Our lab has generated SCA17 mouse model and used for potential therapeutic screening. Three synthesized SOD-like compounds showed increase neurite outgrowth and reduce TBP aggregation on SCA17 cerebellar primary/slice cultures. *In vivo* treatment results showed that mouse body weight and spontaneous activity were unaffected under the treatment of these compounds. The motor coordination of mice on rotarod task was improved after treatment. We also found that Purkinje cell degeneration and mutant protein TBP aggregation were attenuated after the treatment with SOD-like compounds. These results suggest that these SOD-like compounds could be potential for SCA17 treatment.

P03-12

Soluble epoxide hydrolase inhibitor and 14,15-EET differentially regulate NMDA excitotoxicity in cortical neurons

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Soluble epoxide hydroxylase (sEH) is a dual activity enzyme with the C-terminal hydrolase activity mediating metabolic degradation of epoxyeicosatrienoic acids (EETs). Pharmacologic inhibition and genetic deletion of sEH have been shown to reduce infarct size of the cerebral cortex after experimental brain stroke via accumulation of a prosurvival 14,15-EET. The neuroprotective effect of sEH inhibitor was attributed to its anti-inflammatory effect in immune cells, but how its direct effect on neuronal sEH contributes to the neuronal survival remains unclear. In the present study, we used primary rat cortical neurons to investigate the effect of a C-terminal sEH inhibitor 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA) and 14,15-EET on the NMDA-induced excitotoxicity and neurotrophic factor expression. AUDA at 10 μM 14,15-EET at 1 μM were used to pretreat the neuronal culture 30 min prior to the NMDA treatment. Neuronal death was determined by lactic dehydrogenase (LDH) release assay, and viability was determined by WST-1 assay. Brain-derived neurotrophic factor (BDNF) mRNA expression was measured by quantitative RT-PCR. The results show that while 14,15-EET can attenuate NMDA excitotoxicity, 10 μM AUDA surprisingly enhanced 50 μM NMDA-induced excitotoxicity with the increase in LDH release and decrease in neuronal viability. Real-time PCR analysis revealed that AUDA also enhances 50 μM NMDA-induced BDNF mRNA expression. Thus, AUDA and 14,15-EET seem to opposingly affect NMDA excitotoxicity and BDNF expression. Of note, the neurotoxic of AUDA might counteract its anti-inflammation-dependent neuroprotection when applied to brain injuries involving excitotoxicity-induced neurodegeneration. (Supported by Aim for the Top University Plan, Ministry of Education, Taiwan)

P03-13

Evaluation and mechanism studies of potential treatments in SCA17 transgenic mice

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Spinocerebellar ataxia 17 (SCA17) is an autosomal dominant and progressive neurodegenerative disease. Patients with SCA17 have

phenotypes including ataxia, dystonia, parkinsonism, dementia and seizures. SCA17 is caused by CAA/CAG excessive expansion in TATA box Binding Protein (*TBP*) gene which encodes ploy-glutamine (polyQ) in TBP to form detergent-insoluble aggregates. Mutant TBP accumulates in Purkinje cells (PC) that may cause TBP lose its normal function and contribute to PC degeneration. To investigate the disease, our lab has generated SCA17 mouse model and used it to screen for potential therapeutic treatment. To have a quick platform, we also established cerebellar organotypic slice culture, for which is a good semi-*in vivo* system to mimic interaction between different cell types in an organ. We have used the SCA17 cerebellar organotypic slice culture to screen several Chinese herbal extracts and synthetic compounds. We aim to elucidate the molecular mechanism of neurodegeneration in SCA17 through the potential treatment targeting disease proteins in SCA17 transgenic mice.

P03-14

Gasotransmitter H_2S induced neuroprotective effect on Alzheimer's disease models

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The worldwide incidence of Alzheimer's disease (AD) is increasing and creating an unsustainable healthcare challenge due to a lack of effective treatment options. Recent evidence show that H_2S could exert antioxidant, anti-apoptotic, and anti-inflammatory effects. However, the correlation among H_2S, Abeta and tau protein in AD is not been elucidated. In this study, the effects and molecular mechanisms of H_2S in AD were investigated on mouse hippocampal primary culture and APP/PS1/Tau triple transgenic (3 x TG-AD) mice with inescapable foot-shock stress. We found that NaHS pretreatment in hippocampal primary culture reduced neurotoxicity associated with increasing the ratios of pERK/ERK and pS9-GSK3beta/GSK3beta. We then applied the NaHS to the 3 × Tg-AD mice to evaluate the *in vivo* effect. We found the administration of NaHS had no effects on mouse body weight, blood glucose and motor function. Furthermore, NaHS improved the spatial learning and memory of the 3 × Tg-AD mice with stress. Further elucidation of the molecular mechanism, we found that NaHS showed activities in anti-inflammation, anti-oxidationand, and anti-apoptosis. The levels of pS9-GSK3beta, and cholinergic neurons were increased, and tau phosphorylation, $Abeta_{40}$, and $Abeta_{42}$ were decreased. In sum, the administration of the NaHS induced neuroprotection and improved the spatial learning and memory of AD mice.

P03-15

Exposure to a maternal n-3 fatty acid-deficient diet during the brain development enhance the activity and dysregulation of hypothalamic-pituitary-adrenal axis responses to stress in rat offspring later in life

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Brain docosahexaenoic acid (DHA, 22:6n-3) accumulates rapidly during brain development and is essential for normal neurological function. The aim of this study was to examine whether DHA deficiency during brain development leads to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress later in life. Rats were exposed to an n-3 fatty acid-deficient or n-3 fatty acid-adequate diet from embryo to weaning at 3-weeks-old via maternal intake throughout the pregnancy and lactation, and then were changed to chow diet till sacrificed at 10-week-old. We found that the maternal rats fed n-3 fatty acid-adequate diet showed significant higher licking/grooming and arch-back nursing behavior than the maternal rats fed n-3 fatty acid-deficient diet. Exposure to the maternal n-3 fatty acid-deficient diet during the brain development resulted, at weaning, in a significant decrease in hypothalamic and hippocampal DHA levels and a reduced male offspring body weight. DHA deficiency during the brain development significantly increased and prolonged restraint stress-induced changes in colonic body temperature and serum corticosterone levels, caused a significant increase in sensitivity to dexamethasone negative feedback, and enhanced depressive-like behavior in the forced-swimming test and anxiety-like behavior in the plus-maze test in later life. These results suggest that DHA deficiency during brain development leads to excessive HPA responses and blunted HPA negative regulation to stress and elevated behavioral indices of depression and anxiety in adulthood.

P03-16

Ethyl pyruvate ameliorates 3-nitropropionic acid-induced striatal toxicity through anti-neuronal cell death and anti-inflammatory mechanisms

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The potential neuroprotective value of ethyl pyruvate (EP) for the treatment of the striatal toxicity is largely unknown. We investigated whether EP promotes the survival of striatal neurons in a 3-nitropropionic acid (3-NP)-induced mouse model of Huntington's disease (HD). EP (5, 10, 20, and 40 mg/kg/day, i.p.) was daily injected from 30 min before 3-NP intoxication (pretreatment) and from onset/progression/peak point of neurological impairment by 3-NP intoxication. EP produced a neuroprotective effect in dose- and time-dependant manners. EP pretreatment of 40 mg/kg/day produced the best neuroprotective effect among other conditions. Pretreatment of EP significantly attenuated neurological impairment and lethality and prevented formation of lesion area and neuronal loss in the striatum after 3-NP intoxication. This neuroprotection afforded by EP was associated with the suppression of succinate dehydrogenase activity, apoptosis, and microglial activation. The suppressive effect of EP corresponded to the down-regulation of mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-κB) signal pathways, and mRNA expression of inflammatory mediators including tumor necrosis factor-alpha, interleukin (IL)-1 beta, IL-6, inducible nitric oxide synthase, and cyclooxygenase-2 in the striatum after 3-NP intoxication. Interestingly, the intrathecal introduction of inhibitors MAPKs and NF-κB into control mice decreased the lethality after 3-NP intoxication. Our findings indicate that EP may effectively alleviate 3-NP-induced striatal toxicity by inhibition of the MAPKs and NF-κB pathways in the striatum, and that EP has a wide therapeutic window, suggesting that EP may have therapeutic value in the treatment of aspects of HD's disease related to inflammation.

P03-17

Korean red ginseng extract alleviates experimental autoimmune encephalomyelitis in rats

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The preventive and therapeutic mechanisms of Korean red ginseng extract (KRGE) in autoimmune disorders of nervous system are not clear. We investigated whether KRGE has a beneficial effect in a rat model of acute experimental autoimmune encephalomyelitis (EAE). Pretreatment with KRGE from 10 days before immunization with myelin basic protein (MBP) $_{68\text{-}82}$ peptide was effective, while post-treatment after the appearance of symptoms was not. Pretreatment with KRGE significantly attenuated clinical signs and loss of body weight, associated with the suppression of spinal demyelination and glial activation. The suppressive effect of KRGE corresponded to the down-regulation of p38 mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) signal pathways, and mRNA expression of pro-inflammatory cytokines, chemokines, adhesion molecules, and inducible nitric-oxide synthase in the spinal cord after immunization. Interestingly, pretreatment with KRGE significantly reduced population of CD4+, CD4+ /IFN-γ+, and CD4+ /IL-17 + T cells in spinal cords of EAE rats, corresponding with down-regulation of mRNA expression of IFN-γ, interleukin (IL)-17, and IL-23. Also, pretreatment with KRGE significantly increased population of CD4+, CD4+ /IFN-γ+, and CD4+ /IL-17 + T cells in spinal cords of EAE rats, corresponding with down-regulation of mRNA expression of IFN-γ, IL-17, and IL-23. On the other hand, the pretreatment with KRGE increased population of CD4+ /Foxp3 + T cells in spinal cords of EAE rats. The results strongly indicate that treatment of KRGE could delay or diminish the development and progression of EAE by inhibiting the activation of resident immune cells and differentiation/infiltration of peripheral immune cells through down-regulation of p38 MAPK and NF-κB pathway in acute EAE rats.

P03-18

Oriental medicine hyung-bang-pae-dok-san alleviates experimental autoimmune encephalomyelitis in mice

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The preventive and therapeutic mechanisms of in autoimmune disorders of nervous system are not clear. We investigated whether hyung-bang-pae-dok-san (HBPDS), a traditional herbal prescription, has a beneficial effect in myelin oligodendrocyte glycoprotein (MOG) peptides-immunized chronic experimental autoimmune encephalomyelitis (EAE). Onset-treatment with HBPDS (30% ethanol extract) significantly attenuated clinical signs and loss of body weight, associated with the suppression of spinal demyelination and glial activation. The suppressive effect of HBPDS corresponded to the down-regulation of extracellular-regulated protein kinase (ERK) mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) signal pathways, and mRNA expression of pro-inflammatory cytokines, chemokines, adhesion molecules, and inducible nitric-oxide synthase in the spinal cord after immunization. Onset-treatment with HBPDS significantly reduced population of CD4 + , CD4 + /IFN-γ+, and CD4 + /IL-17 + T cells in spinal cords of EAE rats. Also, onset-treatment with HBPDS significantly increased population of CD4 + , CD4 + /IFN-γ+, and CD4 + /IL-17 + T cells in spinal cords of EAE mice. The results strongly indicate that treatment of HBPDS could diminish the development and progression of EAE by inhibiting the activation of resident immune cells and differentiation/infiltration of peripheral immune cells through down-regulation of ERK MAPK and NF-κB pathway in chronic EAE mice.

P03-19

Leptin antagonist alters amyloid beta metabolism and mitochondrial functions in SHSY5Y cells

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Several epidemiological studies have shown the association of decreased level of serum leptin with Alzheimer's Disease (AD). In experimental research, growing evidence indicates that leptin is a pleiotropic hormone that has diverse actions in CNS including hippocampal neurogenesis and synaptic plasticity and also exhibits neuroprotective and antiapoptotic properties against a variety of neurotoxic agents [Doherty et al., 2008; Weng et al., 2007]. Thus, the hypothesis that an absolute deficiency or an impaired signaling of leptin acts as a trigger for sporadic AD pathogenesis appears attractive. In this context we have examined the effects of leptin antagonist (LA), a triple mutant form of the hormone, on amyloid beta metabolism, mitochondrial functions and reactive oxygen species production in SHSY5Y cells. Cells (60–70 % confluent) were exposed to 400 ng/mL of leptin antagonist for up to 48 h followed by the measurement of APP gene expression, Aβ42 content and the activity of β secretase as also mitochondrial membrane potential, and cellular ATP content and ROS load. Our results show that leptin antagonist causes mitochondrial impairment with enhanced production of ROS, increased accumulation of Aβ42, moderate increase in β secretase activity without any remarkable change in APP mRNA expression in SHSY5Y cells. These changes are associated with significant loss of viability and morphological abnormalities of the cells. Our observations tend to suggest that a deficient leptin signaling can mimic several key molecular features of AD pathogenesis like amyloid beta accumulation, oxidative stress and mitochondrial dysfunction in SHY5Y cells.

P03-20

Development of health products to facilitate recovery of nerve function in diabetics

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At present, the diabetic neuropathy is increasing worldwide approximately 75% of diabetic complications. Previous studies

have been reported that many herbal extracts and natural products possessing antioxidant activity could accelerate recovery after nerve injury by enhancing antioxidant enzymes and reducing tissue damage induced by free radicals. The efficacy of therapeutic strategies for diabetic neuropathy is still not in satisfaction level. Recently, it has been reported that substances delivery via transdermal patch can decrease first pass effect giving rise to the decreased therapeutic dose and decreased side effects. Therefore, this study aimed to develop and to evaluate the effect of the health product on functional recovery of the diabetic neuropathy. In this study, Quercetin and Tomato extract were developed as health products as transdermal patch with electrospinning technique and determined enhancing effect on functional recovery of diabetic neuropathy and determined the possible mechanism of transdermal patch. Male Wistar rats were induced diabetes mellitus and crushing the right sciatic nerve. Quercetin-loaded and Tomato-extract-loaded transdermal patches at concentrations of 5, 10 and 15% with the sized of 1x1 cm once daily at the area which the nerve crush injury was performed for 21 days. The evaluation of nerve function was performed using foot withdrawal reflex, De Medinacelli method every 3 days throughout 21-day study period. The results revealed that 5% Quercetin-loaded and 10% Tomato extract-loaded transdermal patches could enhance the functional recovery of lesion nerve. The underlying mechanism of Quercetin-loaded transdermal patch might occur partly via the decreased oxidative stress whereas Tomato extract-loaded transdermal patch might occur via other mechanisms. Therefore, the current data demonstrates the potential as novel health products against diabetic neuropathy.

P03-21

Biochemical, histological and proteomic characterization of contusion and pericontusion during traumatic brain injury

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Traumatic brain injury (TBI) involves pathologically distinct regions of injury called Contusion (injured tissue) and pericontusion (surrounding contused tissue). These regions could be implicated in secondary injury events following injury. We sought to characterize the injured tissue to elucidate the secondary injury pathways. Contusion ($n = 20$) and pericontusion ($n = 16$) tissues obtained from autopsy and from TBI patients undergoing craniotomy. Age matched pathologically normal frontal cortex tissues which were farthest from the site of injury served as controls. Assays for oxidative stress and antioxidant function; immunohistochemistry for GFAP, phosphorylated Neurofilament, ubiquitin and tau; and LC-MS based proteomics followed by bioinformatic analysis was carried out. Study demonstrated extensive oxidative damage evidenced by altered redox markers consistent decrease of antioxidant enzymes such as SOD and GSH metabolic enzymes and ATP depletion in the contused tissues. Total GSH was significantly decreased both in the synaptosomal and mitochondrial fractions both in contusion and pericontusion. Oxidative stress markers such as lipid peroxides, and protein carbonyls were elevated in the contusion. Histological evaluation showed significant demyelination and increased dystrophic neurons in pericontusion. Astrogliosis was prominent in contusion compared to pericontusion. Microglial activation as seen by Iba1 + cells, oedema and axotomy was more in pericontusion compared to contusion. Functional annotation of proteomics data showed down-regulation of synaptic proteins and up-regulation of inflammatory proteins in contusion. Pericontusion showed down-regulation of structural/cytoskeletal proteins and up-regulation of negative cell regulation proteins. We believe that the current data gives insight into the pathology related to secondary damage and subsequently helps in understand the dynamics of injury.

P03-22

Cathepsin C and Cystatin F gene interaction during demyelination

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Cystatin F, a papain-like lysosomal cysteine proteinase inhibitor, and its main substrate, Cathepsin C, have been demonstrated to be crucial factors in demyelinating diseases. It is found that the expression of Cathepsin C and Cystatin F are profoundly elevated and matched with ongoing demyelination/remyelination. However, their accurate functional role in demyelinating diseases is still unclear. To clarify their function in the pathological process of demyelination, we used a spontaneous chronic demyelination mouse model, named heterozygous PLP transgenic 4e ($PLP^{4e/-}$) mice. Meanwhile, Flexible Accelerated –STOP-Tetracycline Operator Knockin (FAST) system is applied to up or down regulate Cathepsin C or Cystatin F gene expression. In situ hybridization revealed that in $PLP^{4e/-}$ mice conditional knock down of Cystatin F gene in microglia lead to the down regulation of Cathepsin C mRNA levels. On the contrary, Cathepsin C gene expression is enhanced by up regulating Cystatin F. It means that Cathepsin C gene and Cystatin F gene interact with each other through some unknown mechanism during demyelination. It has important significance to clarify this mechanism for understanding their function in this disorder. Further study is needed to estimate the possible pathway and substrate of Cystatin F that influences Cathepsin C gene expression.

P03-23

Effect of melatonin on the expression of clock genes in the hippocampus of high-fat diet fed and streptozotocin-induced diabetic rats

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The circadian clock is an endogenous system that acts as an internal time-keeping device, generates approximately 24-hour oscillations

in physiology and behavior known as circadian rhythms. An important function of the circadian clock is to synchronize different metabolic processes in an organism including energy metabolism, sleep-wake cycles, hormone secretion, and cardiac function. Recent evidence have demonstrated that the dysfunction of the circadian clock is associated with the development of several pathological conditions including diabetes. Moreover, there is evidence that a functional clock exists in many parts in the brain including the hippocampus. Therefore, this study aims to investigate the effect of streptozotocin (STZ)-induced diabetes on the clock genes expression in the rat hippocampus, as well as to investigate whether the exogenous melatonin, a major entraining signal for the circadian systems, can restore the expression of clock genes. Our results show that exogenous melatonin provides a protective effect against an alteration of clock genes in the hippocampus of STZ-induced diabetic rats. These data demonstrate the important of circadian clock impairment associated with diabetes which may provide new insights and treatment targets for this disease.

P03-24

Aging and antioxidants modulate amyloid beta metabolism, memory function and survival in rats: implications in the pathogenesis of Alzheimer's disease

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In aging brain varied forms of behavioural and biochemical deficits take place which show many commonalties with Alzheimer's disease (AD) such as increased amyloid beta peptide deposition, mitochondrial dysfunctions, pro-inflammatory reactions and oxidative stress. The aged brain is a useful tool to investigate altered metabolism of amyloid beta peptide that may have implications in the pathogenesis of AD. In the present study, we have shown a significant increase in the amyloid precursor protein (APP) level in the brain cortex of aged rats (22–24 months) without a corresponding increase in the level of APP mRNA. Moreover, the activity of β secretase is elevated (65%) and that of neprilysin diminished (48%) in brain cortex of aged rats compared to that in young rats (4–6 months). All these changes lead to a markedly increased accumulation of Aβ42 in brain cortical tissue of aged rats. Long-term dietary supplementation of rats with α-tocopherol, *N*-acetyl-cysteine and α-lipoic acid has been carried out from 18 months onwards daily till the sacrifice of the animals by 22–24 months. The antioxidant supplementation attenuates all the age-related alterations in amyloid beta metabolism. Further, a significant impairment of spatial learning and memory and survival has been observed in aged rats concomitant with altered brain metabolism of amyloid beta peptide, and the same dietary antioxidant supplementation of aged rats strikingly prevented the former phenomenon. The results indicate the therapeutic potential of this antioxidant combination in ameliorating the amyloid beta load in AD brain.

P03-25

Effect of d-amphetamine on dopaminergic neurons of substantia nigra and expression of tyrosine hydroxylase in the striatum, nucleus accumbens and prefrontal cortex of d-amphetamine treated Wistar rat

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Dopaminergic neurons of the midbrain are the main source of dopamine in the mammalian central nervous system. Dopamine is a chemical messenger active in mesolimbic and mesocortical reward pathways. Dopamine is manufactured in nerve cell bodies in the ventral tegmental area (VTA) and is released in the nucleus accumbens and the prefrontal cortex. To compare dopaminergic neurons of substantia nigra and level of tyrosine hydroxylase (TH) in the striatum, pre- frontal cortex and nucleus accumbens of D-Amphetamine treated wistar mice. 15 wistar rat were injected subcutaneously with amphetamine 10 mg/Kg body weight till 7 days while the controls groups (15 wistar rat) were injected with normal saline in the same dose. On day 7 both the groups were deeply anesthetized, perfused with a fixative of 4% paraformaldehyde in 0.1 sodium phosphate buffer (PBS), PH 7.4. Tissue sectioning was done followed by immunohistochemical (IHC) staining. One way-ANOVA test and *post hoc* Tests was applied. Decreased level of tyrosine hydroxylase was present in striatum, nucleus accumbens and pre-frontal cortex. The percentage of tyrosine hydroxylase in all these 3 areas were highly significant ($p < 0.001$). D-AMPH affects the neuronal cell morphology and decreases expression level of TH. Degeneration of dopaminergic neurons and damaged synaptic connection in substantia nigra, was observed leading to a reduction of stratial dopamine levels. Animal models of Parkinson's disease, Schizophrenia, Alzheimer's disease, introverted personalities, Attention deficit hyperactivity disorder (ADHD) can be designed for the further clinical experiments.

P03-26

Role of caspase-3 in development of neuronal plasticity and memory in rats subjected to prenatal hypoxia

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Caspases (in particular, caspase-3) play an important role in cellular processes underlying brain development, normal cell functioning and apoptosis. Increased caspase activity in pre- and postsynaptic terminals leads to proteolysis of synapse-associated proteins, including cytoskeleton and receptors, and disruption of synaptic activity. As such, inhibition of caspases is considered as a tool for prevention and compensation of various synaptic pathologies. The brain of rats subjected to prenatal hypoxia (E14, O_2 7%, 3 h) is characterised by an increased number of caspase-3-positive neurones and higher activity of this enzyme in the neocortex and hippocampus in the period of intensive synaptogenesis (P20–30) compared to controls. Subsequently, in later life (P30–0) these animals have a reduced number of synaptopodin-positive dendritic

spines in these brain areas accompanied by disruption of cognitive functions. Single i.v. injection of caspase-3 inhibitors (Z-DEVD-FMK or Ac-DEVD-CHO) to hypoxic rats on P18-23 led to a decrease in caspase-3 activity in their brain and improved memory assessed by the novel object recognition test. On the contrary, injections of the inhibitors to control rats resulted in increased activity of the enzymes and disruption of memory. The effects of inhibitors on memory was observed within one month after administration but not detected 2.5 months later suggesting some transitory changes. The data obtained demonstrate the involvement of caspase-3 in normal brain development which is disrupted by prenatal stress and indicate an important role of this enzyme in neuronal plasticity. [Supported by RFBR (13-04-00388) and Program of RAS "Fundamental Sciences to Medicine"]

P03-27

Proteomic analysis of lipid A-activated choroid plexus cell line ECPC4 cells

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Choroid plexus (CP) is the main production site of CSF and responsible for the inflammatory mediators which are thought to play a crucial role in the process of bacterial meningitis. ECPC4 cells are established as choroid plexus epithelial (CPe) cell lines. We studied the intracellular and secreted proteins from lipid A-activated choroid plexus cell line ECPC4 cells. The intracellular and the secreted proteins from Lipid A-treated ECPC4 cells were analysed by the two-dimensional (2D) gel electrophoresis followed by MALDI-TOF MS/MS. There were approximately 350 spots in each SYPRO Ruby-stained 2D gel from ECPC4 cells. The levels of proteins were significantly changed in the intracellular proteins and the secreted proteins of the conditioned medium in ECPC4 cells, respectively. The changed proteins were grouped by functions, which were chaperone, cytoskeleton and structural protein in the intracellular proteins. Thus, the intracellular and secreted proteins might be involved in innate immunity in the brain. In addition, cytoskelton, proteolysis and DNA synthesis nucleoside were changed in the conditioned-medium of ECPC4 cells. We propose that the CPe plays pivotal roles in innate immunity responses and host defense in the brain.

P03-28

Pantaprazole inhibits the growth of glioma cell lines and alters LASS2 expression

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Gliomas are the devastating primary brain tumors and recur even after standard therapy. Acidic tumor microenvironment has been shown to have a role in resistance to chemotherapy, proliferation and metastatic behaviour. Pantaprazole (PPZ) is a proton pump inhibitor widely used for protection against acid related diseases. Recently PPZ is reported to induce apoptosis. The present study elucidates the mechanism underlying PPZ apoptotic effects, using C6 glioma cell line. We observed that treatment with PPZ significantly reduced the proliferation of C6 cell line in a dose and time-dependent manner and induced apoptosis, which was evident from enhanced apoptotic protein expression, PARP cleavage and TUNEL positive cells. PPZ treatment significantly down-regulated TNF-alpha induced NF-kappaB nuclear translocation, NF-kappaB DNA binding activity in C6 cells in a dose-dependent manner. Protein encoded by LASS2/TMSG-1 is reported to interact with the C subunit of V-ATPase, suggesting that LASS2/TMSG1 might inhibit the invasion and metastasis of tumor cells through regulating the function of V-ATPase. We have found decreased or loss of LASS2 expression in high grade GBM compared to control and low grade astrocytoma both at mRNA and protein level. Further we are evaluating the outcome of overexpression of LASS2 in U87 glioma cell line.

P03-29

Increase in astrocytes that express $GABA_B$ receptors in the dentate hilus of mouse during neuronal regeneration

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$GABA_B$ receptors mediate slow and prolonged synaptic inhibition in the brain. To date 2 $GABA_B$ receptor subunits have been identified, $GABA_BR1$ and $GABA_BR2$, and functional $GABA_B$ receptors are heterodimers formed between these 2 proteins. It is broadly accepted that once dentate granule cells are injured, newly generated neurons would be supplied by neuronal progenitor/stem cells in the subgranular layer. It has been demonstrated that the neuronal degeneration in the dentate granule cells caused by the administration of a neurotoxin, trimethyltin (TMT), peaked and regeneration started in parallel within the 2 days after the administration and the regeneration seemed finishing at the 30th day. While mossy fiber, a bundle of axons those originate in the dentate granule cells, would be damaged, cleared by microglia and re-innervated from newly appeared granule cells, however it has not been clear yet. In this experiment, we tried to observe damage in mossy fiber at first. After administration of TMT (2.8 mg/kg i.p.), hippocampal slices were prepared and stained with Fluoro-Jade B (FJB), a marker of neuronal degeneration. In mossy fiber but not pyramidal neurons in the CA3 region, FJB signal was observed and it was sustained until the 30th day at least. Next, the immunochemistry revealed that in the dentate hilus, the GFAP-positive cells, probably astrocytes were increased and $GABA_BR2$ signal was merged with those cells. It was suggested that recovering processes from neurodegeneretaion that induced by TMT are still ongoing at the 30th days. And the process might include modification of $GABA_B$ receptor functions in the astrocytes.