S10.1

The effect of PICK1 PDZ domain inhibitor FSC231 on long-term potentiation of hippocampus in rat

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Aim: FSC231 is the specifical PDZ domain inhibitor of PICK1 which has been suggested as a potential drug target against brain ischemia, pain and cocaine addiction. Our previous study has shown that FSC231 reversed cocaine-induced conditioned place preference, but its exact mechanism is still unknown. In the present study, we explore the effect of FSC231 on long-term potentiation (LTP) in hippocampus of rat *in vivo* and study the cellular and molecular mechanisms of addiction memory. **Methods:** *In vivo* electrophysiological recording was employed to test the effect of FSC231 on the induction of LTP which was produced by high-frequency stimulation (HFS) in dentate gyrus of the hippocampus. **Results:** It was found that intracerebroventricular (ICV) injection of 25 mmol/L FSC231 in 5 μL did not change the basal EPSP and did not affect the paired-pulse facilitation which represents the pre-synaptic changes. Exposure of FSC231 30 min before HFS inhibited the induction of LTP. FSC231 also reduced the amplitude of LTP which has been maintained for 30 min. **Conclusion:** The inhibitory effect of FSC231 on the induction and maintaining of LTP via PICK1 may be the mechanisms for treatment of addiction memory associated with drug abuse.

Keywords: FSC231; PICK1; long-term potentiation; hippocampus

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S10.2

Abnormal changes in voltage-gated sodium channels $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.6$, and in CaM/ CaMKII, within the brains of two genetic epilepsy animal models

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Aim: To analyze both the expression and distribution of the voltage-gated sodium channels (VGSCs) subtypes - $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$, in the hippocampus and in the cortex of the temporal lobe of two genetic epilepsy animal models, the spontaneously epileptic rat (SER) and the tremor rat (TRM). The expression of calmodulin (CaM) and calmodulin-dependent protein kinase II (CaMKII) were also analyzed with the purpose of assessing the effect of the CaM/ CaMKII pathway in these two epilepsy models. **Methods:** The protein expressions of the four VGSC subtypes and CaM/CaMKII were detected by Western blot. Double-labeled immunofluorescence was performed to analyze the distributions and co-localization of these proteins. **Results:** Increased expression of the four VGSC isoforms and CaM accompanied by a decrease in CaMKII was observed in the hippocampus of both the SERs and the TRM rats. However, the change observed in the expression of VGSC subtypes and CaM was decreased with an elevated CaMKII in the cortex of their temporal lobes. In addition, the four isoforms of the VGSC proteins were present throughout the CA1, CA3 and dentate gyrus regions of the hippocampus and temporal lobe cortex and they were co-localized in neurons with CaM. **Conclusion:** These data represent the first evidence of abnormal changes in expression of four VGSC isoforms ($Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$) and CaM/ CaMKII in the hippocampus and temporal lobe cortex of SERs and TRM rats. These changes may be involved in the generation of epileptiform activity and underlie the observed seizure phenotype in these rat models of genetic epilepsy.

Keywords: voltage-gated sodium channels; subtype; calmodulin; calmodulin-dependent protein kinase II; genetic epilepsy

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S10.3

BDNF genetic variability modulates psychopathological traits associated with eating disorders

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Aim: The brain-derived neurotrophic factor (*BDNF*) gene has been shown to impact eating behavior. We aimed to investigate whether *BDNF* genetic variability may influence psycopathological features in patients with eating disorders (ED) and/or modulate the risk for the disorder. **Methods:** A total of 120 unrelated female patients with anorexia or bulimia nervosa (AN, BN) and 125 healthy controls were genotyped for two common *BDNF* single nucleotide polymorphisms (SNP), Val66Met and C-270C, and several selected tag SNPs. Associated psychopathological characteristics were assessed by the EDI-2 and SCL-90R inventories. **Results:** The rs11030119 AA genotype was found to increase the risk for AN [OR=5.23 (1.32–20.98), P=0.02] but the association did not survive correction for multiple testing. The rs16917237 TT genotype was associated with increased weight (60.6±19.3 *vs* 50.4±11.9 kg) and BMI (23.3±7.9 *vs* 19.3±4.1) in the subjects with ED (Bonferroni-P<0.05 *vs* other genotypes in both cases). AN patients who were carriers of the -270CC or rs10835210CC genotypes scored higher in the Interpersonal Distrust and Drive for Thinness scales, respectively (Bonferroni-P<0.05 in both cases). In addition, two *BDNF* haplotypes (**4* and **7*) showed significantly higher scores in several scales of the EDI-2 and SCL-90R inventories than those of the haplotype most commonly found in BN patients. **Conclusion:** Variability in the *BDNF* gene locus may contribute to psychopathological features that are commonly found in these patients

Keywords: anorexia nervosa; bulimia nervosa; BDNF

Acknowledgements: This work has been supported in part by grants GR10122 and GR10022 from Junta de Extremadura, Consejería de Economia, Comercio e Innovacion, Merida (Spain), and grant from Fundación Alicia Koplowitz.

S10.4

Effect of *BDNF* genetic polymorphisms on psychopathological traits associated with anorexia and bulimia nervosa

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Aim: The brain-derived neurotrophic factor (*BDNF*) gene has been shown to affect eating behavior. We aimed to investigate whether *BDNF* genetic variability may influence psycopathological traits in patients with eating disorders (ED). **Methods:** A total of 120 unrelated female patients with anorexia or bulimia nervosa (AN, BN) and 125 healthy controls were genotyped for two common *BDNF* single nucleotide polymorphisms (SNP), Val66Met and C-270C, and several selected tag SNPs. Associated psychopathological characteristics were assessed by the EDI-2 and SCL-90R inventories. **Results:** The rs16917237 TT genotype was associated with increased weight (60.6±19.3 *vs* 50.4±11.9 kg) and BMI (23.3±7.9 *vs* 19.3±4.1) in the subjects with ED (Bonferroni-P<0.05 *vs* other genotypes in both cases). AN patients who were carriers of the -270CC or rs10835210CC genotypes scored higher in the Interpersonal Distrust and Drive for Thinness scales, respectively (Bonferroni-P<0.05 in both cases). In addition, two *BDNF* haplotypes (**4* and **7*) showed significantly higher scores in several scales of the EDI-2 and SCL-90R inventories than those of the haplotype most commonly found in BN patients. **Conclusion:** Variability in the *BDNF* gene locus may contribute to psychopathological features that are commonly found in patients with eating disorders.

Keywords: anorexia nervosa; bulimia nervosa; brain-derived neurotrophic factor (BDNF)

Acknowledgements: The project was supported in part by grants GR10122 and GR10022 from Junta de Extremadura, Merida (Spain); and Fundación Alicia Koplowitz (Barcelona, Spain).

S10.5

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

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Aim: Accumulating epidemiological evidence shows that early life events have long-term effects on the susceptibility to subsequent stress exposure during adulthood.

Here we reported the effects of post-weaning social isolation, an animal model of early life adversities, on the behavioral responses to acute stress in adult mice. **Methods:** At postnatal d 21, mice were randomly assigned to group-housed (GH) or isolated cages for 8 weeks until at the age of 3 months when behavioral tests were performed. **Results:** Socially isolated (SI) mice exhibited a higher spontaneous locomotor activity and more offensive behaviors but not attack number compared with GH mice. 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 NR_{2A} and NR_{2B} without affecting NR_1 subunit of NMDA receptors, PSD-95 and α_2 subunit of $GABA_A$ receptors. Bilateral hippocampal injection of NMDA antagonists reversed acute stress-induced exaggeration of aggressive behaviors in the SI mice. **Conclusion:** These results suggested the involvement of NMDA receptors in the isolation-induced alterations of behavioral phenotypes and NMDA receptor antagonists may be useful to ameliorate child neglect-induced exacerbation of aggressive behaviors.

Keywords: social isolation; aggression; NMDA receptors; acute stress

S10.6

Evaluation on drug dependence of the ethanol extract of *Valeriana jatamansi* Jones

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Aim: To evaluate the drug physical dependence of the ethanol extract of *Valeriana jatamansi* Jones, which is an anxiolytics. **Methods:** Physical dependence mice model was established as positive control through the way of long term usage of diazepam, and the withdrawal symptoms, tailing rate and body weight change were compared. The ethanol extract of *V. jatamansi* can be judged whether it will induce physical dependence. Moreover, gene expression profiling was analyzed to explore the mechanism of drug physical dependence. **Results:** Animal experiment result showed that mice of positive control group appeared obvious withdrawal symptoms, but the *V. jatamansi* groups did not show obvious withdrawal symptoms. Compared with normal mice, the tailing rate of positive control group increased significantly (P<0.01), and their body weight were reduced significantly after drug withdrawal (P<0.01). The tailing rates of *V. jatamansi* groups were always low, and their body weights changed slightly, which had no significant difference compared with normal mice (P>0.05). Gene expression profiling result showed the genes CHRM1, CHRM3, HTR1A, HTR2C, GABRA3 are closely related to drug dependence. GABRG6 were changed significantly in positive control group, which were involved in neuro-active ligand-receptor interaction signaling pathway. And *V. jatamansi* has slight effect on these genes. **Conclusion:** The physical dependence model was successfully established in mice by long-term usage of diazepam. The mice in *V. jatamansi* groups did not show significant symptoms of physical dependence, and *V. jatamansi* has slight effect on these genes related to drug-dependence, which suggests that *V. jatamansi* has low potentiality on physical dependence.

Keywords: *Valeriana jatamansi* Jones; physical dependence; withdrawal symptoms; gene chips; signaling pathway

S10.7

HMGB1 mediates OGD-induced impairment in astrocytic glutamate clearance

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Aim: Astrocytic glutamate transporter GLAST plays a critical role in clearance of extracellular glutamate and prevention of glutamate-induced excitotoxicity. In this study, we used oxygen-glucose deprivation (OGD) as an *in vitro* ischemia model to investigate the role of pro-inflammatory cytokine HMGB1 (high-mobility group protein B1) in ischemia-induced impairment in astrocytic GLAST function. **Methods:** Cultured astrocytes were exposed to OGD for 6 h, and then HMGB1 receptors antagonists FPS-ZM1 were added to culture medium for 18 h. Expression of HMGB1 and GLAST in cultured astrocytes were examined by Western blot, function of glutamate clearance was evaluated by determination of extracellular glutamate concentration by enzyme colorimetric method. Cultured astrocytes were treated with HMGB1 recombinant protein for 24 h, and expression of GLAST was examined by Western blot. **Results:** OGD induced a significant decrease in expression of GLAST and glutamate clearance activity, a significant increase in expression of HMGB1 in cultured astrocytes. The concentration of HMGB1 was markedly increased in OGD-treated astrocytic medium. FPS-ZM1 can effectively inhibit OGD-reduced expression of GLAST and glutamate clearance activity. In addition, HMGB1 recombinant protein significantly reduced expression of GLAST and glutamate clearance activity in cultured astrocytes. These inhibitions could be prevented by FPS-ZM1. **Conclusion:** HMGB1 plays a critically regulatory role in OGD-reduced function of astrocytic glutamate transporter.

Keywords: oxygen glucose deprivation; astrocyte; GLAST; HMGB1

S10.8

The cognitive-enhancing effects of SIPI-SCPd on memory disorder mice and APP/PS1 mice

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Aim: Rhizomes of *Acorus gramineus* has been used as a traditional medicine in China for treating cognitive disorders for hundreds of years. SIPI-SCPd was the bioactive component from rhizomes of *Acorus gramineus*. In this paper, we studied the cognitive-enhancing effects of SIPI-SCPd in memory disordered mice and APP/PS1 transgenic mice. **Methods:** 1) Step-down test, Step-through test and Morris water maze experiments were used. The acquired learning dysmnesia was induced by scopolamine, the memory retention dysmnesia was induced by sodium nitrite, and the memory reappearance dysmnesia was induced by 45% ethanol in mice to imitate the damages of three stages in elementary process. The effects of SIPI-SCPd (70, 35, 17.5, and 8.75 mg/kg, ig) on learning and memory were studied. In order to clarify the mechanism of SIPI-SCPd, we also measured the activities of acetylcholine esterase (AChE) in brains of normal mice. 2) Oxidative dementia was induced by D-galactose in mice to imitate natural aging process, and SIPI-SCP (70, 35, 17.5, and 8.75 mg/kg, ig) were given simultaneously. After 8 weeks, mice's spatial memory was estimated by Morris water maze. The activities of superoxide dismutase (SOD) and the contents of malondialdehyde (MDA) in the brains were also tested. 3) The APP/PS1 transgenic mice was used to imitate the neuropathology features of Alzheimer's disease. The Morris Water Maze was applied to estimate the improvement of spatial memory after SIPI-SCPd (70, 35, and 17.5 mg/kg, ig) treatment. Immunochemistry staining is used to detect the number of β-amyloid peptide, ratio of P_{396}-tau cells and BDNF level in hippocampus and cortical area. **Results:** 1) 17.5 mg/kg and above dosage of SIPI-SCPd had the good effects in improving the learning and memory in mice with memory acquisition disorder, memory retention disorder and memory reappearance disorder. However, SIPI-SCPd didn't influence the activities of AChE in brains of normal mice within the measured dosage range. 2) In mice with oxidative stress damage induced by D-galactose, 17.5 mg/kg and above dosage of SIPI-SCPd could enhance the spatial memory of mice. At the same time, SIPI-SCPd enhanced the activities of SOD and reduced the content of MDA. 3) After 2 months administration, SIPI-SCPd improved the spatial memory in the 6 months of age APP/PS1 transgenic mice at dosage of 70 and 35 mg/kg. The immunochemistry assay showed that SIPI-SCPd of 70 mg/kg significantly reduced the amount of Aβ plaques in the mice brain. SIPI-SCPd 70, 35, and 17.5 mg/kg could significantly increase the BDNF protein level in transgenic mice hippocampus (P<0.05 *vs* APP/PS1 model group), but had no effects in cortical area. **Conclusion:** SIPI-SCPd had the satisfactory effects in improving the learning and memory on memory disordered mice and APP/PS1 mice. Its mechanisms may be related to its abilities of: 1) anti-oxidation; 2) reducing the Aβaggregation; 3) increasing BDNF protein level in hippocampus.

Keywords: Alzheimer's disease; SIPI-SCPd; BDNF; Aβ; APP/PS1 transgenic mice; dementia mice

S10.9

New protection effect on stress induced depression provided by reducing glucocorticoids

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Aim: To explore the effect of adrenalectomy (ADX) on chronic stress-induced depression. Methods: The investigation activity and emotional changes was recorded in 10 and 17 d after stress, respectively. BDNF and GR protein expression in hippocampus and PFC was determined by Western blot. The hippocampus neurons were detected by Nissle. **Results:** ADX can increase the investigation activity of rats in open field test and decrease emotional change in forced swimming test. BDNF and GR protein expression in hippocampus and PFC was

down-regulated in sham group while up-regulated in ADX group after stress. The hippocampus CA1 and CA3 neurons did not show shrinkage in ADX group. **Conclusion:** ADX protect hippocampus neurons from impairment by up-regulating BDNF and GR protein expression in hippocampus and PFC.

S10.10

The selective BACE1 inhibitor VIa reduces Aβ production and ameliorates behavioral deficits in a mouse model of Alzheimer's disease

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Aim: We are pursuing potent BACE1 inhibitors in an effort to identify suitable AD drug candidates. **Methods:** Employing a homogeneous, time-resolved fluorescence (hTRF) technique, we determined the selectivity of compound VIa. We then conducted a cell-based assay using the huAPPswe/huBACE1 transgenic CHO cells to investigate the effect on the β-cleavage of APP. The anti-AD effect of VIa was valued using both acute and subchronic dosing paradigms in PrPhAPPswe/PS1ΔE9 transgenic mice. **Results:** Our results have shown that the novel compound VIa exhibits potent inhibitory effects with and IC_{50}=5.9 nmol/L and displays 30.8-fold, 7500-fold and 17533-fold selectivity against the other aspartic proteases BACE2, cathepsin D and renin, respectively. In cellular assays, VIa moderately reduces Aβ production: $Aβ_{1–40}$ with an IC_{50}=143 nmol/L and 1 nmol/L VIa reduced $Aβ_{1–42}$ by 40.17%. Concomitant with VIa inhibiting the β-cleavage of amyloid precursor protein (APP), VIa increases the production of sAPPα with an approximate EC_{50} of 16.5 nmol/L. In testing this compound's efficacy *in vivo*, the oral administration of VIa resulted in a significant decrease in $Aβ_{1–40}$ and $Aβ_{1–42}$ in the blood of a mouse model of AD by 17.5%–72.44% and 14.5%–80.32%, respectively. **Conclusion:** Although the concentration of VIa in the brain is not sufficient to reduce Aβ production, VIa is able to ameliorate deficits observed in a novel object recognition task. This suggests that the novel compound VIa is a small, potent, selective, and nonpeptidic BACE1 inhibitor.

Keywords: β-site APP cleaving enzyme 1; β-amyloid; inhibitor; Alzheimer's disease

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S10.11

Impairment of inflammatory cytokine-induced manganese superoxide dismutase expression by the PINK1 G309D mutation

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Aim: Parkinson's disease is one of the most common neurodegenerative diseases. Mutation in human *PTEN induced protein kinase 1* (*PINK1*) gene is responsible for the second most common form of recessive Parkinson disease. Here, we investigated the etiology related to PINK1. **Methods:** A stable pool expressing recombinant PINK1 G309D mutant in SH-SY5Y cells was established. We examined the manganese superoxide dismutase (SOD2) expression, protein kinase activation and NFκB nuclear translocation induced by TNFα in the PINK1 G309D mutant cells. **Results:** The SOD2 induction in response to TNFα treatment was impaired by the expression of recombinant PINK1 G309D mutant. TNFα increased SOD2 expression through the NFκB signaling pathway. The phosphorylation of NFκB p65 was inhibited in cells expressing the PINK1 G309D mutant. In addition, the translocation of p65 to nucleus was also decreased in PINK1 G309D mutant cells. **Conclusion:** These results indicate a novel pathway by which the defect of PINK1 kinase domain G309D inhibits the inflammatory cytokine-induced SOD2 production. Impairment of SOD2 production may accelerate the dopaminergic neurodegeneration in Parkinson patients with PINK1 defect.

Keywords: Parkinson's disease; PINK1; TNFα; SOD2

S10.12

Dose-dependent effects of angiotensin converting enzyme (ACE) inhibitors in scopolamine-induced amnesia model of dementia: a biochemical analysis

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Aim: An augmented brain renin angiotensin system (RAS) activity has recently been shown to be involved in pathophysiology of Alzheimer's disease. Drugs that act by manipulating RAS such as ACE inhibitors thus could have potential roles in preventing cognitive deterioration seen in AD and other forms of dementia. In the present study, antioxidant effect and choline acetyltransferase (ChaT) activity of low and high doses of three different ACE inhibitors were studied in scopolamine-induced amnesia model of dementia. **Methods:** Male rats were divided into total of 5 groups. Group I (Control) received 2% gum acacia, group II received 0.225 and 0.45 mg/kg ramipril, group III received 0.225 and 0.45 mg/kg lisinopril and group IV received 0.90 and 1.80 mg/kg fosinopril, orally for 4 weeks, followed by a single dose of scopolamine (1 mg/kg, ip). Group V received single dose of scopolamine 45 min prior to the assay procedures. After 4 weeks, all animals were sacrificed and their brains were isolated for estimation of malondialdehyde (MDA), glutathione transferase (GST), protein thiol and ChaT activity. **Results:** Higher doses of ramipril were shown to significantly attenuate the oxidative stress induced by scopolamine (P<0.05), as evidenced by decrease in MDA level and increase in brain GST and protein thiol level. The effect was less marked in rats treated with lisinopril and fosinopril. Scopolamine treatment was shown to decrease the ChaT activity in adult male rats which was enhanced in rats pre-treated with higher doses of ACE inhibitors (P<0.05). **Conclusion:** AD is associated with oxidative stress and imbalance in ChaT activity. Pre-treatment with ACE inhibitors has been shown to prevent scopolamine-induced oxidative stress as well as increase the ChaT activity, both of which could have a neuroprotective in AD though further studies are warranted to achieve a conclusive result.

S10.13

Experimental study on the sedative and hypnotic effects of WS997

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Aim: WS997 is a novel compound that has the capability to inhibit central nervous system. Experiments were performed to investigate the effects and mechanisms of WS997 on its sedative and hypnotic activity. **Methods:** The sedative activity of WS997 was investigated by recording the spontaneous locomotor activity of mice. The hypnotic property was evaluated by recording the righting reflex latency and duration in mice. A fluorescent indicator, N-[ethoxycarbonylmethyl]-6-methoxyquinolinium bromide (MQAE), was used to measure intracellular chloride concentration ($[Cl^-]_i$) in synaptoneurosomes from mouse cortex. **Results:** WS997 (5 mg/kg, ip) significantly decreased the locomotor activity by 69%, compared with the control group. In WS997 (50 mg/kg, ip) treated mice, the righting reflex was lost after (40±7) min of injection and the duration of sleep was (60±8) min. In addition, the level of synaptoneurosomes $[Cl^-]_i$ was enhanced obviously by WS997. **Conclusion:** These findings intensely indicated that WS997 had potent sedative and hypnotic effects, and the mechanism was likely due to enhancing chloride ion influx, thus resulting in hyperpolarization and decreased cell firing.

Keywords: locomotor activity; hypnotics; chloride ions

S10.14

Targeting SIRT1 in Huntington's disease: rationale and current status

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Huntington's disease (HD) is an autosomal dominant hereditary disease caused by a trinucleotide repeat mutation in the *huntingtin* gene that results in an increased number of glutamine residues in the N terminus of huntingtin protein. Mutant huntingtin leads to progressive impairment of motor function, cognitive dysfunction, and neuropsychiatric disturbance. There are no disease-modifying treatments available. During the past decade, Sirtuin-1 (SIRT1) has been the focus of intense investigation and discussion because it regulates longevity in multiple organisms and has shown beneficial effects in a variety of models of neurodegenerative disorders. Indeed, many exciting connections exist between the deacetylation function of SIRT1 and its role in neuroprotection. Using mammalian models of HD, we demonstrate a neuroprotective role of SIRT1 in HD, indicate key mediators of this protection, and open new avenues for the development of neuroprotective strategies in HD. As a result, pharmaceutical interventions targeting SIRT1 might become promising strategies to combat HD. The identification of therapeutic agents capable of modulating the expression and/or

activity of SIRT1 can be expected to provide promising strategies for ameliorating neurodegeneration in HD as well as other age-related neurodegenerative diseases.

Keywords: Sirt1; BDNF; TrkB; Huntington's disease; neurodegeneration

S10.15

The protective effects of metallothionein-I/II against cerebral ischemia and reperfusion injury

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Aim: Metallothioneins are ubiquitous low molecular weight, cysteine-rich and metal binding proteins in mammals. The major isoforms in CNS are MT-I and-II which localize mainly in astrocytes. Recently significant efforts have been made to the study the biological functions of MT-I/II in the central nervous system. This study is to investigate the role of MT-I/II in cerebral ischemia reperfusion injury in focal ischemia reperfusion model and oxygen-glucose deprivation reperfusion model. **Methods and results**: The focal cerebral ischemia reperfusion model was established with thread embolism of the middle cerebral artery (MCAO) in MT+/+ (wild type) and MT–/– (knock out type) mice for 2 h and 22 h reperfusion. To compare the sensibility of MT+/+ and MT–/– mice to focal cerebral ischemia reperfusion injury, TTC staining, HE staining and TUNEL assays were performed. Moreover, the primary cultured astrocytes from neonatal MT+/+ and –/– were exposed to 6 h oxygen glucose deprivation (OGD) and 24-h reperfusion to evaluate the cell injury as indicated by the morphology changes, apoptosis and LDH leakage. *In vivo* experiments show that MT–/– mice have larger cerebral ischemia injury area, more severe histopathologic changes and apoptosis as compared to MT+/+ mice. In *in vitro* study we found that OGD 6 h/Rep 24 h significantly induced cell injury as evidenced by nuclear shrinkage, vacuolization, apoptosis and higher LDH leakage in astrocytes; comparatively, the MT–/– astrocytes are more sensitive to OGD/Rep induced injury. **Conclusion:** The absence of MT-I/II can aggravate *in vivo* cerebral ischemia reperfusion injury and OGD/Rep induced injury in *in vitro* cultured astrocytes, indicating that MT-I/II protect neurons against cerebral ischemia injury.

Keywords: metallothionein; cerebral ischemia reperfusion; astrocytes; oxygen glucose deprivation/reperfusion; injury

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S10.16

Effects of extracts *Semen Ziziphi spinosae* and *Fructus Schisandrae Chinensis* on amino acid neurotransmitter in rats with insomnia induced by PCPA

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Aim: To observe the effects of extract from *Semen Ziziphus spinosa* and *Fructus Schisandrae Chinensis* on the content of amino acid neurotransmitter in the hypothalamus of insomnia rats caused by DL-P-chlorophenylalanine. **Methods:** The model of insomnia were established by PCPA intraperitoneal injection in rats. After the modeling, all the therapeutic group were treated with corresponding drug for one week. The pathological changes of the rats hypothalamus were observed, Elisa assay the content of GABA and Glu in the hypothalamus was measured by ELISA. GABA and Glu protein expression was detected by immunohistochemical staining. $GABA_AR_{\alpha 1}$ and $GABA_AR_{\gamma 2}$ mRNA expression was determined by RT-PCR. **Results:** Compared with model group, the content of GABA in the hypothalamus of rats increased obviously in the alcohol-water group ($P<0.05$, $P<0.01$), while the content of Glu decreased obviously ($P<0.05$, $P<0.01$). **Conclusion:** The extract from *Semen Ziziphus spinosa* and *Fructus Schisandrae Chinensis* has obviously sedative-hypnotic effect. Its mechanism is related to regulation of the content of amino acid neurotransmitter in the hypothalamus of rats.

Keywords: *Semen Ziziphus spinosa*; *Fructus Schisandrae Chinensis*; PCPA; insomnia; amino acid neurotransmitter

S10.17

Long term regular physical exercise improve chronic unpredictable stress induced depression and cognitive impairment in rats

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Aim: There are increasingly reports of beneficial effects of exercise on depressive disorder and cognitive impairment in humans. Cognitive impairment is one of the main symptoms in depression. However, no study has shown that exercise can improve cognitive impairment in depression. Therefore, this study aim to evaluate the effect of long-term regular physical exercise on depression-induced cognitive impairment in the Chronic Unpredictable stress model. **Methods:** Fifty male Sprague-Dawley rats were randomly divided into five groups according to the performance in Morris water maze (MWM) test, including control (Con) group, chronic unpredictable stress (CUS) group, CUS+fluoxetine (FLX) group, CUS+low intensity running (LIR) group and CUS+moderate intensity running (MIR) group. Rats were subjected to chronic unpredictable stress for 4 weeks, except control group. Open field (OF) test, Sucrose preference (SP) test and novelty-suppressed feeding (NSF) test were used to detect the antidepressant effect of physical exercise. The novel object recognition (NOR) test and MWM test were supplied to evaluate the learning and memory capacity after stress. Levels of p-CREB and BDNF in the hippocampus were detected by Western blotting. The content of cognitive-related inflammatory factors IL-1β and IFN-γ in the hippocampus were measured by Luminex^{200} Milliplex kit. **Results:** 1) CUS rats showed depressive behaviors including anhedonia in SP, decreased locomotor activity in OF and prolonged feeding latency in NSF. Physical exercise reversed these depressive behaviors in CUS rats significantly. 2) In the NOR test, CUS decreased the recognition percentage, while physical exercise reversed this impairment. In the MWM test, the CUS rats still rambled at the familiar quadrant which was used at grouping, but the latency in oriented-navigation test was increased when the plat was set up to a new quadrant. These results indicated that CUS might reinforce the memory capacity during the past period, but damage the learning function. 3) CUS significantly decreased the expression of p-CREB and BDNF in the hippocampus, meanwhile the levels of IL-1β and IFN-γ were raised. Flu, LIR and MIR markedly improved these changes. **Conclusion:** Physical exercise may be an effective approach to improve depressive symptom and cognitive impairment associated with depression; the underlying mechanism may be related to neurotrophic enhancement and anti-inflammatory effects.

Keywords: physical exercise; depression; cognitive impairment; chronic unpredictable stress

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S10.18

An antidepressant from Chinese herbs improves the burst firing of medial prefrontal cortex induced by chronic mild stress

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Aim: Discovery of new antidepressants from traditional Chinese medicine is one of the promising field for the treatment of the major depressive disorders. Furostanol saponins (termed B3 in this study), one type of steroidal saponins of *Rhizoma Anemarrhenae*, were applied to the CMS-induced depressive rats, and its anti-depression mechanism were also investigated by multiple discipline of techniques. **Methods:** CMS-induced depression is a reliable and valid animal model to mimic clinical symptoms of major depressive disorders (MDD). In current study, mice were divided into four groups. i) naïve group; ii) CMS treated group; iii) chronic administration (ig) of B3 and iv) fluoxetine after 3-week CMS administration. The depressive-like anhedonia (assessed by sucrose preference) and behavioral despair (assessed by immobility of tail suspension) were evaluated in the CMS and B3-treated CMS. To examine the effects of B3 on neuronal activities of medial prefrontal cortex (mPFC) and ventral tegmental area (VTA), spontaneous firing was extracellularly recorded *in vivo*. Then the expression of brain-derived neurotrophic factor (BDNF) was tested by Western bolt. **Results:** 1) Mice had depressive-like phenotype after 3-week CMS in the terms of sucrose preference and immobility of tail suspension. We also found that the administration of B3 rapidly reversed depressive-like behaviors induced by CMS within week 2, while fluoxetine needed additional 1 week to improve the depressive-like behaviors. 2) For electrophysiological recording in mPFC, there was a significant decrease in the rate of burst firing observed in CMS group compared to control group.

Furthermore, B3 and fluoxetine could reverse this weakened activity induced by CMS, which was implied by the result that the bursting rates of B3 and fluoxetine-treated groups turned out to be parallel to control group after 3-week intervention. However, the rate of irregular firing showed no significant difference among these groups. Data recorded in VTA showed different results that more neurons fired spikes than control group (53.85% *vs* 50%), while CMS-treated mice displayed a less population (8.33%) of neurons that exhibited markedly higher levels of burst firing. In contrast to mPFC, the administration of B3 and fluoxetine failed to reverse CMS-induced decrease in the proportion of neurons with high levels of burst firing. 3) To better understand the mechanism underlying the protective effects of B3 against CMS, the protein expression of BDNF was examined. BDNF, which can be related to neurotrophin and neurogenesis, showed a robust decreased expression in CMS-treated PFC, compared to control group. However, two stressed groups had elevated BDNF expression in parallel with that of control group after chronic treatment with B3-2 and citalopram. **Conclusion:** These results have revealed a relatively rapid antidepressant-like effect of B3 on CMS-induced depression, demonstrating that the induction of neurotransmission of mPFC might be a target for B3, and BDNF may play an important role in antidepressant-like effect of B3.

Keywords: CMS; depression; neuronal activity; mPFC; VTA; BDNF

S10.19

Targeting nitrosative stress for neurovascular protection

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Aim: NO/ONOO-mediated nitrosative stress accounts for endothelial injury and the subsequent breakdown of the highly structured neurovascular unit. This report is to investigate the mechanisms of nitrosative stress and identify new therapeutic targets. **Methods:** Oxygen-glucose deprivation (OGD) and microsphere (ME)-induced permanent focal cerebral ischemic model were used *in vitro* and *in vivo*, respectively. Cell viability was determined by MTT. JC-1 staining was used for determination of mitochondrial transmembrane potential and monodansylcadaverine was used to evaluate the abundance of the autophagic vacuoles in endothelial cells. Reconstructed plasmid and siRNAs were transfected for molecular biological interferences. Immunoprecipitation, immunoblotting and immunofluorescence were used for related proteins analysis. **Results:** OGD treatment triggered rapid nitrotyrosine production with the decrease in mitochondrial membrane potential, mitochondrial HtrA2 release and PED level, as well as in ME model *in vivo*, and subsequently resulted in cell death, which can be partially rescued by melatonin. In addition, autophagy–lysosome processes were activated under nitrosative stress after OGD or ME treatment *in vitro*/*in vivo*. Moreover, OGD can induce constitutive nuclear import of Keap1 and subsequent disturbance of Nrf2/Keap1 signaling. Conclusion: Determination of the intracellular association between nitrosative stress and the autophagy–lysosome response, the imbalance of HtrA2-PED signaling and the disturbance of Keap1/Nrf2 signaling as well as protective effect of melatonin in endothelial cells following ischemia-like injury, may provide new strategy of targeting nitrosative stress for neurovascular protection.

Keywords: nitrosative stress; endothelium; blood-brain barrier; peroxynitrite; cerebral ischemia; vasoprotection

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S10.20

Neuroprotective effect of 20(*R*)-ginsenoside Rg_3 against delayed neuronal injury induced by transient cerebral ischemia-reperfusion in rats

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Aim: To investigate whether 20(*R*)-ginsenoside Rg_3 (20(*R*)-Rg_3) exhibits neuroprotective effect against delayed neuronal injury induced by transient cerebral ischemia and reperfusion in rats. **Methods:** Adult male Sprague-Dawley (SD) rats were subjected to transient middle cerebral artery occlusion (MCAO) for 2 h, then reperfusion for 72 h. The behavioral disturbance was employed to evaluate the damage to neurological function of rats, and neuronal apoptosis after MCAO was measured with terminal deoxynucleotidyl transferase (TdT)-mediated d-UTP nick end labeling (TUNEL) method. *In situ* hybridization technique was used to detect the expressions of calpain I and caspase-3 mRNA in hippocampal CA1 region. **Results:** 20(*R*)-Rg_3 (10 and 20 mg/kg) significantly improved neurological function of rats, decreased the number of TUNEL-positive cells and reduced the expressions of calpain I and caspase-3 mRNA in hippocampal CA1 region. **Conclusion:** All of these results indicate that 20(*R*)-Rg_3 can reduce delayed neuronal injury induced by MCAO in rats, and the mechanism of neuroprotective effect may be related with suppressing the expressions of calpain I and caspase-3 mRNA.

Keywords: 20(*R*)-ginsenoside Rg_3; apoptosis; calpain I; caspase-3

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S10.21

Neuroprotective and antitremor effect of baicalein on experimental Parkinsonism

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Aim: An attempt has been made to explore the neuroprotective and antitremor effect of baicalein on neurons against experimental Parkinsonism. **Methods:** In *in vitro* experiments, we found that baicalein could significantly ameliorate the 6-OHDA-induced SH-SY5Y cell apoptosis and also promote neurite outgrowth in PC12 cells. In *in vivo* experiments, the rats received unilateral lesions of the medial forebrain bundle by stereotaxic injection of 6-OHDA (4 μg/μL saline, containing 0.04% ascorbic acid). **Results:** Baicalein could significantly attenuate muscle tremor of 6-OHDA-lesioned rats. The frequency of tremors was decreased by 23% and 40% at the doses of 200 and 400 mg/kg (*po*), respectively and the amplitude of tremors was decreased by 7% and 29%, respectively. Moreover, baicalein could suppress mGluR5 and modulate the levels of DA and Glu in basal ganglia (BG). In addition, the number of TH, DAT protein positive cells in substantial nigra of rats treated with baicalein is more than that of model group. **Conclusion:** These results suggest that baicalein could inhibit motor activity or provide symptomatic relief, and it should be a promising candidate for prevention or symptomatic treatment of Parkinson's disease.

Keywords: Baicalein; Parkinson's disease; 6-hydroxydopamine; neuroprotective

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S10.22

Xanthotoxol ameliorates the ischemia/reperfusion-induced impairment of long-term potentiation induction in the CA1 region of the rat hippocampus

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Aim: Cerebral ischemia can impair the induction of activity-dependent long-term potentiation (LTP) in the hippocampus. Here, we investigated the effect of xanthotoxol on the induction of activity-dependent LTP and the expression of NR2A and NR2B subunit NMDA receptor in the hippocampal CA1 region after focal cerebral ischemia/reperfusion injury. **Methods:** Transient focal cerebral ischemia in rats was induced by 2 h middle cerebral artery occlusion followed by 72 h reperfusion. Xanthotoxol (10 mg/kg) was administered ip at 1 h and 12 h after the onset of ischemia. After 72 h of reperfusion, the influence of xanthotoxol on LTP induction *in vivo* and the expression of NR2A and NR2B protein in the hippocampal CA1 region were evaluated. **Results:** The induction of LTP was significantly impaired and the levels of NR2A and NR2B protein were markedly decreased in the CA1 region of hippocampus following focal cerebral ischemia/reperfusion injury. Xanthotoxol treatment attenuated the impairment of LTP induction and alleviated the NR2A and NR2B protein down-regulation induced by cerebral ischemia/reperfusion injury. **Conclusion:** These results suggest that xanthotoxol can ameliorate the ischemia/reperfusion-induced impairment of synaptic plasticity in the hippocampal CA1 region.

Keywords: xanthotoxol; LTP; cerebral ischemia/reperfusion; NR2A; NR2B

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S10.23

n-Butanol extract of Chinese toon fruit ameliorated focal brain ischemic insult in rats via inhibition of oxidative stress and thrombogenesis

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Aim: To evaluate the neuroprotective efficacy of Chinese toon on cerebral ischemic reperfusion model of rats and explored the underlying mechanisms. **Methods:** Rats were subjected to occlusion of the middle cerebral artery (MCAO) by a nylon filament and treated with different doses of n-butanol extract of Chinese toon fruit or the vehicle for 1 week before induction of ischemia, sid. **Results:** n-Butanol extract of Chinese toon fruit reduced the ischemia-induced cerebral infarct and edema volume in a dose-dependent manner and attenuated neurological deficits observed after 6 h. n-Butanol extract of Chinese toon fruit reduced the levels of nitrate, nitrite, lipid peroxidation, cyclooxygenase-1, thromboxane in post-ischemic brain. n-Butanol extract of Chinese toon fruit adjusted the elevation of the activity of glutathione peroxidase and superoxide dismutase in ischemic brain. **Conclusion:** The present study was the first evidence of the effectiveness of n-Butanol extract of Chinese toon fruit in the rat stroke models, as it reduced infarct volume, inhibited the oxidative stress and thrombogenesis.

Keywords: brain ischemia; middle cerebral artery occlusion; oxidative stress; cyclooxygenase; Chinese toon

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S10.24

Companion retards the development of memory deficit in APP/PS1 mice

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Alzheimer's disease (AD) is the most common cause of dementia in aged people and currently no available way to prevent or slow the disease. We assessed memory retention of amyloid precursor protein (APP) and presenilin 1 (PS1) double-transgenic (APP/PS1) mice at the age of 1, 3, 6, 9, and 12 months using contextual and cued fear conditioning paradigms. We found that APP/PS1 mouse's memory is normal at 3 months old and is impaired at the age of 6 months. APP/PS1 mice of 6 months old were randomly co-housed with 1, 3, or 6 month-old wild type (WT) mice for 3 months. Contextual and cued freezing responses of APP/PS1 mice were improved significantly with co-housing with 1-month old most effective. After co-housing with 1 month old WT mice for 3 months, we divided APP/PS1 mice into two groups: freezing responses greater than control group (good memory) and freezing responses less than control group (bad memory). Surface expression of GluR1 in the hippocampus of good memory mice was significantly higher than those of bad memory and APP/PS1 control mice. Furthermore, $A\beta_{42}/A\beta_{40}$ ratio and calpain activity were significantly lower in the good memory than in the bad memory groups. These results suggest companion attenuates the increased $A\beta_{42}/A\beta_{40}$ ratio, calpain activity and rescues the memory deficit in APP/PS1 mice.

S10.25

Acid-sensing ion channels (ASICs) promote rat microglial inflammation and migration

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Aim: Microglia, the main immune cells in central nervous system (CNS), act as the surveillance and scavenger of immune defense and inflammatory response in central CNS. Some primary studies prompted that, there might be close relationship between ASICs and immune inflammation, however, the exact role of ASICs in microglia during immune inflammation remains elusive. In the present study, we identified the existence of acid-sensing ion channels (ASICs) in the rat primary cultured microglia and explored their functions. **Methods and Results:** By using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), quantitative real-time PCR (qPCR), Western blotting and immunofluorescence experiments, we first demonstrated that ASIC1, ASIC2a, and ASIC3 existed in cultured and *in situ* rat microglia, with a different distribution from neurons. ASIC-like currents and acid-induced intracellular calcium elevation were only induced in lipopolysaccharide (LPS)-stimulated microglia, but not in the unstimulated microglia. In neuroinflammatory models, ASIC1 and ASIC2a expressions were up-regulated. The non-specific ASICs antagonist amiloride and specific homomeric ASIC1a blocker PcTx1 reduced the secretion of inflammatory cytokines, including inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) in LPS-stimulated microglia. Furthermore, amiloride and PcTx1 inhibited the migration of primary cultured rat microglia by inhibiting ERK phosphorylation. **Conclusion:** Taken together, these results suggest that ASICs participated in neuroinflammatory response, which provide a novel therapeutic strategy for controlling the inflammation-relevant neuronal diseases.

Keywords: acid-sensing ion channels (ASICs); microglia; inflammatory cytokines; migration

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S10.26

Danggui-Shaoyao-San improves learning and memory in SAMP8

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Aim: Previous studies showed that Danggui-Shaoyao-San (DSS), a traditional Chinese medicinal prescription, could alleviate cognitive dysfunction of Alzheimer's disease (AD) patients. However, the mechanisms remain unknown. We have now examined the effect of DSS on SAMP8 (a famous AD model mouse) and elucidated the possible mechanism. **Methods:** Mice were treated with DSS (1.6, 3.2, and 4.8 g/kg) by intragastric infusion over 2 months, and their passive avoidance ability and spatial learning and memory capacities were measured by the Step-down test and Morris water maze procedure. The estradiol, the excited amino acid and inhibited amino acid, NO and NOS, and monoamine neurotransmitters in blood serum or in hippocampus were detected to explore the possible mechanisms. **Results:** The results show that the ability of passive avoidance and spatial learning and memory inSAMP8 were all impaired compared with age-matched SAMR1, and the impairments were ameliorated by DSS administration, especially in female animals. Additionally, DSS elevated the serum estradiol and NO content in blood and hippocampus. **Conclusion:** These results suggest that DSS could ameliorate deterioration of cognition in SAMP8, and by increasing estradiol and NO.

Keywords: Danggui-Shaoyao-San (DSS); Alzheimer's disease (AD); senescence-accelerated mouse prone-8 (SAMP8); learning and memory

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S10.27

Clerodendron bungei steud relieves pain hypersensitivity and its possible mechanisms in rats with neuropathic pain

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Aim: There are studies demonstrated that neuroimmune system was involved in the generation of neuropathic pain, cytokines are the key molecule in connection of nerve and immune. It is well known that pro-inflammatory cytokines play an important role in the generation and maintenance of neuropathic pain. Since *Clerodendron bungei steud* has anti-inflammatory and analgesic effect, but the effects could not be blocked by naloxone, so we hypothesized that it may be mediated via non-opioid pathways. So this work was designed to explore whether *Clerodendron bungei steud* had the effect on neuropathic pain. **Methods:** Neuropathic pain model was established by L5–L6 spinal nerve ligation (SNL). Pain hypersensitivity was assessed by recording mechanical withdrawal thresholds induced by von Frey filaments and thermal withdrawal thresholds induced by radiant heat. The expression of TNF-alpha, IL-1, and IL-6 are determined by ELISA. **Results:** *Clerodendron bungei steud* can relieve pain hypersensitivity (*ie*, increase in withdrawal threshold) dose-dependently and reduce the expression of cytokines (*ie*, TNF-alpha, IL-1, and IL-6) at spinal cord level. **Conclusion:** These results suggested that mechanisms underlying the inhibitory effects of *Clerodendron bungei steud* on neuropathic pain may be through inhibiting the expression of TNF-alpha, IL-1β, and IL-6 in the spinal cord. This work will also offer experimental evidences for exploring new target and drug for the treatment of neuropathic pain.

Keywords: *Clerodendron bungei steud;* neuropathic pain; cytokine

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S10.28

α7nAchR mediate the protective effect of GSS on brain ischemia/reperfusion injury

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Aim: To explore the protective effects of Genistein Sulfonate Sodium (GSS) on brain ischemia/reperfusion (I/R) injury and its mechanism about cholinergic anti-inflammatory pathway. **Methods:** The rats were divided into four groups randomly, namely sham control group, model group of RI/R injury, low-dosage GSS group and high-dosage GSS group. Normal saline (NS) 1 mL/kg was injected through sublingual vein after common carotid artery (CCA) was dissociated in sham group. Other groups were treated with NS, low-dosage GSS (1 mg/kg) and high-dosage GSS (2 mg/kg) through sublingual vein respectively 10 min after middle cerebral artery occlusion (MCAO). After 1.5-h MCAO and reperfusion for 24 h, the cerebral tissue was stained by triphenyltetrazolium chloride (TTC), and the volume of infarction was investigated. Moreover, the activitiy of lactate dehydrogenase (LDH) in serum was detected by enzyme analysis method, the level of TNF-α in serum was surveyed by ELISA, and the expression of α7nAchR, IL-1β and TNF-α were measured by Western blot assay. **Results:** Compared with the sham group, model group had increased levels of the volume of infarction, activity of LDH in serum, level of TNF-α in serum, and expression of IL-1β and TNF-α, but had decreased expression of α7nAchR. After treatment with GSS, reduction in the volume of infarction, the activity of LDH in serum, the serum level of TNF-α, and the expression of IL-1β and TNF-α, and an elevation in the expression of α7nAchR in cerebral tissue were observed. **Conclusion:** GSS has certain therapeutic effects on cerebral ischemia/reperfusion injury because it up-regulates cholinergic anti-inflammatory pathway.

Keywords: Genistein Sulfonic Sodium; cerebral ischemia/reperfusion; α7nAChR
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S10.29

Neuroprotective effects of plant extracts against the neurotoxicity induced by rotenone, a mitochondrial complex I inhibitor

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Aim: Rotenone, an inhibitor of mitochondrial complex I, has been widely regarded as a neurotoxin because it induces a Parkinson's disease-like syndrome. The plant extracts have been used as traditional medicines in Asia to treat neurodegenerative diseases. Therefore, in this study, we examined the neuroprotective effects of plant extracts in rotenone-treated PC12 cells. **Methods:** PC12 cells were pretreated with plant extracts; the neurotoxic effects of rotenone were assessed by examining cell viability and indices associated with apoptosis, including caspase expression and activation, cellular ATP level, mitochondrial membrane potential, and mitochondrial superoxide level. **Results:** Treatment with rotenone reduced PC12 cell viability and cellular ATP levels, coincident with mitochondrial membrane depolarization; conversely, caspase 3/7 activity, the ratio of Bax to Bcl-2 expression levels, mitochondrial superoxide level, and intracellular calcium (Ca^{2+}) concentration were elevated. Pretreatment with plant extracts significantly increased cell viability and ATP levels; they also attenuated caspase activation, mitochondrial membrane depolarization, and mitochondrial superoxide production. Moreover, confocal microscopy showed that the mitochondrial staining pattern was restored from that of extracts treated cells and that the increase in intracellular Ca^{2+} level was blunted by treatment with the extracts. **Conclusion:** Our results suggest that plant extracts may have the possible beneficial effects in Parkinson's disease by attenuating rotenone induced toxicity.

Keywords: plant extracts; neuroprotective effects; rotenone; PC12 cells; mitochondrial dysfunction
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S10.30

Icariin improve the learning and memory functions in APP/PS1 transgenic mice through NO-cGMP-PDE5 signaling pathway

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Aim: To investigate the effects of icariin (ICA) on learning and memory functions in APP/PS1 transgenic mice and analyze its mechanism. **Methods:** The APP/PS1 transgenic mice (10 months) were randomly divided into APP/PS1 group, ICA group (30, 60 mg/kg) and sildenafil (SIL) group (2 mg/kg) , the wildtype mice (10 months) were randomly divided into blank group and normal administration group (ICA 60 mg/kg). ICA and SIL were given orally bis in die for 4 months, the APP/PS1 group and blank group were given isovolumic distilled water. The learning and memory functions of the mice were tested by Y-maze. The levels of APP, PDE5A, eNOS, iNOS, VEGF, and VEGFR mRNA were evaluated by real ime RT-PCR. The PDE5 activity and cGMP level were determined by ELISA assay. The Griess method was used to detect the level of NO in hippocampus and cortex of APP/PS1 transgenic and wild type mice. **Results:** Y-maze test showed that ICA significantly improved learning and memory functions of APP/PS1 transgenic mice ($P<0.05$). ICA decreased the levels of APP and PDE5 mRNA, but increased the expression of eNOS, iNOS, VEGF, and VEGFR mRNA obviously ($P<0.05$). ICA suppressed the activity of PDE5 and improved the levels NO and cGMP in hippocampus and cortex of APP/PS1 transgenic mice ($P<0.05$). **Conclusion:** These results strongly suggest that ICA can significantly improve the learning and memory function of APP/PS1 transgenic mice, the mechanism may be related with the NO-cGMP-PDE5 signaling pathway.

Keywords: icariin; learning and memory functions; APP/PS1 transgenic mice; NO-cGMP-PDE5 signaling pathway

S10.31

Antinociceptive effect of matrine on vincristine-induced neuropathic pain model in mice

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#The two authors contributed equally to this work.

Aim: Chemotherapeutic drugs induced hyperalgesia and allodynia are common reasons of neuropathic pain, so effective therapeutic strategy is required. In this study, we evaluated the antinociceptive effects of matrine on vincristine-induced neuropathic pain in mice. **Methods:** Vincristine (0.1 mg/kg, ip) was administered once per day for 7 d (d 0–6) in mice. Matrine (15, 30, and 60 mg/kg, ip) was repeatedly administered in early phase (d 0-6) or late phase (d 7–13). Hyperalgesia and allodynia were evaluated by withdrawal response using von Frey filaments, plantar and cold-plate test on 7, 14, and 21 d. **Results:** Injection of vincristine produced mechanical hyperalgesia and cold allodynia. Matrine was found to produce a protective effect in both von frey filaments and cold-plate test. The analysis of the effect supports the hypothesis that matrine is useful in the treatment of vincristine-induced neuropathic pain. **Conclusion:** This study demonstrates that administration of matrine had antinociceptive effect on mechanical and cold stimuli in a mice model of vincristine-induced neuropathic pain.

Keywords: vincristine-induced neuropathic pain; matrine; antinociceptive
Acknowledgements: The study was supported by the National Natural Science Foundation of China (Grant No 81160524). We are indebted to the staff in the animal center and the Science & Technology Centre who provided assistance in the study. The authors would like to thank Dr Ding-feng SU, Prof Wan-nian ZHANG, Miss Jie WANG, Miss Shu-jing WANG, and Yan ZHANG for their help in this study.

S10.32

Oxytocin attenuated methamphetamine-induced neuronal activation

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Aim: Oxytocin (OT) has been known as a hormone synthesized in the paraventricular and supraoptic nuclei in the hypothalamus and released from the posterior

pituitary to elicit milk ejection for lactation and uterine contraction during parturition. OT receptor, however, is present in various regions in the central nervous system, such as amygdale, hippocampus, ventral tegmental area, and frontal cortex. So, OT may have a role of neurotransmitter in the nervous system. Recent evidence indicates OT can reduce methamphetamine (MA) induced self-administration, conditioned place preference and hyperactivity in rats. However, it is unclear how OT was involved in regulation of the reward system. The study was carried out to clarify the central action of OT on the patterns of MA-induced neural activation. **Methods:** Male SD rats (250–300 g) were used in this experiment. All rats received central administration of OT (50, 100, and 200 ng/3 μL) 15 min before injection of MA (2.5 mg/kg, ip). c-Fos-immunoreactive profiles was detected by immunohistochemistry for neuronal activity determination. **Results:** OT significantly inhibited MA-induced c-Fos expression at the motor cortex and the nucleus accumbens at dose-dependent manner. **Conclusion:** OT may eliminate the effects of abused drugs on the reward system.

Keywords: oxytocin; methamphetamine; cFos

S10.33

Sleep and EEG as biological markers in animal models of Huntington's disease

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Although abnormal circadian rhythmicity has been demonstrated in both human patients and in rodent models of Huntington's disease (HD), a comprehensive description of sleep and electroencephalogram (EEG) changes has never been conducted. Here we studied sleep and EEG disturbances in a transgenic mouse model of HD (R6/2 mice). We implanted EEG and electromyogram electrodes into male and female R6/2 mice and their wild-type (WT) littermates and then recorded baseline sleep/wake behaviour at pre-symptomatic, symptomatic, and late stages of the disease. In addition, we treated a subgroup of late stage HD mice with the tricyclic antidepressant amitriptyline or vehicle at dark onset, and recorded subsequent sleep/wake behaviour. We found that sleep and EEG were already significantly disrupted in R6/2 mice at the pre-symptomatic stage. The disturbances worsened with age, so that symptomatic, R6/2 mice were unable to maintain long periods of wakefulness and had an increased propensity for rapid eye movement (REM) sleep. As the disease progressed, an abnormal EEG gamma activity emerged in R6/2 mice, irrespective of sleep states. Treatment with amitriptyline increased non-REM sleep amount, reduced wake and REM sleep amount in both WT and R6/2 mice, and attenuated EEG gamma activity in R6/2 mice. Our results suggest that a decreased monoaminergic activity may be responsible for some of the sleep and EEG symptoms found in R6/2 mice. If similar changes occur in humans, these early and progressive sleep and EEG abnormalities could serve as biological markers of HD.

S10.34

Cerebroprotection by hesperetin is associated with restoration of tryptophan metabolic enzyme system in mice

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Aim: The tryptophan metabolic enzyme systems of indoleamine 2,3-dioxygenase (IDO) and tryptophanyl-tRNA synthetase (TrpRS), which are involved in L-tryptophan catabolism and its use in protein synthesis, respectively, are associated with the neuroinflammation and immune response to acute cerebral ischemia. Hesperetin, a citrus flavanone, is known to help prevent tissue damage from oxidative stress in the brain. In this study we investigated the mechanisms involved in cerebroprotective action of heseperetin in connection with the tryptophan metabolic enzyme systems. **Methods:** Male C57BL/6 mice were anesthetized and subjected to photothrombotic cortical ischemia. Hesperetin was administered ip 1 h after ischemic insult. **Results:** Post-treatment with hesperetin significantly reduced the infarct size including infarct area and volume. Ischemic insult markedly altered the tryptophan metabolic enzyme systems, *ie* an increase in the expression of IDO, CD11b, and CD11c, and a simultaneous decrease in that of TrpRS. Hesperetin significantly decreased the expressions of IDO, CD11b, CD11c, p-JAK2, and p-STAT1 via increasing the expression of TrpRS. Hesperetin significantly restored the tryptophan metabolic enzyme system, inhibiting JAK/STAT signaling activation. **Conclusion:** These results suggest that the cerebroprotective effects of hesperetin might be associated with the increase in the TrpRS expression as well as the suppression of the IDO expression.

Keywords: cerebral ischemia; neuroinflammation; neuroprotection

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S10.35

The effects of flupirtine on acute stress-induced impairment of hippocampal long-term potentiation at the schaffer collateral-CA1 synapse in rats

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Aim: Learning and memory are greatly modified by stress and hippocampal synaptic plasticity such as long-term potentiation (LTP), a putative hippocampal memory mechanism are impaired by acute stress. The present study examined the involvement of Kv7/M channels in modulating of the impairment of LTP in the CA1 area of aneshetized rats after exposure to an elevatated platform stress. **Methods:** Animals were placed on an elvevated platform in the middle of a bright room for 30 min to evoke acute stress. Rats were anaesthetized at the end of stress, the proteins were analyzed at 1, 3, 12, and 24 h after stress by Western blotting. The field excitatory postsynaptic potentials (fEPSPs) from Schaffer collateral to hippocampal CA1 stratum radiatum were recorded *in vivo*. LTP was induced within 180 min after the end of stress. **Results:** A rapid and significant decrease of KCNQ2 and KCNQ3 was evident in stressed rats. Acute stress inhibited the LTP in the hippocampus CA1 region. Flupirtine maleate had no effects on the LTP in unstressed rats, but it inhibited the acute stress-induced supression of LTP. XE-991 (0.3 mg/kg) had no effects on LTP in stressed rat, but blocked the protective effects of flupirtine. **Conclusion:** Kv7/M channels was involved in the acute-stress induced injury and flupirtine inhibited acute stress-induced impairment of LTP in the hippocampal CA1 region.

Keywords: Kv7/M channel; acute stress; flupirtine; long-term potentiation (LTP); XE-991

Acknowledgements: This study was supported by the National Natural Science Foundation of China (No 81173038, 81001425, and 81001432).

S10.36

Icariin decreases APP, Aβ levels and stimulats neurogenesis in the brain of Tg2576 mice

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Aim: Icariin (ICA) is a major fraction of flavonoids derived from the Chinese medical herb *Epimedium brevicornum* Maxim. Our previous studies have shown that ICA can protect neuron from different memory damaged models. This study investigated the effect of ICA on the levels of APP, Aβ and neurogenesis in the brain of Tg2576 mice. **Methods:** Wild type (WT, C57B6) and Tg2576 mice were randomly assigned to four groups: WT, WT+ICA, Tg2576, Tg2576+ICA groups. All these mice at 8 months of age were treated with distilled water or ICA (60 $mg \cdot kg^{-1} \cdot d^{-1}$) for 3 months. The effect of ICA on the spontaneous alternation was observed by Y-maze. ELISA measured the level of insoluble Aβ in the cortex. Western blot was used to analyze the protein expression of APP. Neurogenesis was observed by anti-BrdU and anti-NeuN antibodies double staining in the dentate gyrus (DG) of hippocampus. **Results:** Chronically administration of ICA for 3 months (1) improved the correct ratio of spontaneous alternation of Tg2576 mice to the level of WT mice, (2) reduced the insoluble $A\beta_{40}$ and $A\beta_{42}$ of cortex to, respectively, 69.40% and 53.28% of Tg2576 mice, (3) dramatically down-regulated the protein expression of APP in the cortex and hippocampus, (4) increased the number of BrdU and NeuN double stained-neurons in the DG of hippocampus compared with Tg2576 mice. **Conclusion:** ICA can decrease the levels of Aβ and APP in the brain of Tg2576 mice and stimulate the neurogenesis in the DG of hippocampus. These results supported that ICA could be a potential compound for Alzheimer's disease therapy.

Keywords: Icariin; Aβ; APP; neurogenesis; Tg2576 mice

S10.37

Neuroprotective effects of oxysophocarpine on neonatal rat primary cultured hippocampal neurons injured by oxygen-glucose deprivation and reperfusion

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Aim: In this study we investigated the protective effects of oxysophocarpine (OSC) on neonatal rat primary cultured hippocampal neurons injured by oxygen-glucose deprivation and reperfusion (OGD/RP). **Methods:** Cultured hippocampal neurons were exposed to OGD 2 h/RP 24 h were used as an *in vitro* model of ischemia and reperfusion. The MTT assay and the lactate dehydrogenase (LDH) release were used to evaluate the protective effects of OSC. The concentration of intracellular free calcium $[Ca^{2+}]_i$ and mitochondrial membrane potential (MMP) were determined to evaluate the degree of neuron damage. Morphologic changes of neurons following OGD/RP were observed with inverted phase contrast microscope and fluorescence microscope. The expressions of caspase-3 and caspase-12 mRNA were examined by real-time quantitative PCR during OGD 2 h/RP 24 h. **Results:** Treatment with OSC (1–5 μmol/L) significantly attenuated neuronal damage, with evidence of increased cell viability, and decreased cell morphologic impairment. Furthermore, OSC increased MMP but it inhibited $[Ca^{2+}]_i$ elevation in a dose-dependent manner at OGD 2 h/RP 24 h. At last but not least, OSC also decreased the expressions of caspase-3 and caspase-12. **Conclusion:** OSC have significantly protective effects on OGD/RP-induced neuronal damages in neonatal rat primary cultured hippocampal neurons. The protection against OGD/RP-induced damages appears to be mediated through blocking the decrease in MMP and increase in $[Ca^{2+}]_i$, as well as by decreased the expressions of caspase-3 and caspase-12 mRNA.

Keywords: oxysophocarpine (OSC); hippocampal neurons; oxygen-glucose deprivation and reperfusion; apoptosis

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S10.38

Regulation of neuroinflammation by liver X receptors: differential mechanisms through SHP

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We have previously reported that endogenous (oxysterols) as well as synthetic (GW3965, TO0901317) ligands of Liver X Receptor (LXR) efficiently suppressed STAT1 inflammatory signals in IFN-gamma-stimulated brain astrocytes. And SUMOylation of LXRalpha and beta, mediated by HDAC4 and PIAS1 respectively prevent STAT1 binding to the promoters of target genes. Since target genes of STAT1 inflammatory signals do not have binding sites for STAT1, this inhibitory mechanism is achieved by transrepression. Although our findings unravel a new regulatory mechanisms of LXRs via SUMOylation, the in-depth mechanism how LXRs prevent STAT1 binding to target promoter remains to be solved. In this study we demonstrated how small heterodimer partner (SHP) inhibited STAT1 binding to the promoter of target genes in a LXR subtype-specific manners. It directly interacts with LXRalpha, thus mediates LXRalpha/STAT1/SUMO2,3/HDAC4 complex formation, resulting in the inhibition of STAT1 binding to target gene promoter. But, it potentiates PIAS1 stabilization by inhibiting its ubiquitination, and as a result, it prolongs LXRalpha/STAT1/SUMO1/PIAS1 complex formation, and prevents STAT1 binding to the promoter of target genes. This finding further revealed differential regulation mechanism of LXRalpha and beta through SHP.

Keywords: liver X receptor; small heterodimer partner; STAT1; SUMOylation

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S10.39

Characterization of imidazoline I2 receptors as a novel target for pain management

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Aim: Pain remains a significant clinical challenge and currently available analgesics cannot meet clinical needs. Analgesics with novel mechanisms of action have been attracting numerous efforts in preclinical drug discovery. This study examined the antinociceptive effects of ligands acting on imidazoline I2 receptors. **Methods:** Selective I2 receptor ligands, 2-BFI and BU224, alone or in combination with morphine, were studied in two rat models of acute nociception, 5% NaCl-induced writhing test and warm water tail withdrawal test, and two rat models of chronic persistent pain, chronic constriction injury (CCI)-induced neuropathic pain and complete Freund's adjuvant (CFA)-induced inflammatory pain. Qualitative (writhing test) or quantitative data (tail withdrawal latency or paw withdrawal threshold in tail withdrawal test and chronic pain models, respectively) were collected and analyzed. **Results:** Both 2-BFI and BU224 demonstrated marked antinociceptive effects both in writhing test and in CCI and CFA-induced chronic pain models. Both drugs alone did not produce significant effects in warm water tail withdrawal test, but markedly enhanced the anti-nociceptive effects of morphine, demonstrating clear anti-nociceptive synergy. **Conclusion:** Combined, these data clearly demonstrate marked anti-nociceptive actions of drugs acting on imidazoline I2 receptors and suggest that I2 receptors may represent a novel drug target for the development of effective analgesics.

Keywords: imidazoline I2 receptors; pain; antinociception; rats

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S10.40

Beneficial effects of tetrahydroxy-stilbene glucoside on multiple targets of Alzheimer's disease

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Aim: Alzheimer's disease (AD) is a multifactorial complex disease. The failure of recent development of AD therapeutic agents in clinical trials is partly due to their single-target effect. The purpose of the present study was to investigate tetrahydroxy-stilbene glucoside (TSG), which was extracted from *Polygonum multiflorum*, on multiple targets of AD. **Methods:** Seven kinds of AD-like animal models were used to investigate the effects and their mechanisms of TSG. **Results:** Our studies found that intragastrical administration of TSG significantly improved learning-memory ability in 7 kinds of AD-like animal models; the action mechanisms of TSG included increasing the ratio of choline acetyltransfetase/cholinesterase; decreasing amyloid plaques and Aβ, and inhibiting β-secretase; suppressing α-synuclein over-expression and aggregation, and activating ubiquitin-proteasome system; increasing protein phosphortase 2A, inhibiting tau hyperphosphorylation, and protecting cytoskeleton; inhibiting microglial activation, and decreasing proinflammatory cytokine TNF-α and IL-1β; enhancing mitochondrial function, inhibiting oxidative stress, and decreasing neuronal apoptosis; elevating neurotrophic factor NGF and BDNF and their receptors, and activating neuronal survival signal pathway; increasing synapses and synaptophysin, and elevating synaptic plasticity. **Conclusion:** The results demonstrated that TSG exerted therapeutic effects on multi-targets and multi-pathways in the complex pathogenesis of AD, especially had both neuro-protective and neuro-trophic effects, suggesting that TSG may be beneficial for the early intervention and delaying the progression of AD.

Keywords: Alzheimer's disease; tetrahydroxy-stilbene glucoside; multitarget therapy

S10.41

Chronic restraint stress promotes learning and memory impairments due to enhance oxidative stress in male mice

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Aim: Chronic stress is implicated in many types of neurodegenerative diseases such as Alzheimer's disease (AD). The present study investigated the effects of chronic restraint stress (CRS) on learning and memory impairments and oxidative damage in frontal cortex and hippocampus in male mice. **Methods:** The Morris water maze was used to investigate the effects of CRS (8 weeks) on learning and memory functions. The ROS degeneration was detected by dihydroethidium (DHE) microfluorography. The spectrophotometer methods were used to assay the activity of super oxide dismutase (SOD) and the level of malondialdehyde (MDA). The level of 8-OHdG was measured by ELISA. And the immunohistochemistry and immunoblot were used to analyze the expression of PKCα, p47phox, NOX2, RAC1, GRP78, CHOP, and MANF. **Results:** CRS significantly accelerated learning and memory impairments, and induced ROS accumulation and neuronal damage in frontal cortex and hippocampus CA1. And CRS significantly decreased the activity of SOD, increased the levels of MDA and 8-OHdG. Moreover, CRS significantly

increased the expression of PKCα, p47phox, NOX2, RAC1, CHOP, and MANF, and decreased GRP78 in the frontal cortex and hippocampus. The study suggests that CRS (8 weeks) significantly accelerates learning and memory impairments, the mechanisms may be related to oxidative stress and its associated ER stress in frontal cortex and hippocampus.

Keywords: chronic restraint stress; Alzheimer's disease; reactive oxygen species; NADPH oxidase; endoplasmic reticulum stress

S10.42

Role of molecular chaperone Hsp70 in opioid addiction

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Aim: Hsp70 serves as a powerful chaperone required for the activation and stabilization of proteins, mediating fundamental cellular processes. This study aimed to study the role of Hsp70 in morphine-induced behavioral sensitization closely related to drug addiction. **Methods:** Behavioral sensitization to morphine was induced by the single-dose paradigm in mice and rats. A RT-PCR array and Western blot were used to test changes in Hsp70 gene and protein expression in Nucleus Accumbens (NAc) of mice and rats. Further, we observed effects of transcription (actinomycin D), protein synthesis (cycloheximide), Hsp70 inhibitors (KNK437, methylene blue, and pifithrin-μ) or inducer (geranylgeranylacetone) on Hsp70 expression and behavioral sensitization. **Results:** 1 Behavioral sensitization was evident in mice and rats pretreated with a single morphine injection; 2 The increased expression of Hsp70 gene and protein in NAc of mice and rats was parallel to behavioral sensitization; 3 Transcription and protein synthesis inhibitors could not only block behavioral sensitization, but also decrease Hsp70 expression; 4 Hsp70 inhibitors attenuated behavioral sensitization, while inducer promoted it. **Conclusion:** Hsp70 as a chaperone is critically involved in behavioral sensitization to morphine and the theory of "Chaperone-mediated opioid addiction" is hypothesized.

Keywords: chaperone; addiction; morphine

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S10.43

Effects of potassium channel on neuronal apoptosis induced by oxygen-glucose deprivation with or without astrocytes

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Aim: It was generally believed that excessive potassium efflux was one of the primary causes of neuronal injury after cerebral ischemia, so inhibition of the excessive potassium channels might play a neuroprotective role. The aim of this study was to evaluate effects of potassium channel blocker tetraethylammonium (TEA) on neuronal apoptosis after oxygen-glucose deprivation (OGD) in absence or presence of astrocytes to clarify the role of the potassium channels in the cerebral ischemic injury. **Methods:** Cortical neurons-astrocyte co-culture model was constructed *in vitro*. Neuronal morphology and purity were assayed by immunofluorescence method. Neuronal apoptosis rate after OGD2 h/R24 h and the role of TEA were measured by Hoechst 33342 fluorescence analysis. BDNF levels in astrocytes were detected by Western blot method. **Results:** After being co-culturedwith astrocytes for 10 d *in vitro*, cortical neurons grew well with clear synapses, and its purity reached to 95%. After OGD2 h/R24 h, neuronal apoptosis rate was 34.6%±0.96%, and TEA (5 mmol/L) could significantly reduce the rate to 25.1%±2.87%. Meanwhile, the level of BDNF in astrocytes after OGD 2 h/R24 h was increased, and TEA (5 mmol/L) could further increase BDNF level. **Conclusion:** Potassium channel blocker might attenuate neuronal apoptosis induced by OGD, and increased BDNF level in astrocytes. The mechanisms remained to be studied.

Keywords: potassium channel; cerebral ischemia; neuron; astrocyte; apoptosis

S10.44

The cell models of microglia activation by different stimulators

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Aim: Recently, research of the contribution of activated microglia in Alzheimer's disease (AD) has become a new research hotspot. To further explore the role of reactive microglia in AD, we compare the effects of the stimulators (aggregated and unaggregated $A\beta_{25-35}$, LPS, IFN-γ) on microglial cell line BV-2. **Methods:** $A\beta_{25-35}$ peptides were resuspended in sterile dH_2O (final concerntration, 1 mmol/L) and incubated at 37 °C for 1 week to prepare the aggregated Aβ. The structure of aggregated and unaggregated $A\beta_{25-35}$ was observed using transmission electron microscope. The expression of TNF-α of the BV-2 cells activated by $A\beta_{25-35}$, LPS and IFN-γ were observed by enzyme-linked immunosorbent assay (ELISA). **Results:** The aggregated $A\beta_{25-35}$ showed fibrillar structure, while unaggregated $A\beta_{25-35}$ showed intrinsically disordered structure. The BV-2 cells shriveled significantly when exposed to IFN-γ, and aggregated slightly when exposed to aggregated $A\beta_{25-35}$ and LPS. All of the three simulators, aggregated $A\beta_{25-35}$, LPS and IFN-γ, could increase the production of TNF-α in the BV-2 cells significantly. **Conclusion:** The cell models of microglia activation induced by $A\beta_{25-35}$, LPS and IFN-γ were set up to screen the new drugs targeted to microglia in the prevention and treatment of neurodegenerative diseases, such as AD.

Keywords: microglia; tumor necrosis factor-α; β-amyloid; Alzheimer's disease

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S10.45

Effects of AGAP, a analgesic-antitumor peptide, on voltage-gated ion channels in acutely isolated rat DRG neurons

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Aim: Previous studies showed that analgesic-antitumor peptide (AGAP) has analgesic activities tested. In this study, the effects of AGAP on ion channels including VGSC, VGCC and VGPC were investigated in acutely isolated rat DRG neurons. **Methods:** Whole-cell patch-clamping was used to record the voltage-gated Na^+ currents, voltage-gated Ca^{2+} currents and voltage-gated K^+ currents in acutely isolated rat small DRG neurons. **Results:** It was found that the inhibitory effect of AGAP (3–1000 nmol/L) on Na^+ currents by concentration dependent manner. The max inhibitory rate was 41.14%, and the value of IC_{50} was 11.18 nmol/L. 1000 nmol/L AGAP can not only significantly change the DRG neurons sodium current and current density of the *I-V* curve, making the VGSCs activation curve shift to the left, but also can significantly reduced the TTX-R sodium current and Nav1.8 currents by 44.00% and 53.62%, respectively. The results showed that AGAP reduced the HVA calcium current by 51.92%, which had no significant effect on shift steady-state activation kinetics. It was further suggested that AGAP reduced the LVA calcium current by 48.19%, but produced insignificant effect on delayed rectifier potassium currents. **Conclusion:** Our studies showed that the analgesic effect of AGAP may be related to regulation of the voltage-gated sodium channels and voltage-gated calcium channels, especially Nav1.8 of DRG neurons but not delayed rectifier potassium channels.

Keywords: AGAP; VGSC; Nav1.8; TTX-R sodium channels; VGCC

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S10.46

ER-α36, a novel variant of ER-α, was involved in the progression and acquired tamoxifen-resistance in glioma

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Aim: It is established that glioma, the most common form of primary brain tumor, possess the characteristic of high incidence rate and difficult therapy. Recent studies indicated that Tamoxifen (TAM) could sensitize the glioma cells to radiotherapy and inhibit proliferation, while long-term treatment with TAM would develop acquired resistance and increase the risk of breast cancer. Here, we

studied whether the ER-α36, a novel splicing variant of ER-α, was involved in the pathematology and acquired TAM-resistance of glioma. **Methods:** Primary cultured rodent cortical astrocytes, SH-SY5Y cells and U87-MG cells were employed to evaluate the expression pattern of ER-α36 via Western blot, immunofluorescence and RT-PCR analysis. RNAi technique was developed to allow studies into the possible effects of ER-α36 on proliferation and drug-susceptibility or resistance of glioma. MTT assay was introduced to assess whether ER-α36 was involved in proliferation and acquired TAM-resistance in glioma cells. **Results:** It was found that ER-α36 was expressed in astrocytes and glioma cells, and mainly co-located on the cytomembrane with caveolin-1. Additionally, *ER-a36* gene knockdown inhibited the accelerated proliferation (P<0.01 *vs* Ctrl) but enhanced the sensitization of glioma to TAM (P<0.05 *vs* Ctrl). **Conclusion:** ER-α36 was expressed widely in gliocytes and glioma cells, which might be involved in the progression and acquired TAM-resistance of glioma.

Keywords: ER-α36; tamoxifen; glioma; acquired resistance

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S10.47

Targeting cPLA2 inhibition to reduce lysophosphatidylcholine (LPC) production induced by Cdk5/p25: An anti-neuroinflammatory strategy in neurodegeneration

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Aim: The deregulation of cyclin-dependent kinase 5 (Cdk5) by p25 and neuroinflammation has been linked to the pathogenesis in a number of neurodegenerative diseases such as Alzheimer's disease (AD), ALS and PD. In this report, we aim to delineate the precise mechanism behind the induction of neuroinflammation and its significance in the AD pathogenesis. **Methods:** p25 overexpressin cells and the p25 transgenic mouse model (p25Tg) were used to delineate neuroinflammation *in vitro* and *in vivo*, which was robustly found in the absence of any amyloid or tau pathology. Determination of a neuronal lipid signaling pathway in p25-overexpressing cell was elucidated using HPLC-mass spectrometry. **Results:** We showed activation of neurons via NMDA receptors led to p25 generation and the secretion of a soluble lipid – LPC – which initiates astrogliosis. Increases in level and activity of cPLA2 were also found. Inhibition of cPLA2 with the commercially available inhibitor, AACOCF3 and gene silencing led to reduced astrogliosis. **Conclusion:** Neuroinflammation precedes tau and amyloid pathology. cPLA2 could be a potential therapeutic target for the reduction of neuroinflammation in neurodenegerative diseases.

Keywords: neuroinflammation; Cdk5/p25; cytosolic phospholipase A2; lysophosphatidylcholine; astrogliosis

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S10.48

Madecassoside protects against focal cerebral ischemia reperfusion injury though inhibiting inflammation and apoptosis via suppressing NF-kappaB activation

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Aim: Madecassoside, a triterpenoid derivative isolated from *Centella asiatica*, exhibits anti-inflammatory activities. In this study, we will investigate its neuroprotection effect in a rat model of ischemia-reperfusion (I/R), and its possible mechanism. **Methods:** Madecassoside (6, 12, or 24 mg/kg, iv) was administered at 1 h after the start of reperfusion, and neurological deficit score and infarct volume were evaluated 24 h later. Neuronal apoptosis was assessed by TUNEL staining, and pathological brain damage was estimated by HE staining. mRNA and protein expression of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) were measured by real time RT-PCR and ELISA, respectively; NF-κB p65 expression was determined by Western blotting. **Results:** Madecassoside significantly reduced brain infarct area, relieved neurological deficit, and ameliorated neuronal apoptosis and pathological damage. Madecassoside also significantly reduced levels pro-inflammatory cytokines and NF-κB p65 after madecassoside treatment. **Conclusion:** Madecassoside exerts a potent neuroprotective effect against cerebral I/R injury in rats, which was mediated by anti-inflammatory and anti-apoptotic mechanisms, and these effect might be attributed to its inhibition of the NF-κB activation. These results provide evidence for a novel treatment for ischemic stroke.

Keywords: madecassoside; cerebral ischemia reperfusion; inflammatory; apoptosis; NF-κB pathway

S10.49

The improving learning and studying effect of the polysaccharides of desertliving cistanche herb in *D*-galactose treated mice

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Aim: In this study, we examined the effect of the polysaccharides of desertliving cistanche herb (CDPS) on learning and memory function after the *D*-galactose (D-gal)-induced brain aging in mouse. **Methods:** Mice were given a subcutaneous injection of D-gal (50 mg/kg) and orally administered CDPS (25, 50, or 100 mg/kg) daily for 6 weeks. Experimental groups were subjected to Morris water maze test. Furthermore, the AchE, SOD, MDA were examined, respectively, according to the manufactured kit. The pathological change was examined by HE staining. **Results:** The results showed that CDPS (50, 100 mg/kg) significantly improved spatial learning and memory function in D-gal-treated mice. In addition, CDPS (50, 100 mg/kg) significantly increased the levels of acetylcholine (ACh) and superoxide dismutase (SOD), but decreased the activity of acetylcholinesterase (AChE) and the level of malondialdehyde (MDA) in the brains of D-gal-treated mice. Furthermore, CDPS ameliorated neuronal damage in hippocampus in D-gal-treated mice. **Conclusion:** These results suggest that CDPS possesses anti-aging efficacy and improves the learning and memory function in aging mice.

Keywords: anti-aging; desertliving cistanche herb; learning and studying

S10.50

The inhibitory effect of methyl salicylate 2-*O*-*β*-*D*-lactoside (MSL) on inflammation in LPS-induced microglia

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Aim: To study the inhibitory effect of methyl salicylate 2-*O*-*β*-D-lactoside (MSL) on LPS-induced BV-2 cell. **Methods:** BV-2 cells were treated with LPS (0.1 μg/mL) to establish inflammation model; MTT assay was used to detect the viability of BV-2 cells; nitric oxide (NO) was detected by the method of Griess reaction. ELISA was used to study the release of pro-inflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor-a (TNF-alpha) level. Inducible nitric oxide synthase (iNOS) and cyclooxygenase-1(COX-1) protein expression was examined by the Western blotting analysis. **Results:** Our study showed that MSL (0.05, 0.5, 5, and 50 μmol/L) did not affect cell viability. MSL (0.5, 5, 50 μmol/L) inhibited LPS-induced production of NO in a concentration-dependent manner, aspirin (50 μmol/L) also significantly inhibited the release of NO. MSL (50 μmol/L) and aspirin (50 μmol/L) inhibited LPS-induced production of pro-inflammatory cytokines IL-6 and TNF-alpha. **Conclusion:** This study indicates that MSL inhibits LPS-induced activation of microglia which leads to inflammatory response and its anti-inflammatory mechanism may need further study.

Keywords: MSL; lipopolysaccharides; microglia; neuroinflammation; Alzheimer's disease.

S10.51

Notoginsenoside R1 rescues PC12 cells from hydrogen peroxide-induced oxidative stress via up-regulation of heme oxygenase-1 through PI3K/Akt/Nrf2 pathway

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Aim: Notoginsenoside R1 (NGR1), a novel Nrf2 activator, is a major saponin isolated from *Panax notoginseng*. Although NGR1 has many pharmacological effects including anti-oxidative, anti-apoptotic and neuroprotective properties, little is known about the underlying mechanism. In this study, for the first time, we clearly show that NGR1 has protective effects against H_2O_2-induced cytotoxicity in PC12

cells. **Results:** Pretreatment with NGR1 (12.5 and 25 μmol/L) dramatically inhibited H_2O_2-induced intracellular ROS accumulation and subsequential oxidative stress in PC12 cells. NGR1 pretreatment could also suppress H_2O_2-induced the imbalance of Bcl-2 family proteins, the depolarization of mitochondrial membrane and activation of caspase-3 as well as inactivation of PI3K/Akt/Nrf2 pathway. Furthermore, we clarified a novel underlying mechanism. NGR1 treatment alone could up-regulate expression and activity of HO-1 in dose- and time-dependent manners, and this effect could be abolished by transfection of PC12 cells with Nrf2 siRNA and supplementation with a PI3K specific inhibitor LY294002. NGR1 treatment alone could also induce phosphorylation of Akt, facilitate the nuclear accumulation of Nrf2, and enhance the binding activity of Nrf2-ARE in dose- and time-dependent manners. Most importantly, NGR1-mediated activation of PI3K/Akt/Nrf2 pathway was suppressed by transfection with Akt siRNA and LY294002. Moreover, NGR1-mediated protection was also attenuated by a HO-1 specific inhibitor Snpp and LY294002. **Conclusion:** NGR1 confers protection against H_2O_2-induced oxidative stress by up-regulation of HO-1 through PI3K/Akt/Nrf2 pathway in PC12 cells.

S10.52

Synapse formation in the brain and developmental disorders

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Aim: Synapse formation is the key step in the development of neuronal networks. Precise synaptic connections between nerve cells in the brain provide the basis of perception, learning, memory and cognition. Here, we investigated synapse organizers in the brain. **Results:** Despite the wealth of information on the molecular mechanisms of glutamatergic synaptogenesis proposed by *in vitro* studies using cell culture models, evidence for their relevance to synaptogenesis *in vivo* has been lacking. On the other hand, there is clear *in vivo* evidence that GluRδ2, a member of the δ-type glutamate receptor (GluR), plays an essential role in cerebellar Purkinje cell synapse formation. We have shown that the *trans*-synaptic interaction of postsynaptic GluRδ2 and presynaptic neurexins through Cbln1 mediates parallel fiber-Purkinje cell synapse formation. The assembly stoichiometry of the synaptogenic triad suggests that GluRδ2 triggers synapse formation by clustering four neurexins. We also found that IL1-receptor accessory protein-like 1 (IL1RAPL1), which is responsible for nonsyndromic mental retardation and autism, mediates synapse formation of cortical neurons through *trans*-synaptic interaction with presynaptic PTPδ. **Conclusion:** Failure in synapse formation is implicated in the pathogenesis of mental disorders such as mental retardation and autism.

Keywords: synapse formation; mental disorder; synapse organizer; brain

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S10.53

Transmission regulation of decision-making: pharmacogenetic approaches

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The nucleus accumbens (NAc) plays a pivotal role in reward and aversive learning, learning flexibility and decision-making. Inputs of the NAc are transmitted through 2 parallel direct and indirect pathways and controlled by dopamine (DA) transmitter. To explore how the associative learning is controlled in the NAc, we developed 1) reversible neurotransmission-blocking (RNB), in which transmission of each pathway was selectively and reversibly blocked by the pathway-specific expression of transmission-blocking tetanus toxin and 2) asymmetric RNB, in which one side was blocked by RNB and the other side was pharmacologically manipulated by a transmitter agonist or antagonist. The activation of the direct pathway D1 receptors and the inactivation of the indirect pathway D2 receptors distinctly control reward and aversive learning, respectively. The D2 receptor inactivation is also critical for flexibility of reward learning. Furthermore, decision-making is regulated by the pathway-specific LTP and LTD via selective receptors (NMDA, adenosine A2a and endocannabinoid CBI receptors) characteristic of cortico-accumbens synapses. The dynamic control of the NAc neural plasticity is thus essential for reward-seeking and aversion-avoiding decision-making.

S10.54

Evaluation of tacrine-caffeic acid heterodimers as multi-targeted anti-Alzheimer's disease agents and the mechanism of anti-oxidative stress

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Aim: A series of tacrine-caffeic acid heterodimers as multi-targeted anti-Alzheimer's disease (AD) candidates were designed and synthesized. In this study, the anti-AD activities of these heterodimers were evaluated and the mechanism underlying the anti-oxidative stress was investigated. **Methods:** The multiple anti-AD activities of heterodimers were evaluated through Ellman assay, thioflavine T assay, ultraviolet-visible spectrophotometry, DPPH free radicals scavenging assay, MTT assay and Morris water maze. Besides, the promotion nuclear translocation and transcriptional activity of Nrf2 were analyzed by Western blot, immunofluorescence and dual-luciferase reporter gene assay. The protein and mRNA levels of HO-1, one of the Nrf2 downstream genes, were detected using RT-PCR and Western blot. The role of Nrf2/HO-1 signaling pathway in protection of T3CA was confirmed by using brusatol and ZnPP-IX, inhibitors of Nrf2 and HO-1 respectively, and siRNA targeted HO-1. **Results:** *In vitro*, all these heterodimers were AChE inhibitors. They also inhibit Aβ self-aggregation or AChE-induced aggregation in various degrees, with Cu^{2+} chelation and DPPH free radical scavenging properties, and are capable of protecting HT22 cells against glutamate-induced cell death. *In vivo*, one heterodimer T3CA improves the cognitive impairment induced by scopolamine. T3CA dramatically promotes Nrf2 nuclear translocation, increased its transcriptional activity, and up-regulates the mRNA and protein level of HO-1. Pretreatment with Nrf2 degrader brusatol, HO-1 inhibitor ZnPP-IX, or siHO-1, can partially attenuated the protective effect of T3CA against glutamate-induced cell death, respectively. **Conclusion:** This novel series of heterodimers were with multiple anti-AD activities, and the protection of T3CA was partially through activating Nrf2/ARE/HO-1 signaling pathway.

Keywords: tacrine; caffeic acid; oxidative stress; Alzheimer's disease

S10.55

Ndrg2 plays an important role in the lesions of SK-N-SH cells over-expressing wild type human APP695

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Aim: To elucidate the role of N-myc downstream-regulated gene 2 (Ndrg2) in Aβ production, tau phosphorylation and cell apoptosis in human neuroblastoma SK-N-SH cells over-expressing wild type human APP695 (SK-N-SH APPwt). **Methods:** siNdrg2 was applied to silence Ndrg2 in SK-N-SH APPwt cells and Ad-Ndrg2 was used to overexpress Ndrg2 in SK-N-SH cells. The level of Ndrg2, Aβ, p(Ser199)-tau, p(Thr205)-tau and p(Ser396)-tau were examined by Western blot. Apoptosis was detected by Hoechst staining and cell viability was measured by MTT assay. **Results:** After Ndrg2 knockdown in SK-N-SH APPwt cells, Aβ level was reduced by 60.5% and tau phosphprylation at Ser199, Thr205, and Ser396 epitopes dropped by 74.3%, 59.6%, and 53.5%, respectively. Hoechst staining showed siNdrg2 decreased cell apoptosis dramatically and MTT assays revealed the cell viability was increased by 90.3%. Alternatively, SK-N-SH cells over-expressing Ndrg2 engendered a robust increase of Aβ level by 47.3%. The three phosphorylation epitopes of tau could be clearly observed in SK-N-SH cells tansfected with Ad-Ndrg2, whereas in SK-N-SH cells transfected with Ad-lac Z, they were barely detected. MTT assays displayed the cell viability of SK-N-SH cells transfected with Ad-Ndrg2 was decreased by 48.5% and changes that were indicative of apoptosis could be observed such as nuclear chromatin condensation after Hoechst staining. **Conclusion:** Our study demonstrated that Ndrg2 might increase Aβ production, tau phosphorylation and cell apoptosis in SK-N-SH APPwt cells.

Keywords: Ndrg2; Aβ; tau phosphorylation; apoptosis

S10.56

BDNF expression through nuclear localization of CaMKIIδ3 in the dopaminergic neurons

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Aim: Calcium/calmodulin-dependent protein kinase II nuclear isoform, CaMKIIδ3 is highly expressed in the domapminergic neurons of substantia nigra. CaMKIIδ3 promotes BDNF expression in NG-108 cells (J Neurochem 2002; 82: 316–28). We here investigated the mechanisms underlying nuclear translocation of CaMKIIδ3 and BDNF expression by CaMKIIδ3 in the dopaminergic neurons. **Methods:** CaMKIIδ3 and protein phosphatase 1 (PP1) were co-expressed in Neuro-2a cells. We examined the localization of CaMKIIδ3 and PP1, and expression of BDNF. Using primary cultures of substantia nigral cells, we examined the phosphorylation of CaMKIIδ3 (Ser332) by stimulation of dopamine and chemical LTP. **Results:** CaMKIIδ3 was expressed in both cytoplasmic and nuclear compartments in Neuro-

2a cells, and it translocated into nucleus following co-expression with PP1. The activity of CaMKIIδ3 co-expressed with PP1 significantly increased in nuclear fractions as compared to CaMKIIδ3 activity expressed alone. In substantia nigral dopaminergic neurons, Ser332 phosphorylation significantly increased by chemical LTP, while it was reduced by dopamine stimulation. Co-expression of CaMKIIδ3 with PP1 or expression of CaMKIIδ3 (S332A) in Neuro-2a cells significantly increased BDNF mRNA levels compared to that in CaMKIIδ3-expressed cells. **Conclusion:** CaMKIIδ3(Ser332) is dephosphorylated by PP1, resulting in translocation into nucleus. The nuclear translocated CaMKIIδ3 likely mediates BDNF expression in the dopaminergic neurons.

Keywords: CaMKIIδ3; nuclear translocation; dopaminergic neurons; BDNF
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S10.57

Vulnerable glutamate homeostasis in the nucleus accumbens of heroin-addicted animals

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The transient rewarding qualities of addictive drugs and the enduring vulnerability to relapse that characterizes addiction have been linked to a variety of changes in synaptic transmission in the nucleus accumbens. Numerous studies have revealed that enduring changes in glutamate transmission contribute strongly to relapse, while acute drug-induced increases in dopamine transmission are necessary for drug reward. Using the reinstatement animal model of drug seeking, it has been shown that reinstated heroin or cocaine seeking by cue or drug re-exposure parallels with increased extracellular glutamate in nucleus accumbens, which might result from the spillover of synaptic glutamate. However, this hypothesis lacks direct evidence proving the disability to conquer the spillover of synaptic glutamate in drug-addicted animals. Therefore, we designed protocols to trigger glutamate spillover *ex vivo* or *in vivo* by electrophysiological or biochemical strategies respectively, and evaluated the extent of NMDAR-mediated excitatory postsynaptic current (NMDAR EPSC) by whole cell recording and glutamate kinetics by amperometry as measures of synaptic glutamate spillover and clearance in heroin-addicted rats. We found that activating excitatory afferents escalated glutamate spillover in the NAc in heroin-addicted animals, which is attributed to defect of Na^+-dependent glutamate uptake. And then we verified that the capability of glutamate uptake restored by ceftriaxone is competent to defeat synaptic glutamate spillover, and hence prevented reinstated heroin seeking.

S10.58

Sphingosine kinase 2-mediated autophagy contributes to preconditioning by isoflurane in mouse cortical neurons

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Aim: Sphingosine kinase 2 (SPK2) and autophagy are both involved in cerebral preconditioning, but whether preconditioning-induced SPK2 upregulation and autophagy activation are linked mechanistically remains to be elucidated. In this study, we used *in vitro* and *in vivo* models to explore the role of SPK2-mediated autophagy in isoflurane (ISO) and hypoxic preconditioning (HP). **Results:** In primary cultured mouse cortical neurons, both ISO and HP induced autophagy, as evidenced by an elevated LC3II/LC3I ratio and p62 downregulation. ISO and HP protected against subsequent oxygen glucose deprivation (OGD) or glutamate (Glu) injury, while pretreatment with autophagy inhibitors (3-MA or KU55933) abolished preconditioning-induced tolerance. Pretreatment with SPK2 inhibitors (ABC294640 and SKI-II) prevented preconditioning-induced autophagy. ISO also induced autophagy in the cortex of C57 mice as shown by Western blots for LC3 and p62, immunoflurescence/immunohistochemistry of LC3 and electron microscopy. ISO induced autophagy in mice lacking the SPK1 isoform ($SPK1^{-/-}$), but not in $SPK2^{-/-}$ mice. S1P and the S1P receptor agonist FTY720 did not protect cultured neurons against OGD and did not alter the expression of LC3 and p62, suggesting that SPK2-mediated autophagy and protections are not S1P-dependent. But SPK2 inhibitors abolished ISO-induced disruption of the Beclin1/Bcl-2 association. **Conclusion:** Taken together, these results strongly indicate that autophagy is involved in isoflurane preconditioning both *in vivo* and *in vitro* and that SPK2 contributes to preconditioning-induced autophagy, possibly by disrupting the Beclin1/Bcl-2 interaction.

Keywords: sphingosine kinase 2; isoflurane preconditioning; autophagy; cortical neurons; coimmunoprecipitation

S10.59

B2 induces sedative and hypnotic effects via activating the hypothalamic ventrolateral preoptic neurons in mice

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Aim: B2 is a novel derivative of N^6-(4-hydroxybenzyl) adenine riboside originally isolated from *Gastrodia elata* Blume. The ventrolateral preoptic nucleus (VLPO) is a group of neurons in the hypothalamus that release the inhibitory neurotransmitter γ-aminobutyric acid (GABA) during sleep. It plays an important role in sleep-wake regulation. In this study, we examined the sedative and hypnotic effects of B2 and its influence on the VLPO in mice. **Methods:** The sedative property of B2 was evaluated by assessing locomotor activity and the hypnotic activity of B2 was investigated in pentobarbital sodium treated mice. Immunohistochemistry assay was used to observe the influence of B2 on c-Fos expression in the VLPO. Meanwhile GABA content in the hypothalamus was measured by HPLC-electrochemical detection. **Results:** B2 (5 mg/kg, ip) decreased spontaneous locomotor activity by 93% *vs* the control group, and prolonged the duration of loss of righting reflex induced by pentobarbital sodium from (42.2±1.9) min to (106.3±9.2) min. B2 (5 mg/kg, ip) significantly increased c-Fos expression in the GABAergic neurons of the VLPO, and increased the content of GABA by 39% in the hypothalamus *vs* the control group. **Conclusion:** Altogether, these results indicated that B2 produced exact sedative and hypnotic effects. Such effects might be mediated by the activation of the sleep center VLPO and increase of the inhibitory amino acid GABA content in the hypothalamus.

Keywords: ventrolateral preoptic area; insomnia; hypnotics; sleep; γ-aminobutyric acid

S10.60

Antidepressant effect of Xiaochaihutang in the unpredictable chronic mild stress model via increasing expression of neurotrophin in rat hippocampus

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Aim: Xiaochaihutang (XCHT) has been used in China for thousands of years to treat "Shaoyang syndrome", which involves depressive-like symptoms. Previous studies in our laboratory have showed that XCHT produced antidepressant-like action in rodent behavioral despair models. The present study was aimed to investigate the mechanism(s) underlying the antidepressant-like effect by measuring brain-derived neurotrophic factor (BDNF) and its primary receptor tropomyosin-related kinase B (TrkB) in rat hippocampus. **Methods:** Chronic unpredictable mild stress (CUMS) model was established in rats. The antidepressant effect of XCHT (1.7 or 5 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, for four weeks, *po*) was investigated by detecting food consumption and sucrose preference weekly. The expressions of BDNF and TrkB in hippocampus were measured by using immunohistochemical staining analysis. **Results:** CUMS caused significant decrease in food consumption and sucrose preference in rats, and these depression-like behaviors were significantly improved by XCHT (1.7 or 5 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$). XCHT (5 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$) significantly improved the expressions of BDNF and TrkB in hippocampus, which was decreased in CUMS model rats. **Conclusion:** The results showed that XCHT had protective effects against depression-like behavior induced by CUMS in rats, and the antidepressant-like effect of XCHT is mediated, at least in part, by increasing the expression of BDNF and its receptor TrkB in hippocampus.

Keywords: XCHT; CUMS; BDNF; TrkB; hippocampus
Acknowledgements: This study was supported by the Key Project of National Natural Science Foundation of China (81130071).

S10.61

Effects of MNG on neurogenesis and Wnt signaling after focal cerebral ischemia in rats

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Aim: To investigate the effects of MNG extracted from *Cornus officinalis* on neurogenesis and Wnt signaling after focal cerebral ischemia reperfusion in rats. **Methods:** Focal stroke was induced in adult SD male rats by middle cerebral artery occlusion reperfusion injury (tMCAO/R). Then MNG were administered intragastrically once a day at three doses respectively. Ludmila Belayev's method were used to examine neurological function at 3 d and 7 d post ischemia. Immunohistochemistry staining and Western blotting methods were used to detect the expression of Wnt/β-catenin-related proteins. **Results:** MNG significantly improved neurological function after MCAO and reduced infarct volume both at 3 d and 7 d post ischemia. Immunohistochemical and immunoblot analysis indicated that Wnt signaling pathway might be involved in the mechanisms of neurogenesis enhanced by MNG. **Conclusion:** MNG could increase neurogenesis post ischemia and the possible mechanism were due to activating the Wnt signaling. These results may offer a novel therapeutic approach for cerebral ischemia recovery.

Keywords: MNG; cerebral ischemia; neurogenesis; Wnt signaling

S10.62

An α1-adrenoceptor antagonist:DDPH can efficiently attenuated the decreased expression of HCN1 mRNA in cortex and hippocampal CA1 region in chronic cerebral hypoperfused rats

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Aim: DDPH (1-(2,6-dimethylphenoxy)-2-(3, 4-dimethoxyphenylethylamino) propane hydrochloride), a candidate drug known to be an α1-adrenoceptor antagonist, can efficiently penetrate through blood brain barrier and inhibit the contraction of vascular smooth muscle in the brain. The hyperpolarization-activated cyclic-nucleotide-gated cation non-selective channel (HCN)1 is primarily expressed in the hippocampus and cortex can be regulated in many pathological settings. However, little is known about its change under ischemic conditions and regulation of drugs for it. **Methods:** In the present study, we performed neurophysiological recordings of sham-operated and chronic ischemic rats with hypoperfusion during the resolution of the neurological deficits respectively. *In situ* hybridization methods and reverse transcriptase-polymerase chain reaction (RT-PCR) assays were used to investigate whether and how HCN1 mRNA may be altered in global incomplete chronic cerebral ischemic rat model. **Results:** Our results suggested that HCN1 mRNA declined to 52% and 46% of the control values in the cornus ammon 1 (CA1) regions of hippocampus and neocortex separately after chronic cerebral ischemia. HCN1 mRNA in the hippocampal CA1 region and neocortex was markedly down-regulated by ischemia, reaching 48.90% and 45.80% of the control values respectively in the semi-quantitative RT-PCR experiment. DDPH at 12 mg/kg per day for 30 d can efficiently attenuated the decreased expression of HCN1 mRNA in cortex and hippocampal CA1 region of the rats after bilateral carotid artery ligation. **Conclusion:** The phenomenon opened new insights for further investigation of the physiological and pathological significances of HCN1 in chronic incomplete global cerebral ischemia.

Keywords: DDPH; HCN1 mRNA; rt-PCR; chronic ischemia

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S10.63

The sedative-hypnotic effect of YZG-330 and its influence on the expression of p-CaMKII in mouse hypothalamus

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Aim: Ca/calmodulin-dependent protein kinase (CaMKII) was a critical molecule for the regulation of the wake–sleep cycle. The level of CaMKII phosphorylation (pCaMKII) was positively related with the total percentage of time spent in wakefulness. pCaMKII inhibitor could decrease wakefulness and increase SWS and REM sleep. This study was designed to explore whether YZG-330 had a sedative-hypnotic effect and determine the role of pCaMKII in the regulation of sleep. **Methods:** The effect of YZG-330 on the duration for losing of righting reflex in sodium pentobarbital treated mice was evaluated. The level of pCaMKII expression in mouse hypothalamus was quantified by Western blotting after administration of YZG-330. **Results:** YZG-330 (0.125, 0.5, and 2 mg/kg, ig) respectively prolonged the duration for the losing of righting reflex of mice treated with sodium pentobarbital by 25%, 64%, and 506%, which was dose-dependent. YZG-330 (5 mg/kg, ip) significantly decreased level of pCaMKII expression in mouse hypothalamus by 53.5% compared with control group 30 min later after administration. **Conclusion:** YZG-330 could be a pCaMKII inhibitor inducing a sedative-hypnotic effect, and pCaMKII might play a role in regulating sleep.

Keywords: p-CaMKII; sedative –hypnotic; hypothalamus

S10.64

The mechanism of neuroprotective effect of tacrolimus on rat cerebral ischemia-reperfusion injury

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Aim: To investigate the neuroprotective effect and the mechanism of tacrolimus on rat cerebral ischemia-reperfusion injury. **Methods:** Sprague-Dawley rats were subjected to 60 min of transient middle cerebral artery occlusion (MCAO) followed by 24 h reperfusion. 10 min before reperfusion, animals were intravenously administered tacrolimus (0.01, 0.05, and 0.5 mg/kg) or nimodipine (0.1 mg/kg) or a similar volume of solvent. At 24 h of reperfusion, behavioral score were recorded, brain infarct size were observed, and hematoxylin/eosin staining were performed to evaluate ischemia--reperfusion induced neuronal degeneration. To investigate the potential mechanism of tacrolimus on rat cerebral ischemia-reperfusion injury, perforated whole cell patch were employed to test the effect of tacrolimus on the spontaneous outward currents (STOCs) and big conductance potassium currents (BK currents). **Results:** Tacrolimus (0.05 and 0.5 mg/kg) like nimodipine (0.1 mg/kg) administered 10 min before reperfusion resulted in significant improvement of behavioral score, significant reduction of infarct size at 24 h of reperfusion, and the neuronal degeneration was also attenuated in tacrolimus (0.05 and 0.5 mg/kg) treated group and nimodipine (0.1 mg/kg) treated group. Perforated whole cell patch showed that tacrolimus (1 and 10 μmol/L) could increase STOCs and BK currents which could be blocked by iberriotoxin (100 nmol/L) and ryanodine (10 μmol/L). **Conclusion:** Tacrolimus has protective effect on rat cerebral ischemia-reperfusion injury, which may be induced by the activation of BK currents via ryanodine receptor-BK currents pathway.

S10.65

Leukotriene D4 induces cognitive impairment through enhancement of CysLT1R-mediated amyloid-β generation in mice

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Aim: Amyloid plaques in the extracellular parenchyma mainly consist of amyloid-β peptides (Aβ), one of the pathological hallmarks in Alzheimer's disease (AD). In the present study, we examined neuroinflammation, amyloidogenesis, and memory performance following intracerebral infusions of leukotriene D4 (LTD4) in mice. **Methods:** We carried out behavioral tests 72 h after intracerebral infusions (1 ng/mice), and determined cysteinyl leukotriene receptor 1 ($CysLT_1R$), Aβ, amyloid precursor protein (APP), β- or γ-secretase, and NF-κB of the brain hippocampus and cortex in mice. **Results:** The results demonstrated that intracerebral infusions of LTD4 induced memory impairment determined by Morris water maze test and Y-maze test in mice, and resulted in the accumulations of $Aβ_{40}$ and $Aβ_{42}$ in the hippocampus and cortex of brains through increasing β- and γ-secretase activities accompanied with the increased expression of APP. LTD4 also induced the expressions of $CysLT_1R$ and NF-κB p65 in the hippocampus and cortex of mouse brains. The pretreatment of pranlukast (1.5 ng/mice, intracerebrally), a $CysLT_1R$ antagonist, prevented the LTD4-induced amyloidogenesis and memory dysfunction *in vivo*. Pranlukast (0.6 μmol/L) also prevented LTD4 (20 nmol/L)-induced amyloidogenesis in the cultured neurons *in vitro*. Moreover, the elevated levels of brain $CysLT_1R$ and NF-κB p65 were also inhibited by pranlukast. **Conclusion:** Our results indicate that LTD4 signaling via the $CysLT_1R$ increased Aβ peptide burden,

possibly via effects on the APP level, β- and γ-secretase activities mediated by NF-κB pathway. Our findings identify $CysLT_1R$ signaling as a novel proinflammatory and proamyloidogenic pathway, and suggest a rationale for development of therapeutics targeting the $CysLT_1R$ in neuroinflammatory diseases such as AD.

Keywords: leukotriene D4; cognition; cysteinyl leukotriene receptor 1; amyloid-β peptides; Alzheimer's disease

S10.66

Transient augment of IL-1β promotes seizure susceptibility via upregulating CB1 receptor in prolonged febrile seizures

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Aim: This study was to investigate the role of IL-1β in the enhanced seizure susceptibility after prolonged febrile seizures (FSs). **Methods:** Rats or mice pups (P8) were exposed to a hyperthermal chamber (44±2 °C) to induce FSs with stereotyped behaviors and epileptic EEG. We injected (icv) IL-1Ra to FSs pups and IL-1β to normal pups or IL-$1R1^{-/-}$ mice. Besides, cannabinoid 1 receptor (CB1R) antagonist was injected after FSs or IL-1β treatment and its agonist was injected to IL-1Ra treatment or IL-$1R1^{-/-}$ mice. Seizure susceptibility was tested using maximal electroshock (MES) models when pups became adults. The expression of IL-1β and CB1R were measured by Western blotting. CB1R-targeted siRNA was also used. **Results:** We found that: 1) IL-1β, through IL-1R1, promotes seizure susceptibility after prolonged FSs, while IL-1Ra rescued this process only when given immediately and 12 h delay. 2) Mice with CB1R antagonist or CB1R-siRNA were no longer susceptible to seizures after prolonged FSs. 3) Seizure susceptibility has no change in mice with IL-1Ra and mice lacking IL-1R1 when activated by CB1R. **Conclusion:** Our study identifies the proconvulsant effect of IL-1β on the enhanced seizure susceptibility later in life after prolonged FSs and the existence of a "time window" for IL-1Ra therapy. These effects may through regulating CB1R.

Keywords: prolonged febrile seizures; epileptogenesis; IL-1β; CB1R

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S10.67

Estrogen receptor GPR30 exerts anxiolytic effects by maintaining the balance between GABAergic and glutamatergic transmission in the basolateral amygdala of ovariectomized mice after stress

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G-protein-coupled receptor 30 (GPR30)/G-protein-coupled estrogen receptor is a novel estrogen membrane receptor that localizes to the cell membrane and endoplasmic reticulum. GPR30 is widely distributed and has numerous physiological functions in the central nervous system. We found that GPR30 is highly expressed in the basolateral amygdala (BLA). Additionally, GPR30 expression in the amygdala of ovariectomized (OVX) mice significantly increased after acute stress and was accompanied by anxiety-like behaviors. These effects, however, were reversed by local infusion of the GPR30 agonist (G1) in the BLA. Protein assessments revealed that G1 attenuated the up-regulation of the GluR1 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and NR2A-containing *N*-methyl-D-aspartate receptors (NMDARs) in the BLA of OVX mice using an acute stress paradigm. In the same model, we found that the agonist also blocked the down-regulation of γ-aminobutyric acid A ($GABA_A$) receptors and NR2B-containing NMDARs. Electrophysiological recording showed that the activation of GPR30 increased the inhibitory synaptic transmission in the BLA. Overall, our results indicate that estradiol reduces anxiety-like behaviors induced by acute stress at least partially through GPR30 signaling, maintaining the balance between GABAergic and glutamatergic transmission in the BLA of OVX-stressed mice.

Keywords: estrogen; G-protein-coupled receptor 30; stress; anxiety

S10.68

The role of phosphatidylinositol-linked D1-like receptor agonist SKF83959 in major depressive disorders

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Aim: Growing evidence indicates a relationship between dopamine system and depression, and some dopamine receptor agonists have been proved to produce antidepressant effects. The dopamine receptor that activates the PLC/IP3 pathway has been called the phosphatidylinositol (PI)-linked D1-like receptor (PI-D1 receptor), a novel dopamine D1-like receptor. However, it remains unknown that whether activation of this receptor could modulate depression. **Methods:** Intraperitoneal injection, intracerebroventricular injection, forced swim test (FST), open field test, chronic social defeat stress (CSDS), sucrose preference test, social interaction test, double-labeling immunofluorescence and golgi silver staining were used in this section. **Results:** we found that phosphatidylinositol (PI)-linked D1-like receptor that activates the phospholipase C (PLC)/inositol trisphosphate (IP3) signaling pathway, contributes to stimulation of BDNF signaling and has antidepressant-like effects. We demonstrated that activation of PI-linked D1 receptor exhibited potent antidepressant-like activity through PLC/IP3 pathway, and these effects were mediated by reversing the decreased BDNF signaling pathway. On the other side, stimulation of PI-linked D1 receptor produced no effect on the monoaminergic system and hypothalamic-pituitary-adrenocortical (HPA) system, strengthening that only the BDNF-TrkB system was involved in its antidepressant effects. **Conclusion:** These results suggest that PI-linked D1 receptor plays a role in the developing of depression and may serve as a novel therapeutic target for depression.

Keywords: depression, phosphatidylinositol-linked D1-like receptor; brain-derived neurotrophic factor; chronic social defeat stress

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S10.69

The γ-secretase blocker reduces the permeability of the blood-brain barrier by decreasing the ubiquitination and degradation of occludin during brain ischemia

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Aim: Cerebral ischemia/reperfusion injury induces alterations in many signaling pathways that underlie BBB disruption and lead to neurovascular damage. We investigated the mechanisms of occludin degradation that underlie permanent middle cerebral artery occlusion (pMCAO) in rats. **Methods:** pMCAO was employed as ischemia model and BBB damage was assessed by examining the extent of Evans blue solution leakage from the vessels. Drugs were delivered through intracerebroventricular injection. Analysis of related proteins was performed by co-immunoprecipitation, Western blotting and immunohistochemistry. **Results:** Changes in tight junction proteins and neurovascular damage were observed following pMCAO. DAPT, the γ-secretase blocker, can significantly inhibited BBB disruption and the pMCAO-induced neurovascular damage, whereas ALLM and batimastat, which are inhibitors of calpain and metalloproteinase proteases, respectively, were less effective. pMCAO induces ubiquitination and degradation of occludin, associated with the activation of the E3 ubiquitin ligase Itch in brain microvessels following ischemia. **Conclusion:** The inhibition of Notch1/HES-1 signaling and Itch overactivation may contribute to the neurovascular protective effect of DAPT following pMCAO. These results highlight the importance of the γ-secretase cascade in the progression of neurovascular damage that occurs during brain ischemia.

Keywords: permanent brain ischemia; tight junction; occludin; DAPT; ubiquitination; degradation

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S10.70

Effect of catalpol on HPA disturbance in AD rats

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Aim: To study the effect of catalpol on hypothalamus-pituitary-adrenal axis (HPA) disturbance of AD (Alzheimer's disease) rats. **Methods:** Rats were injected fragment 25–35 of Aβ (β-amyloid protein, Aβ) intracerebroventricularly and D-galactose intraperitoneally. 30 AD rats were randomly divided into 3 groups: catalpol low dose, high dose (5 $mg \cdot kg^{-1} \cdot d^{-1}$ and 10 $mg \cdot kg^{-1} \cdot d^{-1}$) and model (saline). Another 10 healthy rats were as normal controls. Serum levels of hormone CRH, ACTH, and cortisone were detected by radioimmunoassay, expression of CRH and CRH-R1 was determined by immunohistochemistry, ultrastructural changes were observed under electron microscope. **Results:** Cortisol level of model rats was higher, ACTH and CRH levels were significantly lower than normal. Catalpol improved hormone secretion disorder. Hypothalamic structure also changed. Expression of CRH and CRHR1 decreased. Catalpol ameliorated these changes. Catalpol ameliorated the hypothalamic ultrastructure injury, such as dilated rough endoplasmic reticulum and degranulation and swelling of mitochondria. **Conclusion:** Catalpol can improve HPA function and reverse hypothalamus morphological abnormalities.

Keywords: catalpol; Alzheimer's disease; HPA; rats

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S10.71

Noble dendrobium polysaccharides attenuate learning and memory deficits induced by lipopolysaccharide and its mechanisms in rats

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Aim: To investigate the protective effects of noble dendrobium polysaccharides (NDP) on lipopolysaccharide (LPS)-induced learning and memory deficits in rats, and explore the possible mechanisms. **Methods:** Rats were treated orally with NDP (40, 80, and 160 $mg \cdot kg^{-1} \cdot d^{-1}$) for 7 d followed injection of LPS 20 μg (2 μg/μL) into the lateral ventricle to induce learning and memory deficits. The abilities of spatial learning and memory were tested by Morris water maze. The mRNA levels of APP and GSK-3β were detected by real time RT-PCR. **Results:** Compared to sham-treated rats, LPS injection significantly prolonged the escape latency in the navigation test and shortened the adjusted escape latency. The mRNA expressions of APP and GSK-3β in rat hippocampus were increased by LPS. NDP (80 and 160 $mg \cdot kg^{-1} \cdot d^{-1}$) treatments significantly decreased LPS-induced these cytokines at the mRNA levels. Furthermore, NDP attenuated the ability of spatial learning and memory deficits induced by LPS. **Conclusion:** NDP has the protection against rat learning and memory deficits induced by LPS, and this effect may be related to the down-regulation of APP and GSK-3β in hippocampus.

Keywords: noble dendrobium polysaccharides; lipopolysaccharide; learning and memory; APP; GSK-3β; rat

S10.72

Activated mTOR/p70S6K pathway is involved in the cognitive dysfunction of STZ-induced diabetic rats

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Aim: Hyperphosphorylation of tau mediated by mTOR/p70S6K pathway has been thought to contribute to cognitive dysfunction of Alzheimer's disease. Whether mTOR/p70S6K signaling is also involved in cognitive dysfunction of diabetes mellitus still remains unknown. **Methods:** The alteration of mTOR/p70S6K signaling and phosphorylated tau in the hippocampus of STZ-induced diabtetic rats was detected by Western blot at 10, 15, and 20 weeks after STZ injection. The neuronal apoptosis and the expression of Bcl-2 and Bax in the hippocampus were measured by TUNEL and Western blot. The cognitive function was assessed by Morris water maze test. **Results:** A mild cognitive dysfunction was observed at 10 weeks after STZ injection and reached a peak at 15 weeks after STZ injection compared with those of the control group. The phosphorylation of mTOR, p70S6K and tau in the hippocampus of diabetic rats increased slightly at 10 weeks after STZ injection, and showed a significant increase at 15 and 20 weeks after STZ injection. Apoptotic neurons in the hippocampus were significantly increased concomitant with up-regulation of Bax and down-regulation of Bcl-2 expression at 15 and 20 weeks after STZ injection. **Conclusion:** Aberrantly activated mTOR/p70S6K signaling and hyperphosphorylated tau may be related to the neuronal apoptosis in the hippocampus which contributes to the occurrence of diabetes-related cognitive dysfunction.

Keywords: mTOR; p70S6K; tau; cognitive dysfunction

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S10.73

Pinocembrin protects rat against cerebral ischemic injury through soluble epoxide hydrolase and epoxyeicosatrienoic acids (EETs)

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Aim: To investigate the relationship between cerebroprotection of pinocembrin and epoxyeicosatrienoic acids (EETs) and their regulating enzyme soluble epoxide hydrolase (sEH). **Methods:** Rats underwent middle cerebral artery occlusion (MCAO) to mimic permanent focal ischemia, and pinocembrin was administered via tail vein injection at 10 min, 4, 8, and 23 h after MCAO. After 24 h MCAO, rats were re-anesthetized, and the blood and brain were harvested and determined. **Results:** Pinocembrin displayed significant protective effects on MCAO rats indicated by reduced neurological deficits and infarct volume. Importantly, co-administration of 14,15-EEZE (0.2 mg/kg), a putative selective EET antagonist, weakened the beneficial effects of pinocembrin. 14,15-EET levels in the blood and brain of rats after 24 h MCAO were elevated at the presence of pinocembrin. In assay for hydrolase activity, pinocembrin significantly lowered brain sEH activity of MCAO rats and inhibited recombinant human sEH activity in a concentration-dependent manner (IC_{50}=2.58 μmol/L). In addition, Western blot and immunohistochemistry analysis showed that pinocembrin at the doses of 10 and 30 mg/kg significantly down-regulated sEH protein in brain, especially hippocampus CA1 region of MCAO rats. **Conclusion:** Inhibiting sEH and then increasing the potency of EETs may be one of the mechanisms through which pinocembrin provides cerebral protection.

Keywords: pinocembrin; cerebral ischemic injury; epoxyeicosatrienoic acids (EETs); soluble epoxide hydrolase (sEH)

S10.74

Neuroprotection of saponins from *Panax japonicus* against *D*-galactose-induced senescence via activation of Nrf2/ARE pathway

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Aim: Increasing evidence indicates that oxidative stress plays a key role in the senescent development. NF-E2-related factor 2 (Nrf2) is currently considered the master regulator of redox homeostasis through antioxidant response element. *Panax japonicus*, in which the saponins are proved to make up the active major constituents, is a medicinal plant widely used in folk and traditional medicine for its antioxidative activity. The present study was to investigate the neuroprotective effects of saponins from *Panax japonicus* (SPJ) and the potential mechanisms. **Methods:** Male SD rats weighing 220 to 250 g were administered a subcutaneous injection of D-galactose (250 mg/kg per day) and treated with SPJ by daily oral gavage until sacrificed 8 weeks later for behavioral analysis, pathological evaluation, real-time PCR and Western blotting. **Results:** Our results showed that SPJ improved spatial memory performances of aged rats in the water maze as evidenced by decreasing neuronal morphologic abnormalities in hippocampus region and elevating SOD and GSH-Px activities in the serum and brain. Furthermore, our results also showed that SPJ promoted the hippocampal Nrf2 nuclear translocation and enhanced the expressions of Nrf2-dependent antioxidant enzyme genes such as HO-1 and NQO-1 in hippocampus tissue in D-gal-treated rats. **Conclusion:** Our results indicated that SPJ had a potential protect effect on

brain aging induced by D-gal and this effect is at least in part, via modification of Nrf2 antioxidant pathway.

Keywords: saponins from *Panax japonicus*; neuroprotection; Nrf2 signaling

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S10.75

Effects and mechanisms of Wnt signaling pathway on neurogenesis in rats post ischemic brain injury

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Aim: Ischemic brain injury is the leading cause of cerebrovascular disease, which is one of the three diseases of highest incidence. Complex pathology could be induced by ischemic brain injury, therefore leading to diseases such as stroke, vasculardementia and epilepsy. Simultaneously, cerebral ischemia can stimulate stem cell proliferation at low degree, which may not be sufficient for neurorestoration from stroke. Our previous research indicated Wnt signaling pathway might be involved in this process, although most studies of this signaling pathway focus on its effects on the development of embryos and oncology. **Results:** The results of the present study showed that MNG could improve neurological function 3 d post ischemic brain injury, and could alleviate infarct degree which is detected by using *Tesla* MRI. Limited to 1 d post ischemic injury, the expression of Wnt3a was increased in the ischemia-treated rats compared to the sham-operated group. However, MNG treatment at the high dose increased the number of Wnt3a-positive cells in the ischemic ipsilateral side, and made this enhencement last for 7 d. Similar effects of MNG were also observed on the number of cells expressed Pax6, Tbr1, Tbr2, Ngn2, β-catenin, Dvl. **Conclusion:** The results suggest that Wnt signaling pathway could be activated by cerebral ischemia, and this activation might be involved in the neurorestorative mechanisms of MNG in ischemic brain injury.

Keywords: ischemic brain injury; neurogenesis; Wnt signaling pathway

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S10.76

Myricitrin protects against methylglyoxal-induced mitochondrial dysfunction in SH-SY5Y cells via trapping methylglyoxal to inhibit advanced glycation end products formation

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Aim: Methylglyoxal (MGO), a dicarbonyl metabolite, is a major precursor of advanced glycation end products (AGEs). Myricitrin is a flavanoid isolated from the root bark of Myrica cerifera. The present study was to evaluate the effect of myricitrin against MGO-induced cytotoxicity in SH-SY5Y cells and further investigate the mechanism. **Methods:** MGO was added to cultured SH-SY5Y cells after pretreatment for 1 h with different concentrations of myricitrin and co-incubated for another 24 h. The morphological change was observed by microscope and the viability of cells was assayed by MTT method. At the same time, the mitochondrial oxidation-reduction activity of cells was measured by resazurin, the mitochondrial membrane potential was assayed by JC-1 staining, reactive oxygen species (ROS) production in SH-SY5Y cells was measured by H_2DCF-DA, and cellular ATP level was determined using an ATP Bioluminescent Assay. The advanced glycation end products formation from MGO was monitored by fluorescence assay. **Results:** MGO-induced cytotoxicity and mitochondrial dysfunction can be prevented by myricitrin. Furthermore, myricitrin significantly reduced advanced glycation end products formation in a dose-dependent manner *in vitro* and the IC_{50} was 270.6±16.30 μmol/L. **Conclusion:** Myricitrin could alleviate MGO-induced mitochondrial dysfunction in SH-SY5Y cells by trapping MGO to inhibit AGEs formation. Myricitrin maybe offer a promising strategy in diabetic complications and neurodegenerative diseases.

Keywords: myricitrin; methylglyoxal; advanced glycation end products (AGEs); mitochondria; SH-SY5Y cells

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S10.77

Toll-like receptor 4 (TLR4) increases expression of inflammatory mediators in primary sensory neurons via activation of a MyD88-dependent signaling pathway

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Aim: To characterize Toll-like receptor 4 (TLR4) activity in rat dorsal root ganglion (DRG) cells. **Methods:** Acutely dissociated DRG cells were isolated from adult SD rats, and plated on poly-DL-ornithine and laminin-coated tissue culture plates. After 2 d *in vitro*, expression of the TLR4 signalling complex and lipopolysacharride (LPS)-mediated changes in inflammatory mediators were studied. **Results:** 65%± 5% of neurons, but no glial cells, showed cell surface expression of TLR4-ir and co-expression with CD14 and MD-2. LPS produced a TLR4-dependent increase in the expression of IL-1β and TNFα mRNA immediately preceding expression of COX-2 mRNA, with no increase in IFNβ mRNA. These results suggest a role for MyD88-dependent, but not MyD88-independent, signalling in DRG neurons. Although inhibition of NF-κB attenuated LPS-stimulated cytokine expression by 73%, COX-2 expression only decreased by 32%, suggesting an additional route for activating COX-2 gene expression following TLR4 activation. **Conclusion:** DRG neurons express functionally active TLR4 which signals via the MyD88-dependent cell signalling pathway to regulate expression of mediators of inflammation.

Keywords: DRG; TLR4

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S10.78

An effective solution to discover synergistic drugs for anti-cerebral ischemia from traditional Chinese medicinal formulae

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Aim: Combination therapies have been used for more than 2500 years in prescriptions, called formulae, in traditional Chinese medicine (TCM). TCM is gaining attention as a source of new drugs; however, perceived deficiencies in scientific validation of TCM's efficacy impede development of TCM. Understanding optimal drug proportions and synergistic mechanisms of multi-component drugs are critical for developing novel strategies to cope with complex diseases. **Methods:** A new multi-objective optimization algorithm based on partial least squares combined with least angle regression was proposed to construct the predictive model to evaluate synergistic therapeutic effect of the three components, ginsenosides (G), berberine (B) and jasminoidin (J), of a novel combination drug, called Yi-qi-jie-du formula (YJ) in rats impaired by middle cerebral artery occlusion (MCAO)-induced focal cerebral ischemia. This formula came from a TCM prescription which showed the significant clinical effect in treatment of encephalopathy. Optimal proportion of the three was determined via particle swarm optimum. Furthermore, the combination mechanisms were interpreted using PLS VIP and principal components analysis combined with analysis of variance. **Results:** The results showed that YJ had optimal proportion of 3(G): 2(B): 0.5(J), and it yielded synergy in the treatment of adult male Sprague–Dawley rats impaired by middle cerebral artery occlusion induced focal cerebral ischemia. YJ administered under optimal proportion had good pharmacological effects on acute ischemic stroke. The combination mechanisms study demonstrated that the combination of G, B and J could exhibit the strongest synergistic effect. J might play an indispensable role in the formula, especially when combined with B for the acute stage of stroke. All these data in this study suggested that in the treatment of acute ischemic stroke, besides restoring blood supply and protecting easily damaged cells in the area of the ischemic penumbra as early as possible, we should pay more attention to the removal of the toxic metabolites at the same time. Mathematical system modeling may be an essential tool for the analysis of the pharmacological effects of multi-component drug to highlight the complex relationship between drugs and their targets. A powerful mathematical analysis method could greatly

improve the efficiency in finding new combination drug from TCM.

S10.79

Pinocembrin protects brain against ischemia/reperfusion injury by attenuating endoplasmic reticulum stress induced apoptosis

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Aim: Endoplasmic reticulum stress (ER stress) is known to play a vital role in mediating ischemic reperfusion damage in brain. In this study an attempt was made to investigate the modulatory effect on ischemia/reperfusion-induced ER stress of pinocembrin. **Methods:** Focal cerebral ischemia/reperfusion was induced by middle cerebral artery occlusion (MCAO) for 2 h followed by 6 h reperfusion. Pinocembrin was administered in different doses (1, 3, and 10 mg/kg) at the same time of onset of reperfusion. Neurological function and brain infarction were evaluated. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method, and flow cytometer (FCM) were used to investigate cell apoptosis in penumbra cortex. DNA fragmentation assay was also performed using electrophoretic analysis. The expression of ER stress proteins of GRP78, CHOP/GADD153, ATF4, eIF2α phosphorylation was detected by Western blot, and caspase-12 was evaluated by immunohistochemical analysis. **Results:** Our results demonstrate that pinocembrin treatment (3 and 10 mg/kg) significantly reduced neurological deficit scores, infarct volume and neuron apoptosis in the rats. It can also significantly modulate the protein levels by increasing GRP78 (10 mg/kg) and attenuating CHOP/GADD153 expression along with caspase-12 activation (3 and 10 mg/kg). At the same time, eIF2α phosphorylation was restrained and the expression of ATF4 was reduced (3 and 10 mg/kg). **Conclusion:** These results suggest that the attenuation of ER stress may be involved in the inhibitory effects of pinocembrin on neuron apoptosis.

Keywords: endoplasmic reticulum stress; focal cerebral ischemia reperfusion; pinocembrin

S10.80

Aquaporin 4 involves in neuropathic pain through spinal astrocytes activation

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Aim: Spinal glias, including astrocytes and microglias, play an important role in neuropathic pain. Aquaporin 4 (AQP4), the predominant water channel exists in astrocytes, not only permeates water, but also regulates extracellular K^+ homeostasis and glutamate clearance. In the present study, the role of AQP4 in neuropathic pain was investigated. **Methods:** Effects of inhibiting AQP4 function or knocking-out AQP4 gene on acute pain and chronic pain, including neuropathic pain, were observed. Then, whether AQP4 was involved in astrocytes or microglias activation was detected. **Results:** AQP4 knockout or functional inhibition by acetazolamide, a non-selective AQP4 inhibitor had no effect on the acute pain induced by thermal, chemical and mechanical stimuli; however, it attenuated chronic pain, including spared nerve injury (SNI)-induced neuropathic pain and complete Freund's adjuvant-induced inflammatory pain. In SNI model, the changes of spinal AQP4 expression were parallel to astrocytes activation and proinflammatory cytokines release, but not to microglias activation during neuropathic pain processes; whereas AQP4 deficiency abolished astrocytes activation and proinflammatory cytokines release. Furthermore, *in vitro* study revealed that AQP4 was directly involved in astrocytes activation induced by divers stimulating factors via its function of water permeation. **Conclusion:** Our findings firstly demonstrated that AQP4 appears to contribute to chronic pain, but not acute pain. The role of AQP4 in neuropathic pain is due to its involvement in spinal actrocytes activation.

Keywords: aquaporin 4; neuropathic pain; astrocytes activation

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S10.81

H_2S regulates contextual fear memory formation via a protein S-sulfhydration-dependent mechanism

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Aim: Recent advances in redox biology show that Cys residues can be sulfhydrated by hydrogen sulfide (H_2S). Sulfhydration of Cys residue can signal predominantly by altering protein function and mediate various biological processes. However, its role in central nervous system (CNS) is largely unknown. Here we adopted behavioral and eletrophysiological paradigms to test the role of H_2S-linked *S*-sulfhydration in learning and memory. **Methods:** Fear conditioning tasks, measurement of H_2S, electrophysiological recording, modified biotin switch assay for sulfhydration were performed. **Results:** We found that contextual fear conditioning tripled H_2S generation in the hippocampus of rats. Exogenous administration of H_2S donor dose-dependently enhanced, whereas inhibition of H_2S production prevented, contextual fear memory formation. Interestingly, H_2S regulated NMDAR currents via a sulfhydration-dependent manner. Furthermore, H_2S also sulfhydrated and inactivated protein phosphatase type 2A (PP2A), which may be responsible for H_2S-mediated activation of postsynaptic signal pathways that control NMDAR activity. **Conclusion:** Together, our results demonstrated a pivotal role for H_2S-linked sulfhydration in NMDAR-dependent hippocampal memory, providing information for further understanding the redox control of memory formation.

Keywords: hydrogen sulfide (H_2S); sulfhydration; contextual fear conditioning; hippocampus; NR2A-containing NMDA receptor; long-term potentiation (LTP)

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S10.82

Acidic polysaccharose CA4-3 inhibits amyloid β1-42 induced BV2 microglia chemotaxis *in vitro*

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Aim: Acidic polysaccharose CA4-3, extracted from Liuwei Dihuang decoctions, is comprised of galacturonic acid and glycuronic acid, in which the content of aldonic acid is about 96%. In our previous study, CA4-3 was found to improve learning and memory dysfunction in senescence-accelerated mice (SAM) prone 8 (SAMP8) substrain. Further studies revealed that CA4-3 inhibits lipopolysaccharide (LPS)-induced tumor necrosis factor α (TNF-α) secretion in microglia from neonatal mice. This effect might be connected at least partially with the inhibition of activation of nuclear factor κB (NF-κB) and p38. To further explore the effect of CA4-3 on neuroinflammatory reaction, in this study, we investigated the effect of CA4-3 on amyloid β (Aβ) 1-42 induced BV2 microglia chemotaxis *in vitro*. **Methods:** BV2 microglia chemotaxis was detected by xCELLigence system (Roche Diagnostics GmbH). This system bases on dynamic, impedance-based cell analyzer technique. Multiple chemokines secretion in the supernatants was detected with Luminex (Millipore). **Results:** CA4-3 itself could induce BV2 cell chemotaxis, but when Aβ 1-42 is present, it inhibited BV2 cell chemotaxis significantly at 1–100 μg/mL. Then we measured MCP-1, MIP-1α, MIP-1β, RANTES, VEGF, Exodus-2, MCP-5, Fractalkine and IP-10 in the supernatants. It was shown that CA4-3 could decrease MCP-1 and Exodus-2 secretion, but it had little effect on other chemokine secretion in our experiment. **Conclusion:** Acidic polysaccharose CA4-3 could inhibit Aβ 1-42 induced BV2 microglia chemotaxis. Reduction of MCP-1 and Exodus-2 secretion might play an important role in the effect.

Keywords: microglia; BV2; chemotaxis; amyloid β

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S10.83

Effects on the expression of NR2B and PSD-95 in rat cortical neurons by HIV-1 gp120

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Aim: To investigate the mechanism underlying the toxicity of cortex neurons by the glycoprotein 120 of HIV-1. **Methods:** This study applied immunocytochemistry

and Western blot technique to investigate the changes of the expression of NR2B and PSD-95 in primary cortex neurons, which was exposed to gp120. **Results:** MTT assay showed gp120 had the toxic effect on cortex neurons and the effect could be blocked by memantine. Further results showed that gp120 up-regulated NR2B and down-regulated PSD-95 protein. **Conclusion:** The results suggest that NR2B and PSD-95 are involved in HIV-associated dementia. NR2B and PSD-95 are potential targets for prevent HAD.

Keywords: gp120; HAD; NMDAR; PSD-95

S10.84

The mechanisms of Rongshuan Capsule improving cognitive function induced by acute cerebral hypoxia ischemia in C57 mice

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Aim: Rongshuan capsule is made from *Pheretima asperfillm,* which is reported to improve children' recovery with cerebral palsy in clinical cases. In this study, the effects of Rongshuan capsule on cognitive function of mice induced by acute cerebral hypoxia ischemia (HI) and the action mechanism were investigated. **Methods:** C57 mice were subjected to left carotid artery ligation and mixed gas of O_2 and N_2 (8:92) for 15 min. Rongshuan capsule at different doses (100, 200, or 400 mg/kg) or saline were administered ig at 24 h after HI. Long-term outcomes were measured by water-maze performance and degree of cerebral injury at 30 d after HI. The expression of neuron specific enolase (NSE) and microtubule-associated protein (MAP)-2 and caspase-3 in brains were examined. Meanwhile, regional cerebral blood flow (r-CBF) was assessed when Rongshuan capsule at different doses (100, 200, or 400 mg/kg) or saline were duodenally administered at 10 min after HI. **Results:** A significant increase in r-CBF was observed in Rongshuan capsule group compared with saline. Immunostaining revealed MAP-2 expression was up-regulated and caspase-3 as well as NSE expression was down-regulated in injured brain by Rongshuan capsule (200 and 400 mg/kg) compared with saline. Water-maze performance showed that saline-treated mice demonstrated significantly prolonged latency time and path length to locate a submerged platform using peripheral navigational cues compared with Rongshuan capsule-treated mice (200 and 400 mg/kg). **Conclusion:** Rongshuan capsule plays an important role in the improvement of cognitive function following acute cerebral HI in mice. Rongshuan capsule can elevates r-CBF in acute cerebral HI injury via inhibiting neural injury and apoptosis.

Keywords: Rongshuan capsule; cognitive function; acute cerebral hypoxia ischemia; mechanisms; C57 mice

S10.85

Expression of A30P mutant α-synuclein impairs macroautphagy in PC 12 cells

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Aim: α-Synuclein is the major component of Lewy body which is the pathological hallmark of Parkinson's disease (PD). Recent studies indicated that A30P mutation of α-synuclein causes familial PD. In this study, we investigated the effect of over-expression of A30P α-synuclein mutant on marcoautophagy pathway in PC12 cells. **Methods:** Stable PC12 cell lines expressing A30P mutant human α-synuclein were generated. Macroautophagy related proteins LC3II and p62 levels were detected by Western blot. **Results:** PC12 cell lines stable expressing A30P mutant human α-synuclein showed dramatically decreased LC3 II compared with the control group. Meanwhile, the level of macroautophagy substrate p62 was significantly increased in cell lines expressing A30P mutant human α-synuclein compared to the control group. **Conclusion:** Expression of A30P mutant human α-synuclein impairs macroautophagy in PC12 cells.

Keywords: A30P mutant; α-synuclein; macroautophagy

S10.86

Molecular imaging of disease-related pathology in neurodegenerative disorders

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Aim: Alzheimer's disease (AD) and other neurodegenerative dementia belong to a family of protein mis-folding diseases. These diseases are characterized by the accumulation of insoluble protein aggregates containing an enriched β-sheet structure. AD is pathologically characterized by the extensive deposition of amyloid-β (Aβ) plaques and neurofibrillary tangles. Aβ is a 39–43 amino acid protein product derived from the proteolytic cleavage of the amyloid precursor protein. The abnormal accumulation of Aβ has been implicated as an initial event in the pathogenesis of AD and precedes the cognitive deterioration. Noninvasive monitoring of Aβ plaques promises to be a useful technique for the pre-symptomatic detection of AD-related pathology and for the preventive intervention and assessment of therapeutic effects. **Methods:** For this purpose, many β-sheet binding radiotracers for molecular imaging have been developed for positron emission tomography (PET). **Results:** Amyloid PET studies in human subjects have shown a robust difference between the retention pattern in AD patients and healthy controls. Abnormal tracer retention in the neocortical areas predicts the progression of cognitive decline in the subjects with mild cognitive impairment and cognitively normal individuals. In contrast with the successful imaging of Aβ plaques, no specific radiotracer is available for *in vivo* imaging of tau and α-synuclein fibrils. Thus, an increasing interest has been focused on searching for novel imaging probe targeting at these protein fibrils. In this talk, our recent progress will be presented in developing novel molecular imaging technology for early detection of disease-specific pathology including Aβ, α-synuclein and tau in the brain.

Keywords: PET; amyloid Aβ; α-synuclein; tau protein; molecular imaging

S10.87

Protective effects of NAD against amyloid beta-peptide neurotoxicity in rat cortical neurons

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Aim: One major pathological hallmark of Alzheimer's disease (AD) is the aggregation of amyloid beta-peptide (Aβ), which could induce neuronal apoptosis. Nicotinamide adenine dinucleotide (NAD) is an important cofactor for cellular energy production and signal transduction. However, whether NAD replenishment may exert neuroprotection against Aβ toxicity and, if so, its underlying mechanisms remain unknown. Therefore, the aim of this study is to test the following hypothesis: NAD confers neuroprotection against Aβ-induced DNA damage in cortical neurons. **Methods:** Primary rat cortical culture was treated with $A\beta_{25-35}$ or $A\beta_{1-42}$ to mimic AD *in vitro.* Cell survival was determined by MTT assay, Hoechst staining, and immunocytochemistry. The extents of DNA fragmentation were quantified by the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining. DNA single-stranded break was quantified by the DNA polymerase I-mediated biotin-dATP nick-translation (PANT) assay. **Results:** We found that NAD at 50 mmol/L enhanced neuronal survival against Aβ toxicity and reduced the extents of DNA fragmentation and DNA single-strand breaks in primary rat cortical culture. **Conclusion:** These results provide evidence that exogenous NAD has neuroprotective effects against Aβ-induced DNA damage *in vitro.*

Keywords: Alzheimer's disease; apoptosis; DNA damage; DNA fragmentation; oxidative stress

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S10.88

Role of astrocyte-released neutrophin-3 in a mouse model of Fragile X syndrome

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Fragile X syndrome (FXS) is a neurodevelopmental disorder caused by lack of fragile X mental retardation protein (FMRP) due to the mutation of the fragile X mental retardation 1 (*Fmr1*) gene. Recent studies suggest the role of astrocytes in neuronal growth. However, the mechanisms involved in the regulation process of astrocytes from FXS remain unclear. In this study, we found that astrocytes derived from a Fragile X model, the *Fmr1*-knockout (KO) mouse which lacks FMRP expression, inhibit the proper elaboration of dendritic processes of neurons *in vitro*. Furthermore, astrocytic conditioned medium (ACM) from KO astrocytes inhibits proper dendritic growth of both wild type (WT) and KO neurons. Inducing expression of FMRP by transfection of FMRP vectors in KO astrocytes restored dendritic morphology and levels of synaptic proteins. Further experiments revealed that KO ACM contained elevated levels of the neurotrophin-3 (NT-3). Addition of high concentration of exogenous NT-3 to culture medium reduced dendrites of neurons and synaptic protein levels, whereas knockdown of NT-3 expression in KO astrocytes by shRNAs normalized these measures. Finally, we demonstrated that excessive astrocyte-derived NT-3 in KO ACM exhibited elevated levels of two oxidative stress measures, MDA and ROS, and that knockdown of

NT-3 partially restored levels to that seen in WT ACM. This study indicates that excessive NT-3 from astrocytes contributes to the neuronal developmental disorder of FXS and astrocytes could be a potential therapeutic target for FXS.

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S10.89

Antidepressive mechanism of phosphodiesterase4D4/5 knock-down in a chronic stress mice model

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Aim: Elevating intracellular cAMP via inhibition of cyclic AMP-specific phosphodiesterase-4 (PDE4) produces antidepressant-like effects in animal model. Our previous studies indicated the antidepressant-like effects of PDE4D4/5 knock-down (4DmiR) in unimpaired mice. Therefore, the aim of present research is to investigate whether 4DmiR of the prefrontal cortex mediate antidepressant-like effects and explore the mechanism of action in chronic stress mice. **Methods:** Fifty male BALB/c mice were randomly divided into five groups: 1) NC+Vehicle (Veh; 2.5% DMSO) group; 2) NC+Veh+chronic stress (CUS) group; 3) NC+rolipram (Rol, 1.25 mg/kg)+CUS group; 4) 4DmiR+Veh+CUS group; 5) 4DmiR+Rol+CUS group. Lentiviral vectors containing NC or 4DmiR were infused into prefrontal cortex at a rate using a microinjection pump before CUS. Mice were given chronic stress for 4 weeks except non-chronic stress groups. The sucrose preference test and serum corticosterone level were regarded as the success criteria for model creation. The tail-suspension test (TST) and the forced-swim test (FST) and the novelty suppressed feeding (NSF) test were used to detect antidepressant-like effects. Levels of PDE4D3/4/5, p-CREB, and p-ERK in the prefrontal cortex were detected by Western blotting. Intracellular cAMP concentration and serum corticosterone level were respectively analyzed by time-resolved FRET immunoassay and enzyme-linked immunosorbent assay. **Results:** Microinfusions of 4DmiR lentiviral vectors down-regulated PDE4D4 and PDE4D5 and exhibited antidepressant-like effects in the chronic stress mice model, however rolipram treatment did not affect that actions. Western blot analysis demonstrated that CUS significantly decreased the protein levels of p-CREB and p-ERK, whereas 4DmiR treatment significantly reversed this effect. CUS mice had the decreased cAMP concentration in the prefrontal cortex, and this effect was reversed by 4DmiR and/or rolipram treatments. These results suggested that knock-down PED4D4/5 in the prefrontal cortex produced antidepressant-like effects on behaviors by up-regulating cAMP signaling and MAPK signaling. **Conclusion:** These results suggest that PDE4D4/5 splice variants play an essential role in the antidepressant-like effects of rolipram and the mechanism may be related to cAMP signaling pathway and MAPK signaling pathway. PDE4D4/5 splice variants may be the promising targets for the development of PDE4 variant-selective inhibitors as the new pharmacotherapies for depressive disorders.

Keywords: PDE4D4/5 konck-down; antidepression; chronic stress

S10.90

Epigenetic modulation of coping strategy to stress

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After repetitive exposure to the same stressor, varied behavioral responses can occur and are often maintained for a long time. We investigated epigenetic modulation of individual differences in coping strategies established in response to stressful stimuli in rats. We focused on the role of mGluR5 in this study and discovered that repeated exposure to restraint stress caused variably altered mGluR5 protein expression levels in the hippocampus. The low mGluR5 protein expression group showed increased methylation sites on the CpG island of the mGluR5 gene, decreased mGluR5 mRNA expression, and unaltered basal theta electroencephalogram power and corticosterone blood concentrations, suggesting positive behavioral adaptation. In contrast, the high mGluR5 protein expression group showed the opposite results, suggesting negative adaptation. These individual differences were abolished by injection with the mGluR5 antagonist MPEP. This study suggests that coping strategies to a facing stressor are critically regulated by epigenetic modulation of the mGluR5 gene.

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S10.91

Protective effect of uric acid on cerebral ischemia and its regulatory action on NOS and XO

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Aim: To investigate the neuro-protective effects and underlying mechanisms of uric acid on rat cerebral ischemia/reperfusion injury *in vivo*. **Methods:** Middle cerebral artery occlusion was introduced as an *in vivo* ischemic model in male SD rats. After ischemia impairment, uric acid (6.25 mg/100 g per day, 9.375 mg/100 g per day) was administered by ip for 7 d in treated group. Neurological deficits evaluations were carried out at 6 h after operation. TTC staining method was used to determine the area of cerebral infarction. Levels of MDA and xanthine oxidase (XO), activity of SOD and glutathione peroxidase (GPX) in cortex were detected by spectrophotometry. Expressions of nNOS, eNOS, iNOS, and XO mRNA were measured by RT-PCR techniques. **Results:** 1) Uric acid improved neurologic scores and reduced infarct area suffered from ischemia/reperfusion. 2) A decline of the activity of SOD and GPX and an elevation in the level of MDA and XO were shown in ischemia group, uric acid reversed these changes. 3) The expression of nNOS, iNOS and XO mRNA were increased significantly in ischemia group, while eNOS mRNA was decreased. Uric acid down-regulated the expression of nNOS, iNOS and XO mRNA, but up-regulated the eNOS mRNA expression. **Conclusion:** Uric acid can significantly alleviated cerebral ischemia/reperfusion injury, its mechanism may be related to the inhibition of oxidative stress, the down-regulation of XO, iNOS, and nNOS mRNA expression, up-regulation of eNOS mRNA during cerebral ischemia/reperfusion .

Keywords: uric acid; cerebral ischemia; NOS; XO

S10.92

Tenuifoliside A inhibits corticosterone-induced neurotoxicity of rat glioma cells via ERK pathway

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Aim: To investigate the neuroprotective effects of Tenuifoliside A (TFSA) on corticosterone-induced injury in rat glioma cells and explore the potential mechanism. **Methods:** The injury model was established by treating C6 cells with 0.8 mmol/L of corticosterone (CORT) by 48 h; C6 cells were pretreated with CORT for 30 min followed by co-treatment with 30, 10, and 3 μmol/L of TFSA for 48 h. Cell viability was detemined by Cell Counting Kit (CCK) assay; Hoechst 33258 staining was used to identify the neuroprotective effects of TFSA against neurotoxicity induced by CORT; the method of Western blot was used to detect the expression of ERK, Bcl-2 and Bax protein. Caspase-3 activity as a specific apoptotic marker in C6 cells exposed to CORT alone or co-treated with TFSA was also assayed. **Results:** Compared with the normal control group, CORT significantly decreased the viability of C6 cells to 69.58% (P<0.01). Western blot results showed that the protein levels of ERK and Bcl-2 decreased significantly. In contrast, the expression of Bax and the activity of caspase-3 increased markedly (P<0.01). However, TFSA can increase the viability of C6 cells damaged by CORT up to 84.48% (P<0.01). Moreover, TFSA increased the protein level of ERK, Bcl-2 and decreased the protein level of Bax and the activity of caspase-3 in C6 cells exposed to CORT (P<0.01). **Conclusion:** TFSA protects C6 cells from CORT-induced damage, and the neuroprotective mechanism may be associated with the activation of Bcl-2 and suppressing Bax and the activity of caspase-3 via ERK pathway.

Keywords: tenuifoliside A; *Polygala tenuifolia*; ERK; apoptosis; neuroprotective; C6 cells

S10.93

Antinociceptive effects of oxymatrine from Sophora flavescens, through regulation of NR2B-containing NMDA receptor-ERK/CREB signaling in a mice model of neuropathic Pain

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Aim: In this study we investigated anti-nociceptive effects of oxymatrine through regulation of NR2B-containing NMDA receptor-ERK/CREB signaling in a chronic

neuropathic pain model induced by chronic constrictive injury (CCI) of the sciatic nerve. **Methods:** The von Frey and plantar tests were performed to assess the degree of mechanical and thermal changes, respectively. Immunohistochemistry assay was used to evaluate the expressions of NR2B. Western blotting assay were used to evaluate the expressions of NR2B, tERK, p-ERK, tCREB, and p-CREB. **Results:** The intraperitoneal administration of OMT (160 and 80 mg/kg) could prevent the development of mechanical allodynia and thermal hyperalgesia induced by CCI. Intraperitoneal administration of OMT decreased the mean IOD of NR2B in the dorsal horn and expression of NR2B, p-ERK, and p-CREB protein. **Conclusion:** Regulation of NMDA NR2B receptor-ERK/CREB signaling may be the targets for the antinociceptive effects of OMT on a chronic neuropathic pain model induced by CCI of the sciatic nerve.

Keywords: oxymatrine from *Sophoraflavescens*; NR2B; p-ERK; p-CREB; neuropathic pain

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S10.94

Effect of 3'-daidzein sulfonate sodium on gene expression profile of cerebral ischemia-reperfusion injury in rats

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Aim: To investigate the effect of 3'-daidzein sulfonate sodium (DSS) on gene expression profile of ischemia-reperfusion injury in rat brain. **Methods:** Male SD rats were randomly divided into sham operation group, cerebral ischemia-reperfusion injury model group and DSS treatment group. The total RNAs were extracted respectively from the rat brain in each group. All the RNAs were purified into mRNA and then reverse-transcribed into double-stranded cDNAs. The cDNAs was used for the Illumina rat genome-wide chip hybridization. The changes in the gene expression profile among three groups were analyzed by using bioinformatics. **Results:** According to the cDNA microarray analysis, a total of 1868 differential genes were found between cerebral ischemia-reperfusion injury model group and DSS treatment group, of which 1084 were up-regulated and 784 were down-regulated. DSS caused multiple gene expression changes, especially for oxidative stress-related gene and nerve growth-related genes, which can be restored to normal tissue expression level. **Conclusion:** The study suggests that DSS may play a role in the signal transduction pathways related to oxidative stress and nerve growth. It will be helpful to looking for the drug target genes and further research on the molecular mechanism of DSS.

Keywords: 3'-daidzein sulfonate sodium; cerebral ischemia-reperfusion injury; gene expression profile; gene chip

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S10.95

Rescuing the ischemic brains by inducing ER stress

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Aim: The contributions of endoplasmic reticulum (ER) stress in cerebral ischemia/reperfusion (I/R)-induced injury are under debate. In the present study, the effects of ER stress inducers tunicamycin (TM) and thapsigargin (TG) on ischemic brains were investigated. **Methods:** TM and TG at different dosages were administered to transient middle cerebral artery occluded (tMCAO) mice and oxygen-glucose deprivation/reperfusion (OGD/Rep) treated neurons. The brain infarct volumes and cell apoptosis ratio were determined by TTC staining and TUNEL assay, respectively. In primary cultured neurons, the eIF2α was knocked down by shRNA. **Results:** Both TM and TG showed significant protection against ischemia-induced brain injury *in vivo* and *in vitro*, as revealed by reduced brain infarct volume and apoptosis ratio. In OGD/Rep-treated neurons, 4-PBA, the ER stress releasor, counteracted the neuronal protection of TM and TG. Further, the eIF2α silencing almost completely blocked the neuroprotection of TM and TG. **Conclusion:** Taken together, the present investigation suggested a neuroprotective role of ER stress in transient cerebral ischemia, and shed light on the possiblity to treat transient cerebral ischemia by enhancing ER stress during recovery.

Keywords: cerebral ischemia; ER stress; tunicamycin; thapsigargin; eIF2α

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S10.96

The effect of ginkgolides on Alzheimer's Disease-like model rats and its mechanism

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Aim: In this study an animal model of AD was established and the effect and mechanism of ginkgolides on the AD-model rats was investigated. **Methods:** Okadaic Acid (OA) was injected into hippocampal CA1 region of rats every two days for four times to establish Alzheimer's Disease-like model, then ginkgolides was injected into the abdominal cavity. The learning and memory capabilities of rats was recorded through Morris Water Maze behavioral test. The senile plaques (SP) and neurobrillary tangles (NFT) of the rats hippocampus were observed by Bielschowsky stain. The expression of β-amyloid protein ($A\beta_{1-40}$) and neuronal nitric oxide synthase (nNOS) of the rats hippocampus were observed by immunohistochemistry. **Results:** Compared with model rats, the rats after three weeks treatment with ginkgolides showed that: (1) Their average escape latency was shortened in place navigation test ($P<0.05$ or $P<0.01$) and their swimming time in the third quadrant in spatial probe test was prolonged after the platform was removed ($P<0.01$). It indicates that ginkgolides improved the learning and memory capabilities of rats. (2) SP and NFT were reduced or disappeared in hippocampal CA1 region of the rats. (3) Expression of $A\beta_{1-40}$ in hippocampus CA1 region was reduced or disappeared ($P<0.01$). (4) Expression of nNOS in hippocampus CA1 region was increased ($P<0.05$). **Conclusion:** Ginkgolides can alleviate the damage of hippocampus neurons induced by OA and improve the learning and memory capabilities of the model rats, possibly because they reduce the formation of $A\beta_{1-40}$ and inhibit the formation of SP and NFT. They accelerate the expression of nNOS and synthesization of NO in hippocampus and protect the neurons of hippocampus.

Keywords: Alzheimer's disease; ginkgolides; learning and memory; amyloid beta-protein; nitric oxide synthase

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GanDanLiangYiTang exerts sedative and hypnotic activities by elevating amino acids neurotranmitters in mice

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Aim: To study the sedative and hypnotic effects of GanDanLiangYiTang (GDLYT) and the potential mechanism in mice. **Methods:** The KM mice were divided into seven groups: the control group, the diazepam group, the BaiLeMian group, and GDLYT 5.2 2.6, 1.3, and 0.65 g/kg group. GDLYT was administered once per day. The models including the mice were treated with pentobarbital sodium at threshold value. The locomotion activity was estimated, and the rotarod test was used to evaluate the effects of GDLYT. The contents of amino acids neurotranmitters including aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), γ-aminobutyric acid (GABA) in mice brain were determined by HPLC-FL. **Results:** The results showed that administration of GDLYT at 2.6 and 1.3 $g \cdot kg^{-1} \cdot d^{-1}$ significantly reduced the latency period and prolonged the duration period of sleep ($P<0.05$, $P<0.01$). Besides, the locomotion activities were significantly reduced by GDLYT (1.3 and 2.6 $g \cdot kg^{-1} \cdot d^{-1}$, $P<0.05$, $P<0.01$) while the coordination of motion had no change in mice after GDLYT treatment. The results of HPLC indicated that GDLYT 5.2 g/kg can significantly increase the GABA, Asp and the Glu content ($P<0.05$, $P<0.01$), and the 2.6 g/kg could increase the GABA and Gly content significantly ($P<0.05$, $P<0.01$), meanwhile the 1.3 g/kg could significantly increase the content of GABA ($P<0.01$) in the brain tissues of mice. **Conclusion:** Our results suggested that GDLYT exerted sedative and hypnotic activities, and the mechanisms may be correlated with the effects of increasing the contents of amino acids neurotransmitters GABA and Gly.

Keywords: GanDanLiangYiTang; mice; sedative hypnotic; central neurotrasmitter

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Effect of Mongolian medicine Garidi-13 on neurological score, pathological changes, serum levels of NSE, TNF-α, IL-1β, IL-6, IL-8 at different phases during cerebral ischemia in rats

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Aim: To study the effect of Garidi-13 on neurological score, pathological changes, serum levels of NSE, TNF-α, IL-1β, IL-6, IL-8 at different phases during cerebral ischemia in rats and investigate protective mechanism of Garidi-13. **Methods:** Male SD rats were randomly divided into vehicle control group, sham-operated group, Gridi-13 large dose group, Gridi-13 middle dose group and Gridi-13 small dose group. Each group was divided into the ischemia 1, 6, 12, 24, 72, and 120 h subgroups. Rats were given Garidi-13 once a day till d 27, then cerebral ischemia was induced with suture method. Neurological score was recorded, the pathological changes of brain was observed with HE staining. The level of NSE, TNF-α, IL-1β, IL-6, IL-8 was determined in serum. **Results:** (1) neurological score: Compared with the vehicle control group, Gridi-13 treated groups had significantly decreased neurological score (P<0.01). There was no significant difference between each Garidi-13 treatment groups (P>0.05). (2) HE staining: In sham operation group, nerve degeneration and necrosis was rare. In model group, neuron rarefaction and cell spaces were accreted and there existed a lot of degenerated neurons. Cell body shrinkage and karyopycnosis appeared and chromatospherite vanished. But each drug treatment group had more survival neurons and the degree of injury was obviously lessened. (3) Compared with the vehicle control group. Gridi-13 all doses could significantly decrease the content of NSE in serum (P<0.01). There was no significant difference between each Garidi-13 group (P>0.05). (4) Compared with the vehicle control group, Garidi-13 at high and middle doses could significantly decrease the content of TNF-α, IL-1β, IL-6, IL-8 in serum (P<0.01, P<0.05). Garidi-13 S could significantly decrease the content of inflammatory factor in serum at 12 h-120 h (P<0.05); The serum content of inflammatory factor in Garidi-13 M group showed significant difference at 1 h and 6 h compared with Garidi-13 L group (P<0.05). **Conclusion:** After cerebral ischemia, the nerve function of rat was impaired, that caused increase in neurological function score and brain tissue pathological changes and severely led to the elevation of serum levels of NSE and the expression of TNF-α, IL-1β, IL-6, IL-8. Garidi-13 improved the neurological function of rats, reduced the pathological changes, and decreased the content of NSE and inflammatory cytokines in serum. Garidi-13 is exerts it protective effects against cerebral ischemia injury possibly through inhibiting the expression of inflammatory cytokines at different phases.

Keywords: Mongolia drug Garid-13; cerebral ischemia; TNF-α; IL-1β; IL-6; IL-8; NSE

S10.99

The pharmacological basis of anti-cerebral ischemic action induced by salvianolic acids

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Salvianolic acids (SA), isolated from *Salvia mitiorrhiza* is a new anti-cerebral ischemic agent approved by state food and drug administration of China recently. It is also the first injection of TCM since 2005. The present paper reports its pharmacological characteristics and mechanism of action. Stroke is an acute neurodegenerative disease which has multiple pathogenesis occurring in the early, middle and later stages. These pathological changes will certainly aggravate ischemic brain injures. A good anti-cerebral ischemic drug must suppress or delay all of these pathogenesis firstly. Secondly, drug can reach central nervous system to play regulatory role. Thirdly, SA can activate neuroprotective mechanism. Our researches showed that SA could pass through blood-brain barrier and accumulated in the CNS, increased regional cerebral blood flow in ischemic hemisphere with no steal blood and without hypotension, inhibited platelet aggregation and thrombosis formation, but had no hemorrhage risk. It decreased intracellular calcium overload and excessive production of free radicals and excitoamino acids, inhibited apoptosis and inflammation. In addition, SA improved energy metabolism, raised ATP, reduced lactic acids, increased neurogenesis, angiogenesis and anti-oxidant enzyme activity, as well as activated neuroprotective signal transduction pathway. As we know, no any drug that used in clinic has so much pharmacological actions as those of SA. For further evaluating SA, comparison of SA with the available drugs was investigated. For example, aspirin has been widely used as a prevention drug for decreasing incidence of cardio and cerebral-vascular diseases. SA could inhibit the increase of TXB_2 in MCAO rats but has no apparent effect on 6-keto-PGF1α, *ie*, TXA_2 was inhibited and PGI_2 was not affected. This is different from aspirin which can inhibit both TXA_2 and PGI_2. More interestingly, SA has anti-thrombosis effect, but has no influence on coagulating system of normal animals. This property of drug may avoid hemorrhage risk. EGb761 has approved to be marked as therapeutic dietary supplements to counteract a variety of neurological disorders including stroke and AD. We carried out a serial of experiments to test free radicals ($O_2^{\cdot-}$ and ·OH) scavenging effects, anti-cytotoxicity, anti-apoptosis, anti-fibril formation of Aβ between SA and EGb761. Results showed SA and EGb761 have similar pharmacological activities on above parameters, but the concentration of SA was far lower than that of EGb761 with the similar effect was obtained. *In vivo* study, we compared the anti-cerebral ischemic effects in MCAO rat between EGb761 and SA. Results indicated that the effective dose for GEb761 ranges from 50 to 100 mg/kg, while the effective doses for SA was reduced to 7.5–15 mg/kg. Oxidative stress caused by reactive oxygen species (ROS) is believed to be a primary factor both in neurodegenerative diseases and in normal process of aging. SA was proved to inhibit lipid peroxidation, lower MDA content and scavenge superoxide anion and hydroxyl radicals. The effective concentration of SA in scavenging free radicals was 10^{-9}–10^{-10} mol/L, while the scavenge concentration for Vit C, Vit E, melatonin and edaravone were 10^{-5}–10^{-6} mol/L indicating that SA was much stronger than those of the above well-known anti-oxidants. Taken together, SA has multi-target effects and unique mechanism of action, suggesting that in addition to stroke, it can be used for treatment of AD, aging, galactose cataract, diabetics and so on.

S10.100

Beneficial effect of cornel iridoid glycoside on neural protection and regeneration

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Aim: Cornel iridoid glycoside (CIG) is the main component extracted from *Cornus officinalis*, which is a traditional kidney tonic Chinese herb. The aim of this study was to investigate the beneficial effect of *Cornel iridoid* glycoside on neural protection and regeneration. **Methods:** The drugs were intragastrically administered to the animal models for 7 d to 2 months respectively. We detect the effect of CIG on a variety of animal models. **Results:** (1) In fimbria-fornix transection (FFT) rat model of AD, CIG intragastric administration for 28 d improved the learning and memory capacity of FFT rats; attenuated the loss of neurons in hippocampus; increased the expression of synaptophysin; enhanced the expression of several neurotrophic factors, including NGF and its receptor TrkA, BDNF and its receptor TrkB; increased the content of growth-associated protein 43 (GAP-43), reduced the content of axon growth inhibitory factors Nogo A and chondroitin sulfate proteoglycan (CSPG); enhanced the expression of Bcl-2 and inhibited the expression of Bax and cytochrome c in the hippocampus of FFT model rats. (2) In gerbil model of global cerebral ischemia (2VO), 7 d after ischemia, CIG intragastric administration increased the expression of Bcl-2, reduced the expression of Bax and caspase-3, decreased apoptosis of neurons in hippocampus. 28 d after ischemia, CIG improved learning and memory ability of the gerbil model, increased the number of neurons in the hippocampus, enhanced the expression of several neurotrophic factors, including BDNF, bFGF and VEGF, and also the expression of PI3K and Akt in signal transduction of neuron survival. (3) In MCAO rat model, CIG was taken by intragastric administration three hours after the surgery and several kinds of tests were performed 7, 14, and 28 d after the surgery, respectively. CIG was able to improve the neural injury of the rat model.CIG was also able to increase the number of endogenous proliferating cells in subventricular zone (the SVZ) and the number of precursor cells of the cerebral cortex and striatal; to promote neural precursor cells to differentiate into neurons and reduce its differentiate into astrocyte; to increase the number of neovascularization around the infarct and the expression of vascular endothelial growth factor (VEGF) and its receptor Flt-1 in cerebral cortex at both mRNA level and protein level, and to increase the expression of BDNF and its receptor TrkB. (4) In MCAO model constructed 7 d after CIG intragastric administration, CIG could reduce the neural injury of the rat model, reduce the infarct volume, increase the number of survival nerve cells in the infarct zone, inhibit the activation of microglia and astrocytes, and reduce IL-1β and TNF-α levels in the cerebral cortex. CIG could also inhibit the apoptosis of neurons in the infarct area by increasing the expression of Bcl-2 and by inhibition of Bax, caspase-3 and cytochrome *c*. When CIG intragastric administration was done 6 h after ischemia, dynamic changes of the infarction were observed 2, 7, 14, and 28 d after cerebral ischemia by magnetic resonance. The imaging results showed that CIG could significantly reduce the

cerebral infarction volume of the rat model. Twenty eight days after the surgery, TTC staining showed that cerebral infarction volume was significantly reduced in CIG treated rats compared with that in the control rats. And the mortality of the CIG treating group (37.5%) was significantly lower than control group (65.0%). (5) In Free-fall hitting (Feeney method) traumatic brain injury rat model, behavioral testing was performed immediately after the model was constructed and brains were collected 24 and 72 h after injury. CIG could improve the behavior scores of the rats, reduce the neuronal death of the cerebral cortex around the injury area, and reduce the expression of TNF-α and IL-1β, increase Bcl-2/Bax ratio. CIG could also reduce the content of caspase-3 and the content of s100β in brain and serum. (6) In complete and oppressive spinal cord injury rat model, CIG intragastric administration for one month could improve the movement capacity of the rat model and maintain the relative proportion of the gray matter and the white matter in impaired spinal segments. It could also protect the anatomy structural integrity and the residual neurons in the impaired spinal cord, ensures more physiological functional nerve fibers passing through the impaired spinal cord segments, and promote newborn of nerve fibers and protect the growth of newborn fibers. CIG treatment could also disturb the inhibition process of the myelin regeneration initiated by Nogo-A, inhibits the expression of p75NTR and ROCK II, and provides a more suitable microenvironment for regeneration of nerve fiber. (7) Neural stem cells were isolated from the hippocampus of neonatal 1 day rat. When such cells were cultured under EGF-free and bFGF-free condition, CIG promoted the survival and proliferation of neural stem cells. When being cultured under fetal bovine serum-free condition, CIG facilitated the differentiation of neural stem cells into neurons. **Conclusion:** CIG has significant effects on a variety of neuro-injury animal models, which suggested that CIG could act on multi-targets and multi-pathways in complex pathogenesis of many diseases. Its dual role in neuroprotective and neurotrophic/ regeneration suggests that CIG has good prospects in treating AD, ischemic brain injury, traumatic brain injury, spinal cord injury and other related diseases.

Keywords: cornel iridoid glycoside; neural regeneration; neuro-protection

S10.101

Antidepressant, anxiolytic and anti-PTSD effects of YL-IPA08, a potent ligand for the translocator protein (18 kDa)

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Aim: The translacator protein (18 kDa) (TSPO), previously called peripheral benzodiazepine receptor, is the key element of the mitochondrial import machinery supplying the substrate cholesterol to the first steroidogenic enzyme (P450scc), which transforms cholesterol into pregnenolone, the precursor of all neurosteroids. TSPO have been demonstrated to play an important role in stress-response and stress-related disorders, such as posttraumatic stress disorder (PTSD), anxiety and depression, by means of affecting the production of neurosteroids. In the present study, we performed detailed investigation of the pharmacological characteristics of N-ethyl-N-(2-pyridinylmethyl)-2-(3,4-ichlorophenyl)-7-methylimidazo[1,2-a] pyridine-3-acetamide hydrochloride (YL-IPA08), a novel TSPO ligand synthesized by our institute, *in vivo* and *in vitro*. **Methods:** First, we determined the binding profile of YL-IPA08, *ie*, its affinity for rat TSPO and CBR. Then, in order to clarify the compound mechanism at the cellular level, the concentrations of neurosteroids were evaluated in the conditioned medium from YL-IPA08-treated astrocytes. Furthermore, we investigated the antidepressant, anxiolytic and anti-PTSD effects of YL-IPA08 in various mouse and rat models. To confirm the role of TSPO in the behavioral action of YL-IPA08, we evaluated the effects of TSPO inhibitors on the behavioral effects of YL-IPA08. Finally, we investigated its potential to cause the side effects normally associated with conventional benzodiazepines. **Results:** YL-IPA08 showed high affinity for TSPO in the crude mitochondrial fraction prepared from rat cerebellum (IC_{50}=0.23±0.04 nmol/L), but only negligible affinity for the central benzodiazepine receptor. We also found the concentrations of pregnenolone and progesterone were increased in the conditioned medium from YL-IPA08-treated astrocytes. Moreover, YL-IPA08 produced antidepressant, anxiolytic-like and anti-PTSD effects in series of mouse and rat models on behaviors. In addition, the antidepressant-like behavior of YL-IPA08 was totally blocked by TSPO antagonist PK11195 (a TSPO antagonist) in tail suspension test, and the anxiolytic effect was also blocked by PK11195, but not by flumazenil (a CBR antagonist) in elevate plus maze test. Furthermore, compared with the CBR agonist diazepam, YL-IPA08 had no myorelaxant effects, did not affect the motor coordination, memory or hexobarbitone-induced sleep in mice. **Conclusion:** Overall, these results indicate that YL-IPA08 is a more selective and potent TSPO ligand, which exerts antidepressant, anxiolytic and anti-PTSD effects that are mediated by TSPO, but does not has the side effects normally associated with conventional benzodiazepines, suggesting that TSPO ligands may represent a therapeutic target for the promising novel drugs of stress-response and stress-related disorders.

Keywords: the translocator protein 18 kDa; YL-IPA08; anxiolytic; antidepressant; neurosteroids; posttraumatic stress disorder

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S10.102

Effects of HSW on mice model of Parkinson's disease induced by MPTP

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Aim: To observe the effects of HSW on behavior and content of dopamine (DA), DOPAC and HVA in the striatum of mice treated with 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). **Methods:** C_{57}BL mice were treated with MPTP. The pole test and Rota-Rod test were conducted to determine the motor harmony; spontaneous motor activity was determined to test the excitability of mice. The contents of DA, DOPAC and HVA in striatum of mice were determined with high performance liquid chromatograph (HPLC). **Results:** Model mice showed disability in pole test and Rota-Rod test and decreased spontaneous motor activity. HSW increased the spontaneous motor activity, shortened the climbing time of pole test, and increased the content of dopamine in striatum. **Conclusion:** Mice treated with MPTP manifested Parkinsonism behavior. HSW can ameliorate abnormal behaviors of model mice and may have a good perspective in the treatment of PD.

Keywords: PD; C_{57}BL mice; behavior test; dopamine

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S10.103

A transmembrane accessory subunit that modulates kainate-type glutamate receptors

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Glutamate receptors play major roles in excitatory transmission in the vertebrate brain. Among ionotropic glutamate receptors (AMPA, kainate, NMDA), AMPA receptors mediate fast synaptic transmission and require TARP auxiliary subunits. NMDA receptors and kainate receptors play roles in synaptic transmission, but it remains uncertain whether these ionotropic glutamate receptors also have essential subunits. Using a proteomic screen, we have identified NETO2, a brain-specific protein of unknown function, as an interactor with kainate-type glutamate receptors. NETO2 modulates the channel properties of recombinant and native kainate receptors without affecting trafficking of the receptors and also modulates kainate-receptor-mediated mEPSCs. Furthermore, we found that kainate receptors regulate the surface expression of NETO2 and that NETO2 protein levels and surface expression are decreased in mice lacking the kainate receptor GluR6. The results show that NETO2 is a kainate receptor subunit with significant effects on glutamate signaling mechanisms in brain.

S10.104

Protocatechuic acid protects primary astrocytes against MPP^+-induced oxidative stress and apoptosis death

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Aim: Protocatechuic acid (PCA), a phenolic compound, has been reported to exert

neuroprotective effects, but its effect on astrocytes has not been studied. The aim of this paper is to investigate whether PCA could protect primary astrocytes against MPP^+-induced oxidative stress and apoptosis death. **Methods:** The viability of cells was determined by the MTT reduction and lactate dehydrogenase (LDH) assay. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and the content of malondialdehyde (MDA) were assayed using biochemical methods. The possible protective mechanism was investigated via detecting apoptosis by Hoechst staining, Bcl-2 and Bax protein expression by ELISA, caspase-3 activation and mitochondrial membrane potential by immunofluorescence, caspase-3 mRNA expression by PCR method. **Results:** The results showed that PCA was effective in reducing apoptotic death induced by oxidative stress, mainly through increasing SOD, GSH-PX activities and reducing MDA content, inhibiting the loss of mitochondrial membrane potential and activation of caspase-3, down-regulating of apoptosis-related protein Bax and up-regulating Bcl-2. **Conclusion:** PCA could inhibit MPP^+-induced oxidative stress and apoptosis death on primary astrocytes, therefore PCA may be used in the treatment of neurodegenerative diseases associated with oxidative stress.

Keywords: protocatechuic acid; oxidative stress; apoptosis; mitochondrial membrane potential; astrocytes

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S10.105

Hypnotic effect and mechanism of quercetin in rats

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Aim: Quercetin is commonly found in plants and exerts a variety of biological activities. In the present study, we investigated the effect of quercetin on the sleep of freely moving rats and the potential neural mechanisms. **Methods:** Rats were intragastricly treated with quercetin (200 mg/kg) at 9:00 pm for 3 d. On the first and third day, electroencephalography (EEG) and electromyography (EMG) were recorded for 6 h from 9:00 pm to 3:00 am in freely moving rats. Sleep parameters were analyzed with SleepSign software. The levels of neurotransmitters were detected with HPLC-ECD 3 h after administration of quercetin. **Results:** Quercetin (200 mg/kg, ig) significantly increased non-rapid eye movement (non-REM) sleep in rats on both first and third day, while it did not affect REM sleep. After 3 d treatment of quercetin NE levels were decreased in ventral tegmental Area (VTA), posterior pretectal nucleus (PPT) and dorsal raphe nucleus (DRN); DA levels were decreased significantly in laterodorsal tegmental nucleus (LDT) and locus coeruleus (LC), and 5-HT levels were decreased in VTA, VTM and LC. Moreover, DOPAC levels were significantly decreased in VTA and LC while 5-HIAA levels were significantly decreased in LC and increased in DRN. **Conclusion:** These results suggest that hypnotic effect of quercetin may be related to its regulatory activity on the neurotransmitters NE, DA and 5-HT in CNS.

Keywords: quercetin; hypnotic effect; NE; DA; 5-HT

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S10.106

Anti-apoptotic and neuroprotective effects of oxysophoridine on cerebral ischemia both *in vivo* and *in vitro*

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Aim: In this study we investigated the neuroprotective effect of oxysophoridine (OSR) on ischemia and ischemia-like insults. **Methods:** Protection by oxysophoridine (OSR) was studied *in vivo* level using a model of middle cerebral artery occlusion (MCAO) in mice and *in vitro* level using primary rat hippocampal neuronal cultures exposed to oxygen-glucose deprivation (OGD), a model of ischemia-like injury. The behavioral test was performed by using the neurological scores. The infarction volume of brain was assessed in the brain slices stained with 2,3,5-triphenyl tetrazolium chloride. The neuron apoptosis was evaluated by Hoechst 33342 staining. Morphological change in the neurons was examined using a Transmission Electron Microscope (TEM or EM). To evaluate neuron apoptosis, caspase-3, -9, and -8 activities were measured using assay kits with an ELISA reader. Western blotting assay was used to evaluate the release of cytochrome *c* and expression of caspase-3, Bcl-2, and Bax proteins. Quantitative real-time PCR assay was used to evaluate the release of cytochrome *c* and the expression of caspase-3 mRNA. **Results:** OSR-treated groups (62.5, 125, and 250 mg/kg) markedly reduced neurological deficit scores and infarct volumes. Treatment with OSR (5, 20, and 80 μmol/L) markedly attenuated neuronal damage, with evidence of decreased cell apoptosis and decreased cell morphologic impairment. Furthermore, treatment with OSR could effectively down regulate the expression of cytochrome *c*, caspase-3 in both mRNA and protein levels and Bax in protein level, induce an increase of Bcl-2 in protein level, the caspase-3, -9, and -8 activities were also inhibited. **Conclusion:** These findings suggested that OSR may be a potential neuroprotective agent for cerebral ischemia injury.

Keywords: oxysophoridine (OSR); Cerebral ischemia; middle cerebral artery occlusion (MCAO); oxygen-glucose deprivation (OGD); apoptosis

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S10.107

Xanthotoxol suppresses the inflammatory response and protects against ischemic brain injury following permanent focal cerebral ischemia

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Aim: Our previous study has demonstrated that xanthotoxol possesses neuroprotective properties following transient focal cerebral ischemia. The present study was designed to investigate the effect of xanthotoxol on the permanent focal cerebral ischemia-induced brain damage and determine the mechanisms of xanthotoxol neuroprotection. **Methods:** Male adult Sprague-Dawley rats underwent permanent middle cerebral artery occlusion (pMCAO) and received xanthotoxol (5 and 10 mg/kg, ip) or vehicle at 1 h and 12 h post-MCAO. After 24 h following pMCAO, infarct size, brain edema, blood-brain barrier (BBB) disruption and the production of pro-inflammatory mediators such as IL-1β, IL-6, TNF-α, iNOS, and COX-2, as well as MMP-9 expression were determined. **Results:** Xanthotoxol treatment following pMCAO resulted in a significant decrease in lesion size, brain edema and BBB permeability as compared with vehicle. Meanwhile, xanthotoxol significantly suppressed the pMCAO-induced up-regulation of IL-1β, TNF-α, IL-6, iNOS, and COX-2, as well as MMP-9 protein expression in the ipsilateral hemisphere. **Conclusion:** The results suggest that xanthotoxol is neuroprotective in permanent cerebral ischemia, and this effect might be related to the suppression of specific aspects of the inflammatory response.

Keywords: xanthotoxol; permanent focal cerebral ischemia; inflammatory response

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S10.108

Effects of xanthoceraside on memory impairments and central cholinergic system in rats by lesions of bilateral nucleus basalis of Meynert with quinolinic acid

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Aim: The present study is to investigate the effect and mechanisms of xanthoceraside on memory impairments and central cholinergic system in Alzheimer's disease (AD) rat model conducted by lesions of bilateral nucleus basalis of Meynert (NBM) with quinolinic acid (QA). **Methods:** The improving effects of xanthoceraside on learning and memory impairment in rats were tested by Y-maze test and Morris water-maze test. Western blot, immunohistochemistry and ELISA were used to detect the expression and activity of choline acetyltransferase (ChAT) and the content of acetylcholine (ACh) in hippocampus and cortex of rats. **Results:** Xanthoceraside increased the alternation percentage in Y maze test, and reduced the latency to find the terminal platform in Morris water maze test compared with the model rats impaired by lesions of bilateral NBM with QA. Xanthoceraside markedly increased the activity and expression of ChAT and the content of ACh. **Conclusion:** Xanthoceraside could effectively improve cognitive performance in rats impaired by lesions of bilateral NBM with QA. The mechanism may be associated with the improvement of central cholinergic system function.

Keywords: xanthoceraside; Alzheimer's disease; learning and memory; Meynert's basal nucleus; central cholinergic system

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