动物实验伦理审查表

The Tab of Animal Experimental Ethical Inspection

编号(No)：20170702

申请人填写的相关信息 (Concerned information wrote by applicant)	申请单位 (Name of organization)：Department of Human Anatomy, Histology and Embryology, The Fourth Military Medical University		
	申请人学历 (Education of applicant)：Ph. D., M. D. 技术职称 (Professional title)：Professor		
	实验名称 (Study title)：The caudal nucleus of the solitary tract mediates visceral hypersensitivity induced by chronic pancreatitis in rats		
	实验目的 (Aim of experiment)： To investigate the plastic changes of excitatory transmission within the caudal nucleus of the solitary tract under the condition of chronic pancreatitis pain as well as the pain-facilitating role of the caudal nucleus of the solitary tract in the progress of chronic pancreatitis pain, which may shed light on new clues for clinical treatment of visceral hypersensitivity induced by chronic pancreatitis		
	拟进动物情况	动物来源 (Source of animal)：Experimental Animal Center of the Fourth Military Medical University	
		品种品系 (Species or strain) SD rats　　等级 (Grade)：SPF level	
		数量 (Number)：81, male	申请日期 (Application date)：2017/07/01
		进驻日期 (Entering date)：2017/09/01	结束日期 (Ending date)：2019/03/01

实验要点，包括实验方法、观测指标、实验结束后处死动物的方法等
(Outline of experiments, experimental methods, observational index, executing animal method et. al):

A total of 81 male Sprague-Dawley rats (250-280 g) were used in the present study. Every measure was taken to minimize the discomfort of the animals. The animals were acclimatized to laboratory conditions (23 ℃, 12 h/12 h light/dark, 50% humidity) for two weeks prior to experimentation, and provided water and food ad libitum 12 h before and after induction of pancreatitis, during which food and water were withdrawn. Animals for tissue collection were euthanized by an overdose of 2% pentobarbital sodium (i.p., 100 mg/kg) while those for surgery were deeply anesthetized by 2% pentobarbital sodium (i.p., 40 mg/kg).

Experiment 1: Evidence of increased expression of FOS-immunoreactive (-ir) neurons within NTS in CP rats: 8 rats were equally divided into 2 groups (sham and TNBS groups). Pancreatitis was induced by intraductal infusion of 0.4 ml 2% trinitrobenzene sulfonic acid. Sham rats received equal volume of saline. On post-operation day (POD) 14, rats were perfused with 4% paraformaldehyde (PFA) with brain tissues sampled for FOS immunostaining

Experiment 2: Effect of painful CP on excitatory synaptic transmission within NTS. 18 rats were equally divided into 2 groups (sham and TNBS groups), which were exposed to intraductal saline or TNBS treatment. At POD 14, rats were sacrificed and brainstem sections containing NTS were performed for electrophysiological recordings.

Experiment 3: Effect of painful CP on the expression of vesicular glutamate transporters (VGluTs), NMDAR subunit NR2B, and AMPAR subunit GluR1 within NTS. 12 rats were equally divided into 4 groups (sham group, TNBS POD 7, POD 14 and POD 28 groups), which were exposed to intraductal saline or TNBS treatment. All TNBS-treated rats were sacrificed according to aforementioned time points and processed for NTS sampling for VGluT1, VGluT2, NR2B and GluR1 immunoblotting.

Experiment 4: Evidence for painful CP induced membrane insertion of NR2B and GluR1 within NTS. 6 rats were equally divided into 2 groups (saline and TNBS groups), which were exposed to intraductal saline or TNBS treatment. On POD 14, rats were perfused with 4% PFA with brainstem sampled for NR2B and GluR1 electron microscopy.

Experiment 5: Effects of CNQX (AMPAR antagonist) and AP-5 (NMDAR antagonist) microinjection into NTS on visceral hypersensitivity of CP rats. 19 rats were divided into 3 groups (6 in saline group, 6 in CNQX group and 7 in AP-5 group) and received cannula implantation. 7 days later, all the rats were exposed to intraductal TNBS treatment. 14 days later after TNBS treatment, CNQX and AP-5 were microinjected into the brain and abdomen mechanical threshold was measured. After behavior tests, the rats were perfused and brain tissues were harvested.

Experiment 6: Effects of activation or inactivation of NTS excitatory neurons on visceral hypersensitivity of CP rats. To explore the role of NTS excitatory neurons in painful CP, 18 rats were equally divided into 3 groups (CaMKIIa-Gq, CaMKIIa-mCherry, and CaMKIIa-Gi groups) and received virus injection. 7 days later, all the rats were exposed to intraductal TNBS treatment. 14 days later after TNBS treatment, CNO were i.p. administrated and abdomen mechanical threshold was measured. After behavior tests, the rats were perfused and brain tissues were harvested.

申请人签名 (Signature of applicant):

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审查依据 (Inspection contents)	该项目是否必须用实验动物进行实验，即能否用计算机模拟、细胞培养等非生命方法替代动物或用低等动物替代高等动物进行实验(Does laboratory animal must be used in the project? Could other methods such as computer simulation, cell cultivation or using the low-grade animal instead of the high-grade animal)? 持久严重的内脏痛是慢性胰腺炎的重要特征，给慢性胰腺炎病人带来难以忍受的痛苦，也给社会带来巨大的医疗负担，因此探索慢性胰腺炎内脏痛机制具有重要的临床意义。TNBS诱导的大鼠的慢性胰腺炎模型具有人类胰腺炎的基本形态特征，可诱导长期的内脏痛觉敏感状态，造模方法简单，模型形成率高，可重复性高。因此本研究选用大鼠作为实验动物。 表中所填申请人资格和所用动物的品种品系、质量等级、规格是否合适，能否通过改良设计方案或用高质量的动物来减少所用动物的数量(Are the qualification of applicant, species or strain, grade and specifications of animals suitable? Could the quantity of animals be reduced by improving the study design or using high quality animals)? 申请人资格和所用动物的品种品系、质量等级、规格符合要求，此研究已尽量减少动物数量来保证研究的顺利进行。 能否通过改进实验方法、调整实验观测指标、改良处死动物的方法，来优化实验方案、善待动物(Could the study design and animal treatment be refined by ameliorating experimental method, adjusting observational index, executing animal method)? 此研究采用腹腔注射2%的异戊巴比妥的方法进行动物麻醉和安乐死，所有实验操作已都尽最大努力减少动物的痛苦。
审查结果 (是否同意申请人的实验方案) (Results of inspection)	课题负责人意见 (Study director): ☑ 同意 (Agree) ☐ 不同意 (Disagree) 签名 (Signature)
	动物实验部意见(Director of Dept. of Lab Animal Science): ☑ 同意 (Agree) ☐ 不同意 (Disagree) 签名 (Signature)
	动物中心实验动物福利与伦理委员会& Welfare Committee): ☑ 同意 (Agree) ☐ 不同意 (Disagree) 签章(Stamp) 福利与伦理委员会
备注(Supplement):	