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Orexins control intestinal glucose transport by distinct neuronal, endocrine and direct epithelial pathways

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Abstract

摘 要

实验目的: 研究小鼠食欲素A/B对肠道葡萄糖转运的影响。

实验设计和方法:

口服葡萄糖实验，注射食欲素降低血糖水平。

通过Ussing chamber实验分析空肠的葡萄糖转运情况。

结论:

注射10 nmol/l OxA or OxB后葡萄糖吸收被抑制。

神经抑制剂TTX, CCK2R拮抗剂使OxA诱导的抑制作用降低。

SB334867（OX受体抑制剂）能够使OxB的浓度效应曲线的显著右移。

前 言

- 背景知识
- 葡萄糖转运过程
- 研究目的

背景知识

orexins, A and B

食欲肽是下丘脑神经元分泌的一种神经递质或神经调质，主要作用是促进摄食，增加体重。食欲肽分为2 个类型: 食欲肽A 和食欲肽B，由相同的基因编码，食欲素A有33个氨基酸，食欲素B有28个氨基酸。

OX_1R and OX_2R

两种食欲素受体，与G蛋白偶联的细胞表面受体。OX_2R与OxA/OxB结合的概率相同，而OX_1R更容易与OxA结合。

背景知识

Ussing chamber

尤斯室,Ussing灌流室，主要功能是通过微电极检测整个细胞膜离子通道变化的电流信号,来反映肠道物质吸收、通透性和分泌情况的变化。

CCK

是一种由胃肠道黏膜细胞分泌的多肽类激素，在体内分布非常广泛，具有多种生物学功能。在消化方面，具有刺激胰液分泌和胆囊收缩、延缓胃排空等作用;在中枢及外周神经系统方面，具有抑制摄食、降低体温和对抗吗啡和内啡肽的镇痛效应。CCK所有的生物学效应均是通过作用于相应受体来实现的。CCK 受体分为CCKA和CCK-B受体2 种亚型，两者都属于G 蛋白偶联受体。

葡萄糖转运过程

exogenous glucose

↓ SGLT1

the blood stream and tissues

↓ GLUT2

interstitial space

研究目的

The study was conducted to determine whether orexins A and B modulate intestinal glucose transport.

材料与方法

- 材料与试剂
- 口服葡萄糖耐量试验
- 组织准备和短路电流测量
- 上皮细胞分离和RT-PCR分析

研究材料

实验动物

雄性Wistar鼠体(240 - 280克), 标准实验室条件饲养，提供自来水和常规食品,12 h / 12 h光/暗周期 温度21–23℃。

Chemicals

Orexins A and B 、 Tetrodotoxin (TTX) 、 OX1R specific antagonist SB334867

研究方法

口服葡萄糖耐量试验

- 使用有意识的鼠, 禁食18 h。
- 食欲素A/B（55 μg/kg）用0.9%NaCl溶液稀释，腹腔注射，设置对照组。
- 5min后所有组灌喂30%葡萄糖溶液，按1g/kg体重灌喂
- 在15，30，60，120min后尾静脉取血，用血糖仪测定血液葡萄糖含量。

研究方法

组织准备和短路电流测量

- 禁食16h的鼠腹腔注射过量的戊巴比妥(精神类药物)杀死
- 解剖小肠，用盐溶液冲洗，剥去边缘的肠系膜.
- 选取四个邻近的组织样本安装到Ussing chamber装置中
- 组织浸泡在由(in mmol/l) NaCl 115.4, KCl 5,$MgCl_2$ 1.2, NaH_2PO_4 0.6, $NaHCO_3$ 25, $CaCl_2$ 1.2 and 甘露醇（mannitol）10组成的电解液中。
- 充气95% O_2-5% CO_2 恒温37°（pH 7.4）

上皮细胞分离和RT-PCR分析

◆ 上皮细胞分离

鼠的肠上皮细胞由含EDTA的裂解液震荡获取

非上皮细胞组织由剥离上皮细胞得到

上皮细胞分离和RT-PCR分析

◆ RT-PCR分析

- 总RNA用Trizol提取
- 反转录合成cDNA，进行RT-PCR
- 引物

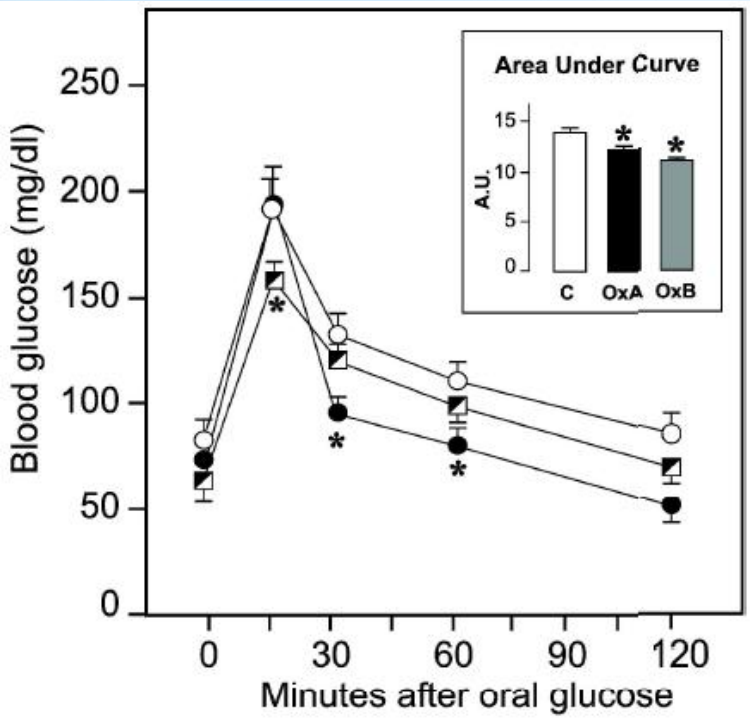
  OX_1R F (5′-CCTGTGCCTCCAGACTATGA-3′)
  R (5′-ACACTGCTGACATTCCATGA-3′)
  OX_2R F (5′TAGTTCCTCAGCTGCCTATC-3′)
  R (5′CGTCCTCATGTGGTGGTTCT-3′)
  GADPH F (5′TGAAGGTCGGAGTCAACGGATTTGGT-3′)
  R (5′CATGTGGGCCATGAGGTCACACAC-3′).

- 94° 1 min,62° 1 min, 72° 1 min，35个循环，

Results
结果

- 口服葡萄糖耐量试验结果
- 食欲素A/B在体外对肠葡萄糖转运的影响
- 食欲素B抑制葡萄糖吸收的途径
- 食欲素A抑制葡萄糖吸收的途径
- 食欲素受体mRNA在肠粘膜上的分布情况

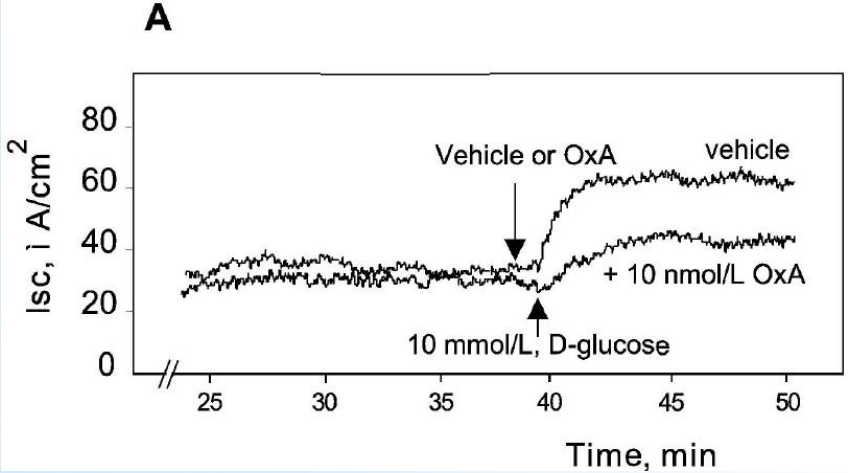
口服葡萄糖耐量试验结果



Rats were injected i.p. with saline (control,), or with 55 µg/kg OxA (X) or OxB () five min before they were challenged by oral administration of a 30% D-glucose solution. Results are presented as mean ± SEM. n= 6-10. *P<0.05. Area under the curve (insert) is expressed in arbitrary units

Figure 1. Oral glucose tolerance test (1 g/kg) in rats.

食欲素A/B在体外对肠葡萄糖转运的影响



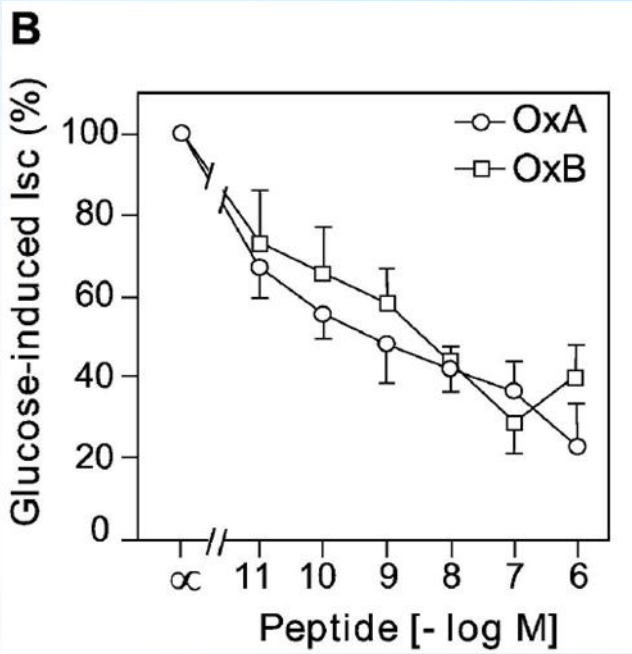
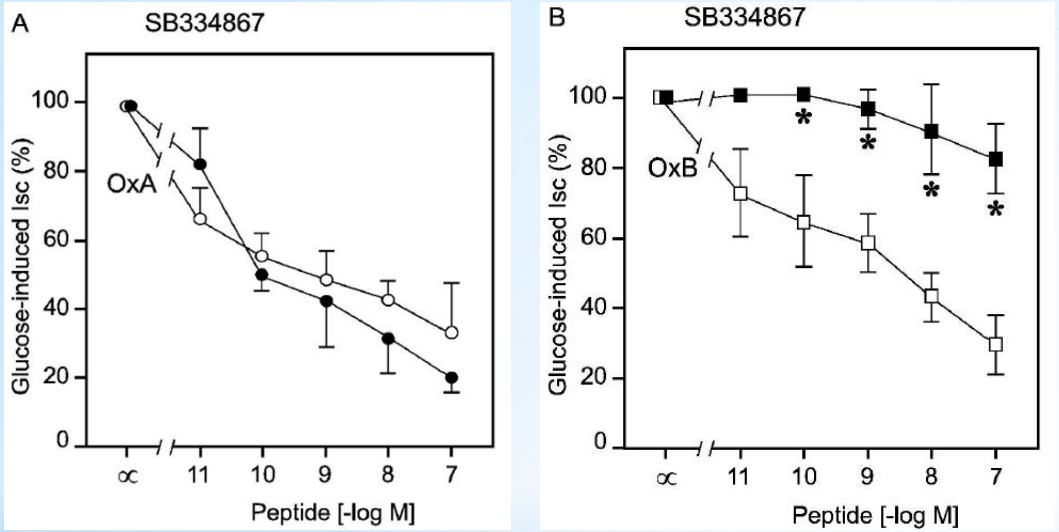


Figure 2. Effect of OxA and OxB on glucose-induced short-circuit current (Isc).

食欲素A/B在体外对肠葡萄糖转运的影响



No significant effect of the OX1R antagonist was observed for OxA. By contrast, the antagonist markedly inhibited the inhibitory response induced by OxB at allconcentrations.

Figure 3. Effect of OX_1R-antagonist SB334867 on inhibition of glucose-induced Isctriggered by OxA or OxB.

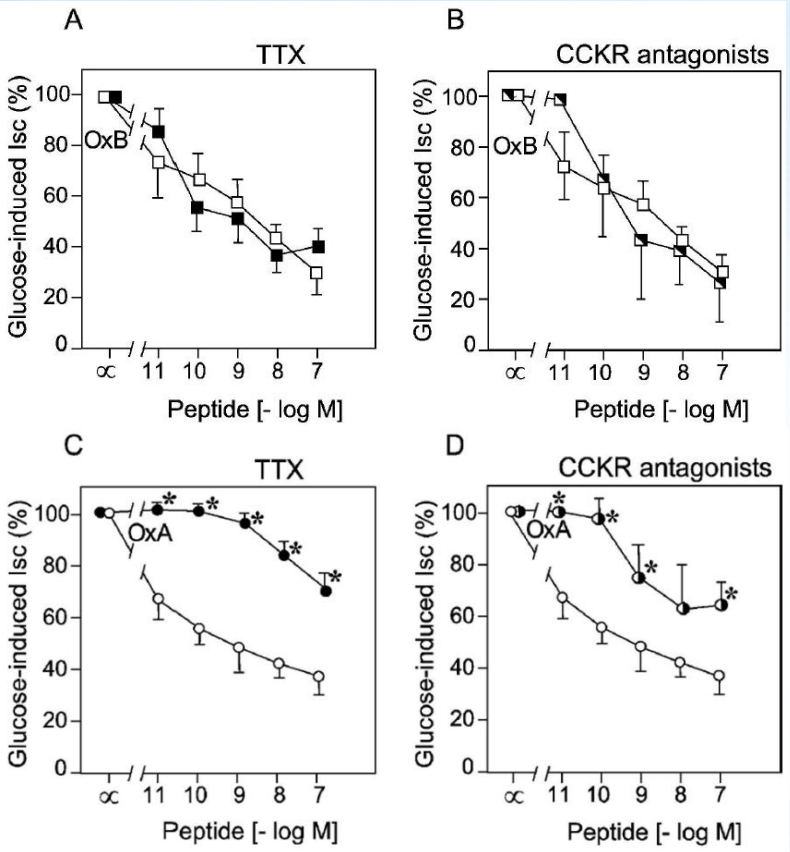


Figure 4. Effect of tetrodotoxin (A, C) and CCK receptor antagonists (B, D) on OxBor OxA-induced inhibition of glucose transport.

Analysis of the pathway involved in the inhibitory action of OxB and OxA on glucoseinduced Isc.

As shown in Fig 4A, TTX had no effect on OxB-induced inhibition,indicating that neuronal cells do not play a significant role in this effect.

As shown in Fig 4B, CCKR antagonists did not modifythe effect of OxB on glucose-induced Isc.

As shown in Fig. 4C, dose-effect curve for OxA was markedly shifted to the right in the presence of TTX.

As shown in Fig 4D,the inhibition of glucose transport induced by OxA was reduced by a mixture of both CCK1R and CCK2R antagonists.

食欲素A抑制葡萄糖吸收的途径

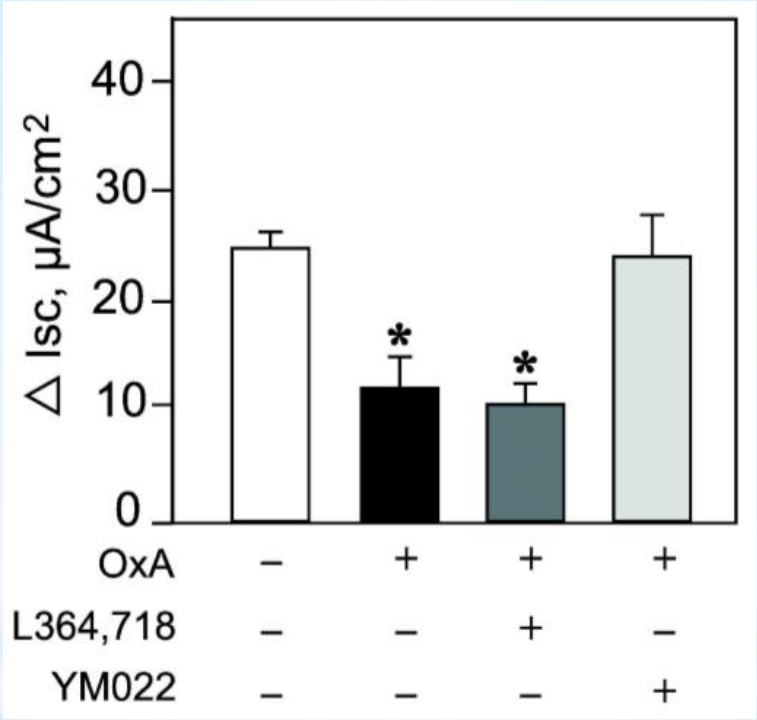


Figure 5. Effect of CCK receptor antagonists on OxA-induced inhibition of glucose transport.

Inhibitory effect of OxA (10 nmoles/l) was studied in presence of CCK1 receptor antagonist, L-364,718 (1 nmoles/l) or CCK2 receptor antagonist, YM022 (1nmoles/l). n=4 - 5 differents tissues. YM022 alone had no effect.

食欲素A抑制葡萄糖吸收的途径

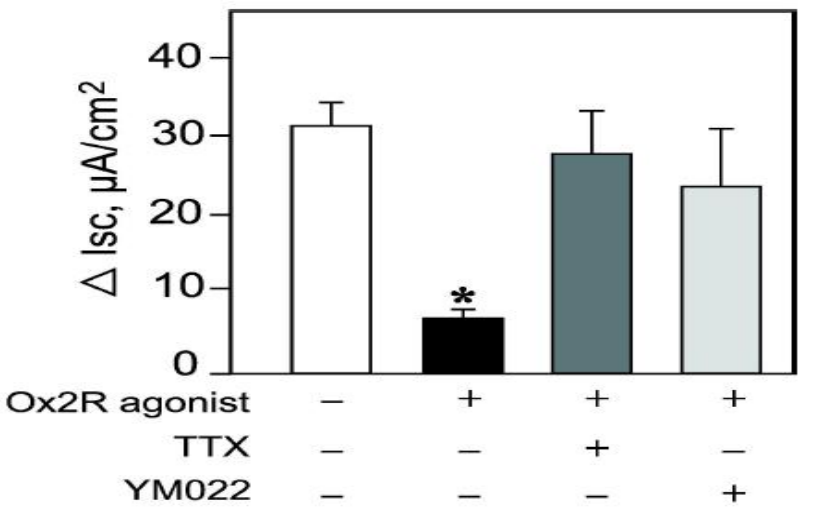


Figure 6. Effect of the OX_2R agonist Ala_{11}, D-Leu_{15}OxB on inhibition of glucose transport.

Inhibitory effect of OX2R agonist (10 nmoles/l) was studied alone or in presence of TTX (5 μmoles/l) or in the presence of CCK2 receptor antagonist, YM022 (1 nmoles/l).

食欲素受体mRNA在肠粘膜上的分布情况

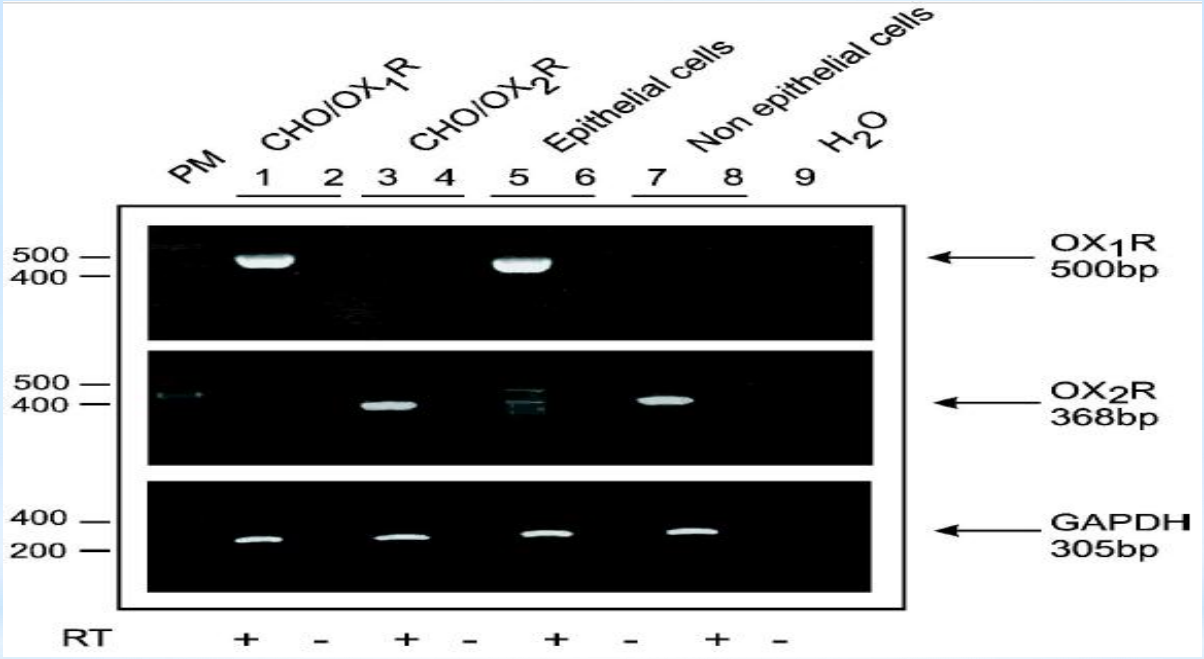


Figure 7. RT-PCR analysis of OxR expression in epithelial and non-epithelial fractions of rat jejunal mucosa.

Results

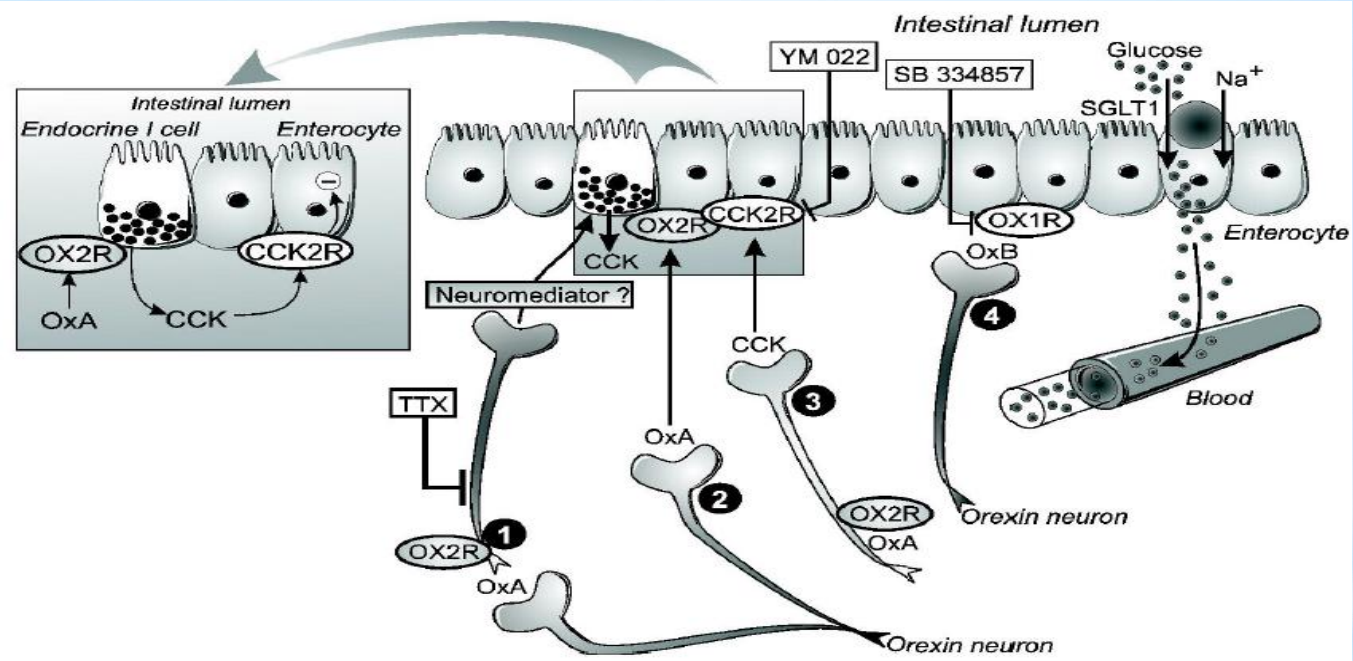


Figure 8. Schematic drawing of inhibitory pathways involved in OxA and OxB inhibition of glucose absorption by enterocyte.

Discussion
讨 论

Our results demonstrate that OxA and OxB acutely inhibit the active absorption ofluminal glucose mediated by SGLT-1.

Both orexins reduced significantly blood glucose when administered by i.p. route.

The relevance of the effect of endogenous orexins on glycemia should be considered in this physiological context.

The nature of the peripheral neural circuitry through which signals from the homeostatic pathways may be integrated into the regulation of energy balance, appetite, locomotor control and body weight is not yet fully understood.

启发与思考

- ✓ 食欲素
- ✓ 实验设计

THANKS!

恳请各位老师批评指正！