

读书报告

报告人：闫潇



2017.4.2



A

1 ACATGGGGAGGGGAGAGCAGAAATGAATGTGAAGATCTTGACGCTGGTGATTGTGCTGGTGGTTTCTCTGCTGTGT

1 M N V K I L T L V I V L V V S L L C

77 TCAGCCAGTGCTGGTCCAATGGCCTCCACCGATCATAGCAAAGAGCTGGAGGAGGTGGGCAGCGTGAGGACTCCT

19 S A S A G P M A S T D H S K E L E E V G S V R T P

152 TTGCGGCAGAATTCGCTCGAGCCGGCCGGAGCCAAAGACCTTCTGGCTGGAGGAGGAGACGCCCTCGACCCCGC

44 L R Q N S A R A G R S Q R P S G W R R R R P R P R

227 CTCTCCATAAGGGGCCATGCCATTTTAGAGCAAGGCACAGCTTAGAGTGCCCTCCCCCTTGGTTCTGATTTCT

69 L S H K G P M P F *

302 ATGCTCTTCCTTTGCCTTGGGGGGCCTTGCAACAAGGGGCCTTTGCTAGCCCAGCCTGAAGAGTGATGTCCATAG

377 CACCTGCTGGTTCTTCAAGCAACTCTTCTTGTCCACAAAAAACCAGGACAAGCCACTCACCCCTCAGCTCTAC

452 GAAGAATTGTGGGCCAGGAACGGGGGTGGGGGTATTGAGCCCGAGCTATCAGATTCTGTCTCTCTGTCTGTCTC

527 TCTCCTCCTGTGGTTTTCTCATTGCTTTCTAGAGCCCTTTCATGGCCACACTTCGCATGATGTGTGAGCAGTG

602 GGGAGCCTGGTCTAAACAGTTATAGTCTGAAGCTTAAGGATAAAGCCACAGTGTCTCATTATCAAATCACACTC

677 CACTTCATAAGCGTGAGATCATCGTCCTGAGCCCTTCTCCTCCCCCATGACACACGCTGCTTTACAGCAGAG

752 GTACATGACTGTAACTGGCCAGGGTGATCTGCTTCACACGGCGGACCATCTCTCCTTTAATGAAGTGCCAAAT

827 AGCAAATCTACTCCACAGTGCTGCCCGGAGAAGAAAAGTAAACATGTTACCTTTATCTTGGGGGAAGCATAACA

902 TCTAACCATATGTGCGCATTTTGCCATTTGTTTGAACATCCAATGTAAAGGCAACGGTAGCATGGTTTCAAGACA

977 TTAGACAAAAA

Apelin

全长：1001bp

ORF：231bp

编码77个氨基酸

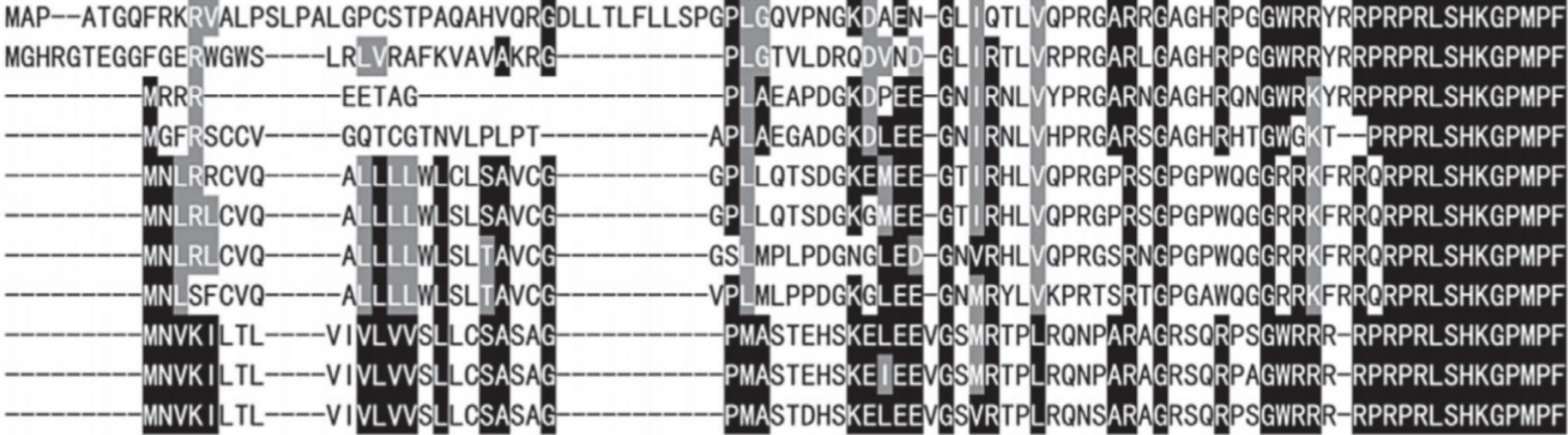
N端有一个22个氨基酸组成的信号肽。

Apelin

- 游隼
- 虎皮鹦鹉
- 扬子鳄
- 中华鳖
- 牛
- 虎鲸
- 人类
- 褐家鼠
- 鲫鱼
- 斑马鱼
- 齐口裂腹鱼

B

Falco peregrinus
Melopsittacus undulatus
Alligator sinensis
Pelodiscus sinensis
Bos taurus
Orcinus orca
Homo sapiens
Rattus norvegicus
Carassius auratus
Danio rerio
Schizothorax prenanti



基因克隆

A

```
1 ACATGGGGGCATTCTGACCTGAGCACATCACAAGCTGACTTCTCTGTGATCATTTAGGTACCGATACTTTAAA
75 CACTGAAAGATCCTTGGAGGAATGAATGCCATGGACAAAATGACTGCTGACTATAGCCAAGATGACTACGACGAG
1 M N A M D K M T A D Y S Q D D Y D E
150 ACATTGAACAACCTCAATGTGTGATTTTGATGAGTGGGAACCGTCATATTCGCTGATTCCTGTGCTCTACATGCTC
19 T L N N S M C D F D E W E P S Y S L I P V L Y M L 1
225 ATCTTCATCCTGGGCCTCACTGGGAATGGTGTGGTCATCTTCACCGTATGGCGGGCTCAGTCCAAACGGAGAGCT
44 I F I L G L T G N G V V I F T V W R A Q S K R R A
300 GCAGACATCTACATTGGAAACCTGGCTCTTGCTGACCTGACCTTTGTGGTGACCCTGCCTCTGTGGCCGTCTAC
69 A D I Y I G N L A L A D L T F V V T L P L W A V Y 2
375 ACCGCCCTGGGATACCACTGGCCGTTTGGCGTGGCCCTCTGCAAGATCAGCAGCTATGTGGTGTGCTCAACATG
94 T A L G Y H W P F G V A L C K I S S Y V V L L N M 3
450 TACGCCAGTGTCTTCTGCCTCACCTGCCTGAGTCTGGACCGTACATGGCCATCGTTCCTCCCTCACCAGCACA
119 Y A S V F C L T C L S L D R Y M A I V H S L T S T
525 CAGCTGCGGACCAGAGGACACATGCGGGCCTCGCTGGCCACCATCTGGCTCCTCTCAGGTGTGCTGGTGCACCC
144 Q L R T R G H M R A S L A T I W L L S G V L A A P 4
600 ACGCTGTGTTTCGCACCACGGTGTACGACGCTGAGACCAACCGTACCTCCTGCGCCATGGACTTCAGCCTGGTG
169 T L L F R T T V Y D A E T N R T S C A M D F S L V
675 GTGAGCAAACCGGGTCAGGAGACCTTTTGGATCGCCGGCCTCAGCATCTCCTCCACTGCTCCTGGCTTTCTGATC
194 V S K P G Q E T F W I A G L S I S S T A L G F L I 5
750 CCCCTTCTGGCCATGATGGTGTGCTACGGATTCATCGGCTGCACCGTCACACGTCACTTCAACAGCCTGCGCAAG
219 P L L A M M V C Y G F I G C T V T R H F N S L R K
825 GAGGACCAGCGCAAACGCCGCTGCTCAAGATCATCACCACATTGGTGGTGGTGTGCTGCGTGGTGGATGCCC
244 E D Q R K R R L L K I I T T L V V V F A A C W M P 6
900 TTCCACGTCGTGAAGACCATGGACGCTCTTTCGTACCTGAACCTTGCTCCTGACTCCTGTACCTTCTGAACCTC
269 F H V V K T M D A L S Y L N L A P D S C T F L N L 7
975 CTTCTTCTGGCTCATCCCTATGCAACCTGCCTGGCGTACGTCAACAGCTGCCCCAACCCGCTCCTCTACGCCTTC
294 L L L A H P Y A T C L A Y V N S C P N P L L Y A F
1050 TTCGACCTCCGCTTCCGCTCCAGTGCCTCTGCCTCCTCAACCTGAAGAAAGCCCTTACGCCAGTCCCTGCCAGC
319 F D L R F R S Q C L C L L N L K K A L H A S P A S
1125 TCCCTTTCTTACAGAAGACTGAGGCCAGTCTCTGGCTACGAAGGTGTGAGGAGG
344 S L S S Q K T E A Q S L A T K V *
```

APJ

ORF : 1080bp

编码359个氨基酸

7个跨膜域

Table 2
Comparison of Ya-fish apelin and APJ sequences with other known vertebrate sequences.

		Carassius auratus	Danio rerio	Salmo salar	Anguilla japonica	Alligator sinensis	Pelodiscus sinensis	Falco peregrinus	Melopsittacus undulatus	Bos taurus	Orcinus orca	Rattus norvegicus	Homo sapiens
Apelin		97.1%	95.1%	-	-	52.0%	51.0%	30.4%	44.1%	53.9%	52.9%	52.0%	50.0%
APJ	(A)	-	79.7%	79.4%	77.4%	50.1%	47.1%	52.9%	50.1%	42.3%	47.1%	47.8%	47.3%
	(B)	-	95.8%	81.8%	-								

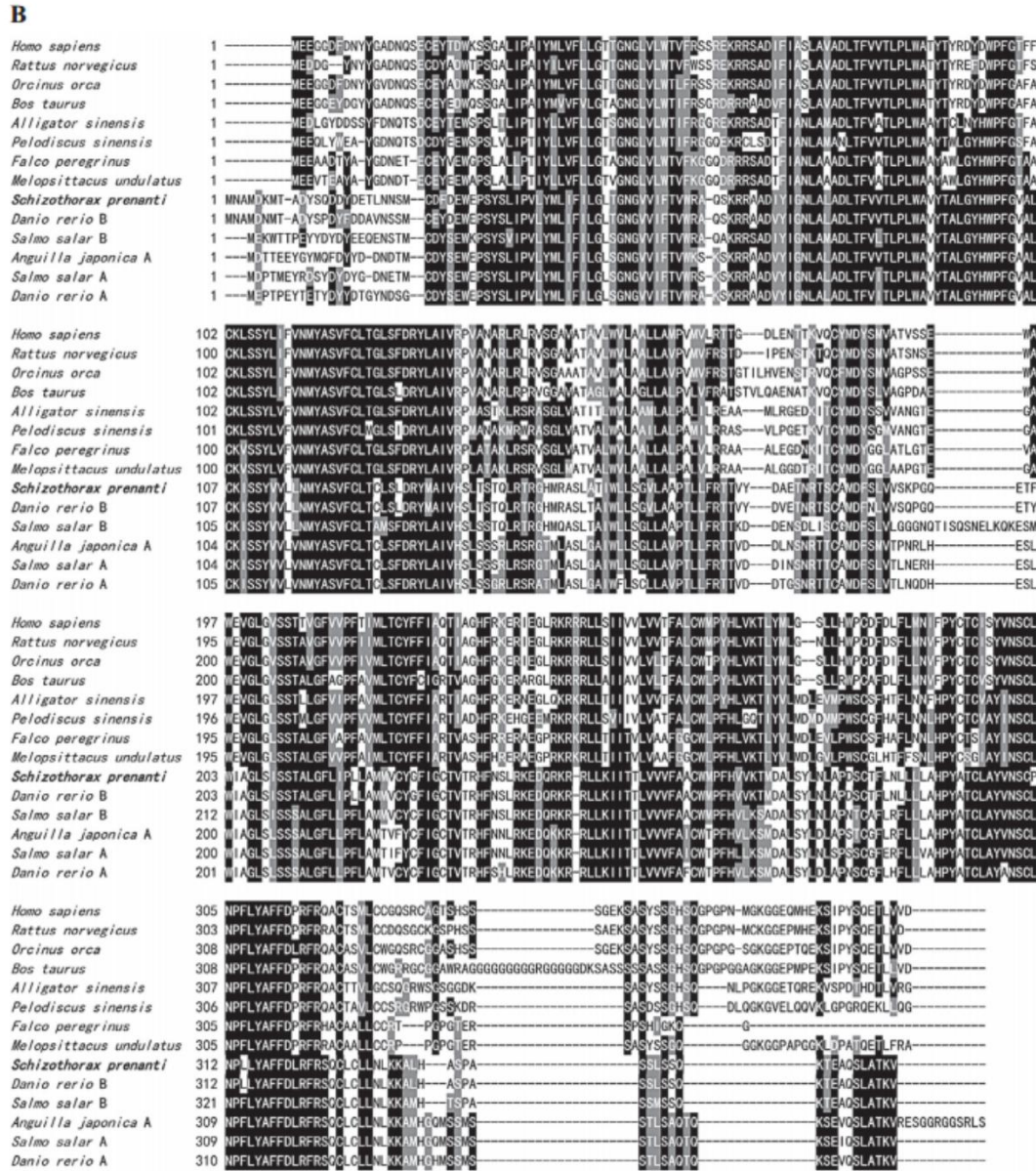
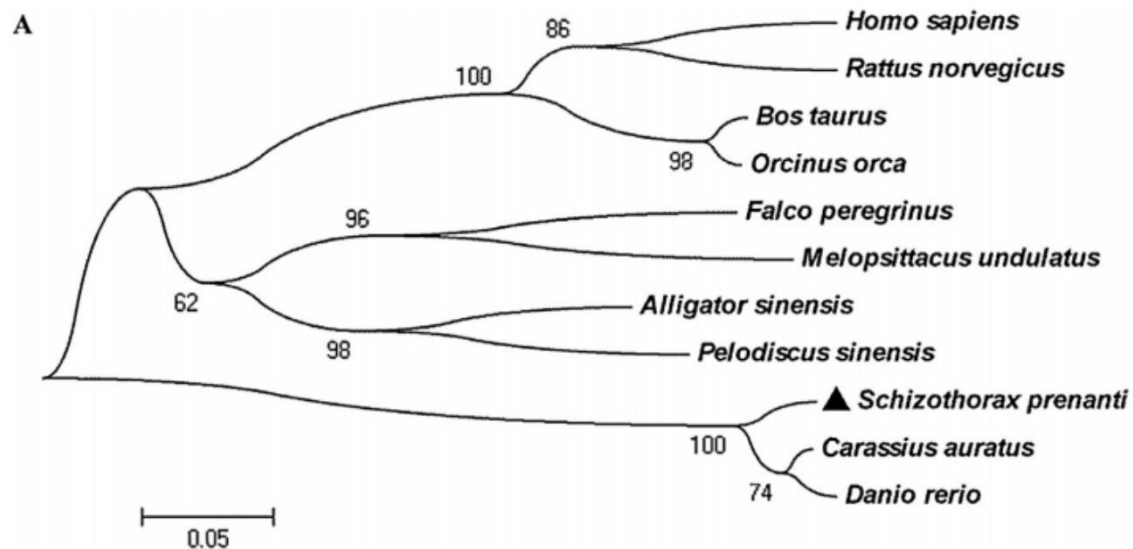


Fig. 2. (continued)

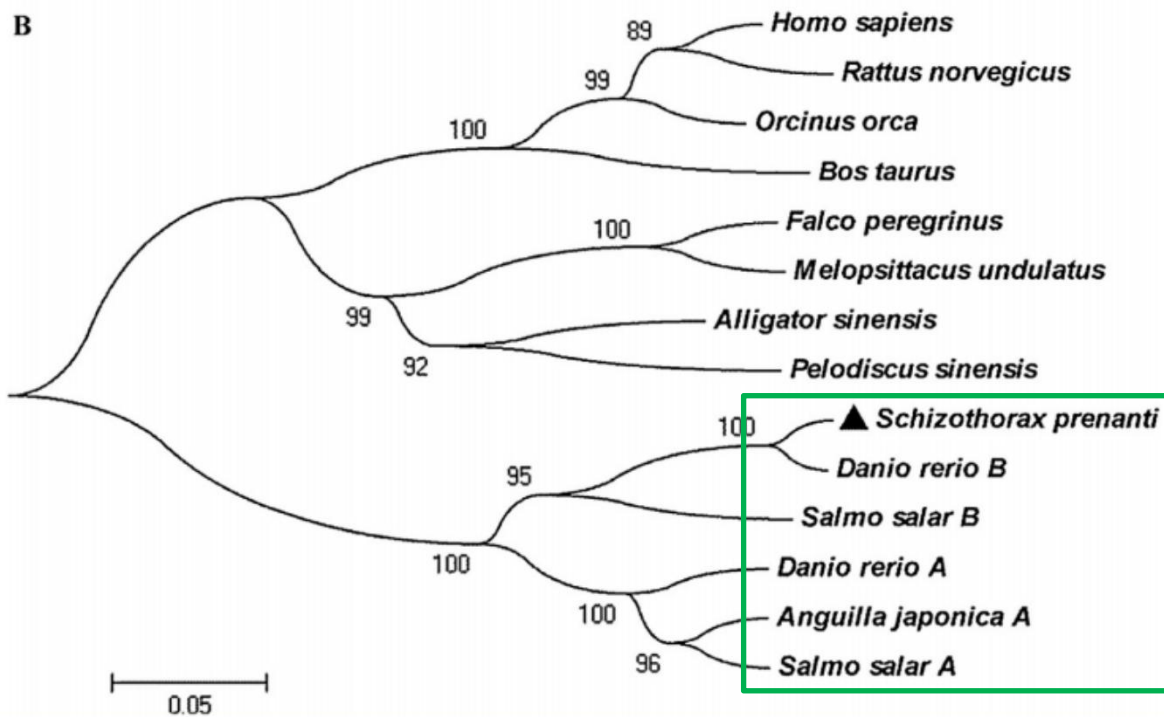
Apelin



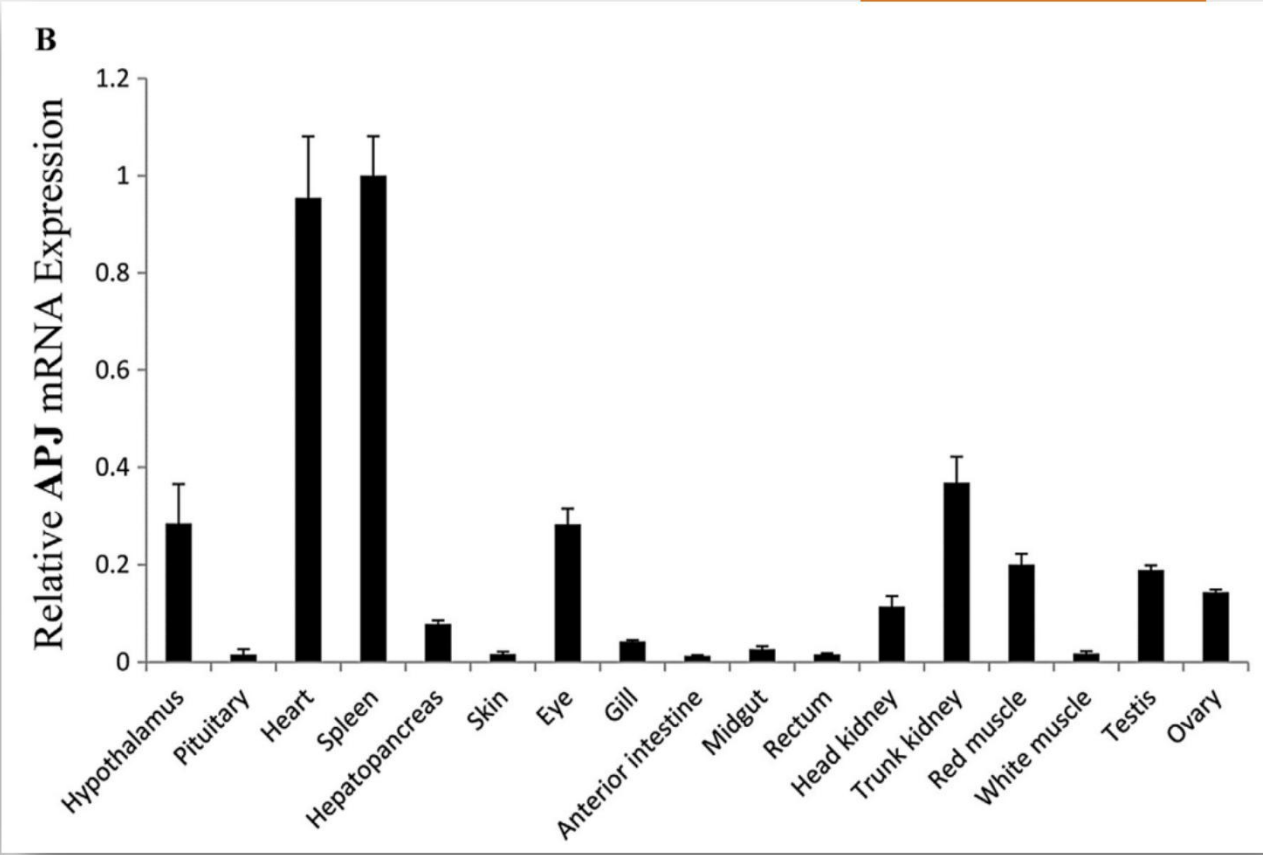
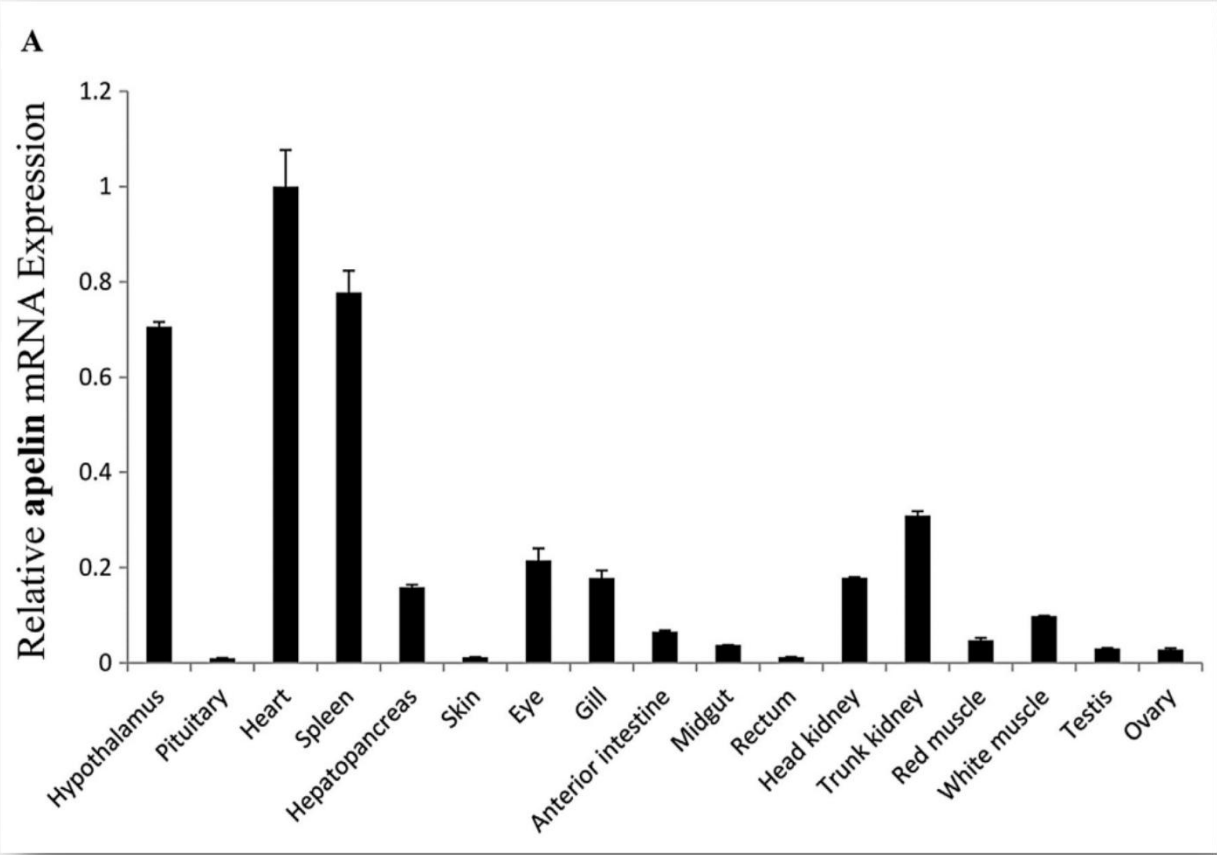
人类
褐家鼠
牛
虎鲸
游隼
虎皮鹦鹉
扬子鳄
中华鳖
齐口裂腹鱼
鲫鱼
斑马鱼

聚类分析

APJ

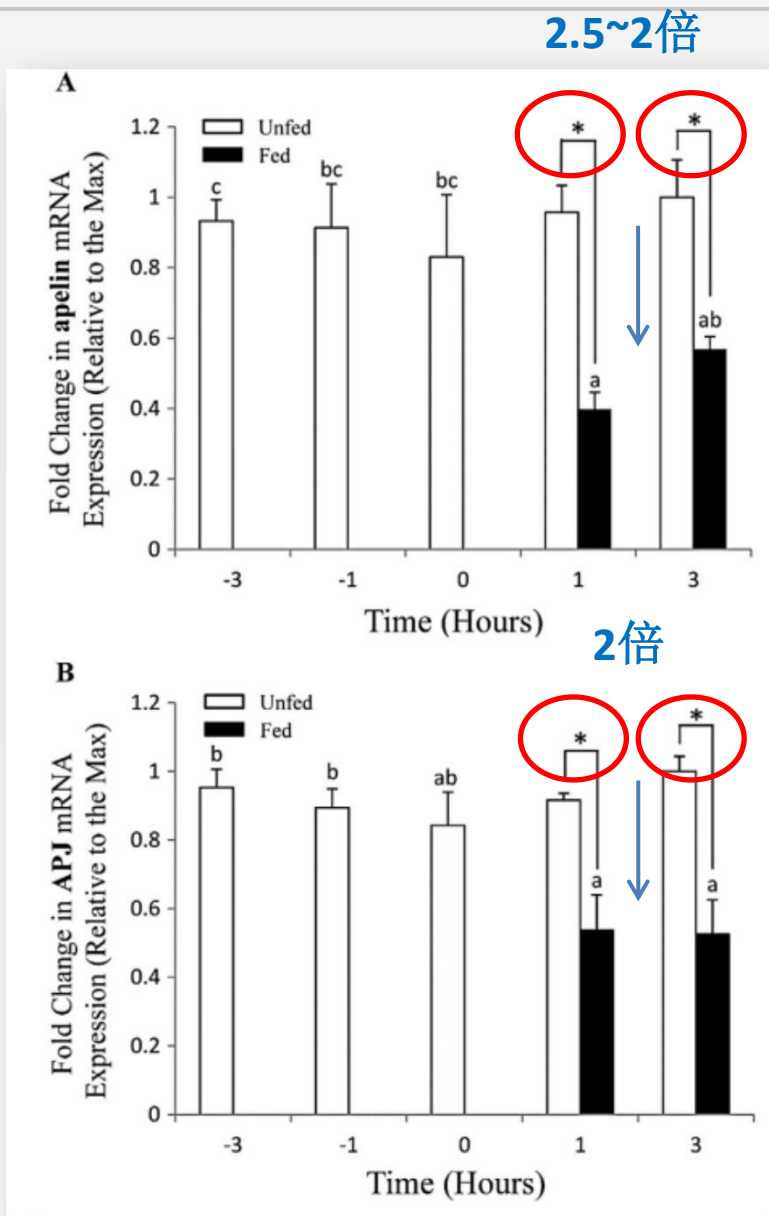


齐口裂腹鱼
斑马鱼
大西洋鲑
斑马鱼
鳗鲡
大西洋鲑

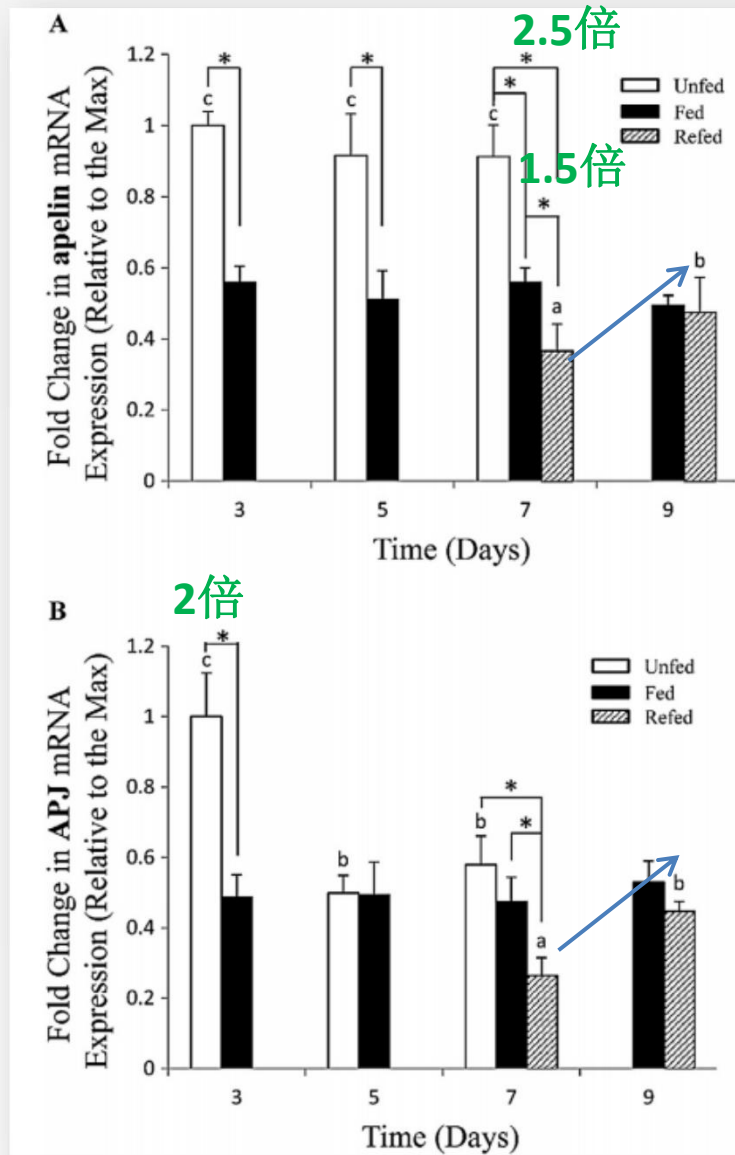


下丘脑、垂体、心、脾、肝、皮肤、眼、鳃、前肠、中肠、后肠、头肾、肾脏，红肌，白肌，睾丸和卵巢
apelin: 心脏 > 脾脏 > 下丘脑 > 肾脏

Apelin



1.8倍

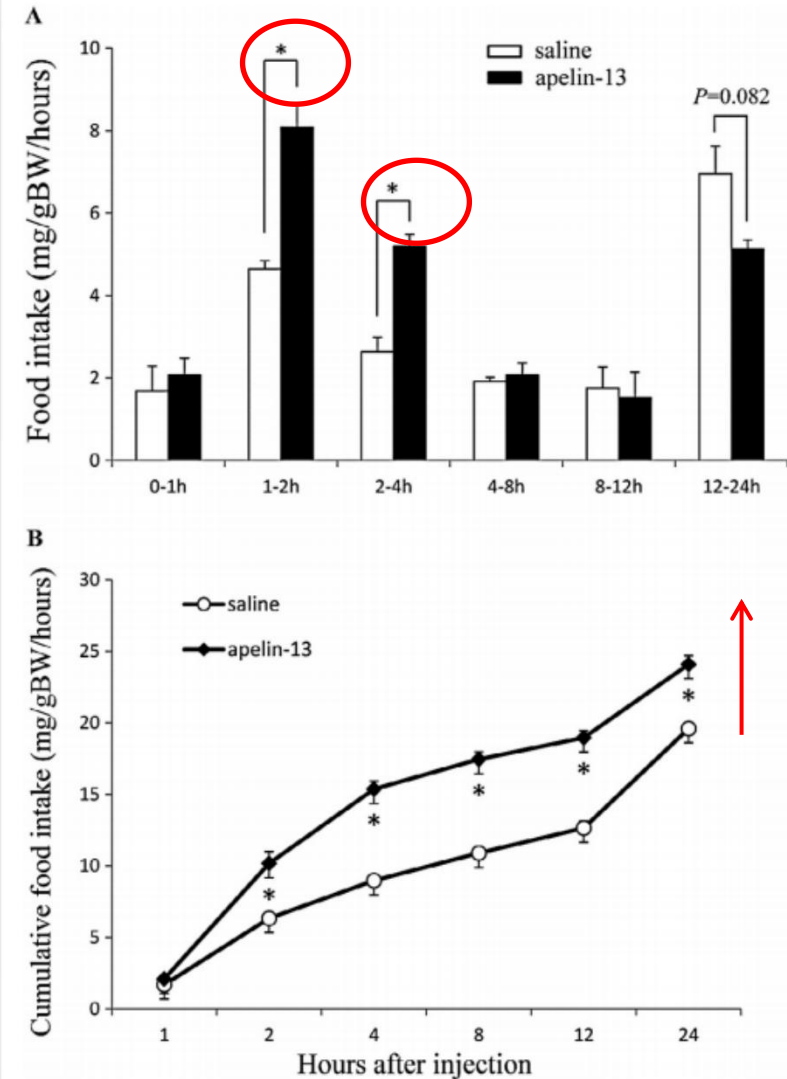


饥饿实验

下丘脑

11:00

注射实验



Apelin-13由上海生工合成，
通过HPLC和质谱确定纯度：
>98.81；
溶解于淡水鱼生理盐水中，
对照组注射生理盐水。
100 ng/g body weigh

Apelin-13对食物摄取的影响

结果总结：

- Apelin和APJ mRNA在测试的所有组织中均有表达，在心脏、脾脏、下丘脑和肾脏具有相对较高的表达水平；
- 短期饥饿APJ mRNA表达显著增加，但是第5、7饥饿组合对照组没有显著性差异；
- 短期饥饿Apelin mRNA表达在7天的饥饿过程中持续呈现增加状态；
- 相对于注射盐水组，注射apelin能够显著增加齐口裂腹鱼的摄食；

Apelin-APJ在鱼类中的广泛分布表明其可能具有多种生理调节作用。

APJ和apelin饥饿实验中的差异，可能是由于另一受体（APJa）介导apelin对喂养的远期影响。

Apelin起到食欲因子的作用，通过与喂养相关的激素和途径之间的相互作用来调节摄食。



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Apelin in goldfish (*Carassius auratus*): Cloning, distribution and role in appetite regulation

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ABSTRACT

Apelin is a recently discovered peptide produced by several tissues including brain and adipose tissue. In mammals and zebrafish, apelin regulates cardiovascular functions. Recent evidence in mammals suggest that apelin might also regulate food intake. In this study, we cloned a cDNA encoding apelin and examined apelin mRNA distribution within the brain and in peripheral tissues. We also assessed the effects of fasting on apelin brain mRNA abundance. Apelin mRNA was expressed throughout the brain as well as in several peripheral tissues including brain, spleen, heart and fat. Apelin mRNA abundance in both hypothalamus and telencephalon was significant higher in fasted fish than in fed fish. In order to further characterize apelin in goldfish, we assessed the effects of central (intracerebroventricular, icv) and peripheral (intraperitoneal, ip) injections of apelin-13 on food intake in goldfish. Apelin injected ip at a dose of 100 ng/g or icv at a dose of 10 ng/g induced a significant increase in food intake compared to saline-injected fish. Our results suggest that apelin acts as an orexigenic factor in goldfish. Its widespread distribution in the brain and the periphery also suggests that apelin might have multiple physiological regulating roles in fish.

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基因克隆

```
(A) 1 cttgcggcgctgtcacacacttgacacagacatacaaacacattcagccacgcacacacacacatagacacacgcgcacactgaaaagcaaacagtctc 100
101 tctctctctcgctctcgctcaccactctcctctccctcccatccacacacgcacaccactacagtatatcagctagcgactggcagggaaacagagggg 200
201 agagcagaa ATG AAT GTG AAG ATC TTG ACG CTG GTG ATT GTG CTG GTG GTG TCT CTG CTG TGT TCA GCC AGT GCT 275
1 M N V K I L T L V I V L V V S L L C S A S A 22
276 GGT CCA ATG GCC TCC ACC GAG CAT AGC AAA GAG CTG GAG GAG GTG GGC AGC ATG AGG ACT CCT TTG CGG CAG AAT 350
23 G P M A S T E H S K E L E E V G S M R T P L R Q N 47
351 CCT GCT CGA GCC GGC CGG AGC CAA AGA CCT TCT GGC TGG AGG AGG AGA CGC CCT CGA CCC CGC CTC TCC CAC AAG 425
48 P A R A G R S Q R P S G W R R R R P R P R L S H K 72
426 GGG CCA ATG CCA TTC TAG agcaaggcacagcttagagtgcctcccccttggttctgatttctatgctcttcccttgccctggggggccttgca 519
73 G P M P F * 78
520 acaaggggcctttgctagcccagcctgaagagtgatgtccatagcacctgctggttcttcaagcaactcttcttctgtccacaaaaaaccaggacaagc 619
620 cactcatcctcagctctgcaaagaattgtgggccaggaatggggatgggggtattgagcccagctatcagattctatctctctctgtctgtctctctc 719
720 ctctgtggttttctcatttgctttctagagccttttcatggccacactttgcatgacgtgtgagcagtgaggagcctggtcagttatagtccgaaagc 819
820 gtcactttcttctccgaggtgtgctagtgccattctctccagcgtgaaggtgaccacagcgtttgtccattgctatgtgtcggcaactcttagcttat 919
920 acagtaagtgggtgtcatgagagaagaaagtggcagggaaactaaagtaaaggaactaaatgctcaggcccattatgaacaggtcagcaactgttttaca 1019
1020 tcttgcaaaattttaccatctgcctataactaaaaatggacagttagctttataatagcatcagtgagtgacatttacatgacaaaactgccaacaaaacgc 1119
1120 aattatcacttatcaaaaatgtattctaaaaaccttacagtaaaaaaaaaaaaaa 1175
```

Apelin

全长：1175bp

ORF：231bp

编码77个氨基酸

N端有一个22个氨基酸组成的信号肽。

```
(B) rat      MNLSFCVQALLLLWLSLTAVCGVPLMLPPDGKGL EE-GNMRYLVKPRTSRTGPGAWQGGR 59
    human    MNLRLCVQALLLLWLSLTAVCGGSLMPLPDGNGLED-GNVRHLVQPRGSRNGPGPWQGGR 59
    xenopus   MNLRLWALALLLFILTLTSAFGAPLAEGSDRND-EE-QNIRTLVNPKMVRNSAPQRQANR 58
    zebrafish MNVKILTLVIVLVVSVLLCSASAGPMAS TEHSKEIEEVGSMRTPLRQNPARAGRSQRPA GW 60
    goldfish  MNVKILTLVIVLVVSVLLCSASAGPMAS TEHSKELEEVGSMRTPLRQNPARAGRSQRPSG W 60
          ** : : . . : : * . * : . . : : . : * : . : * : . . * . . .

    rat      RKFRRQRPRLSHKGPM PF 77
    human    RKFRRQRPRLSHKGPM PF 77
    xenopus   RKLIRQRPRLSHKGPM PF 76
    zebrafish RR-RRPRPRLSHKGPM PF 77
    goldfish  RR-RRPRPRLSHKGPM PF 77
          * : * *****
```

氨基酸水平:

- 金鱼VS斑马鱼: 97%
- 金鱼VS爪蟾: 43%
- 金鱼VS哺乳动物: 35-38%

核酸水平 (ORF):

- 金鱼VS斑马鱼: 95%
- 金鱼VS爪蟾/哺乳动物: 15%

组织分布

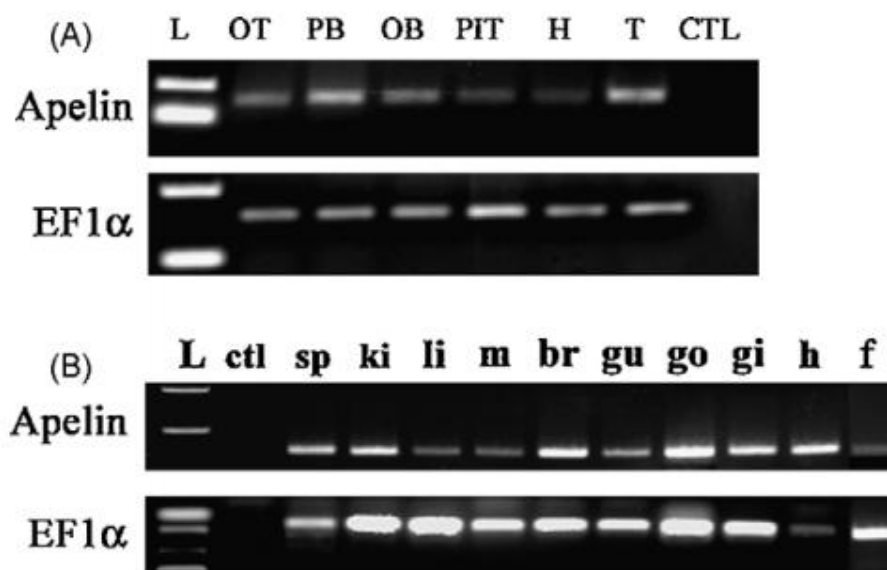


Fig. 2. RT-PCR distribution of apelin (108 bp) and EF-1 α (104 bp) in different brain regions (A) and peripheral tissues (B) of goldfish. L, ladder; OT, optic tectum/thalamus; PB, posterior brain/cerebellum; OB, olfactory bulbs and tract; PIT, pituitary; H, hypothalamus; T, telencephalon; ctl: control; sp, spleen; ki, kidney; li, liver; m, muscle; br, brain; gu, gut; go, gonad; gi, gill; h, heart; f: fat.

L, ladder;

OT, optic tectum/thalamus;视顶盖/丘脑

PB, posterior brain/cerebellum;后脑/小脑

OB, olfactory bulbs and tract;嗅球

PIT, pituitary; 垂体

H, hypothalamus; 下丘脑

T, telencephalon; 端脑

ctl: control; 对照

sp, spleen; 脾

ki, kidney; 肾

li, liver; 肝

m, muscle; 肌肉

br, brain; 脑

gu, gut; 肠

go, gonad; 性腺

gi, gill; 鳃

h, heart; 心脏

f: fat.脂肪

全脑没有显著性差异

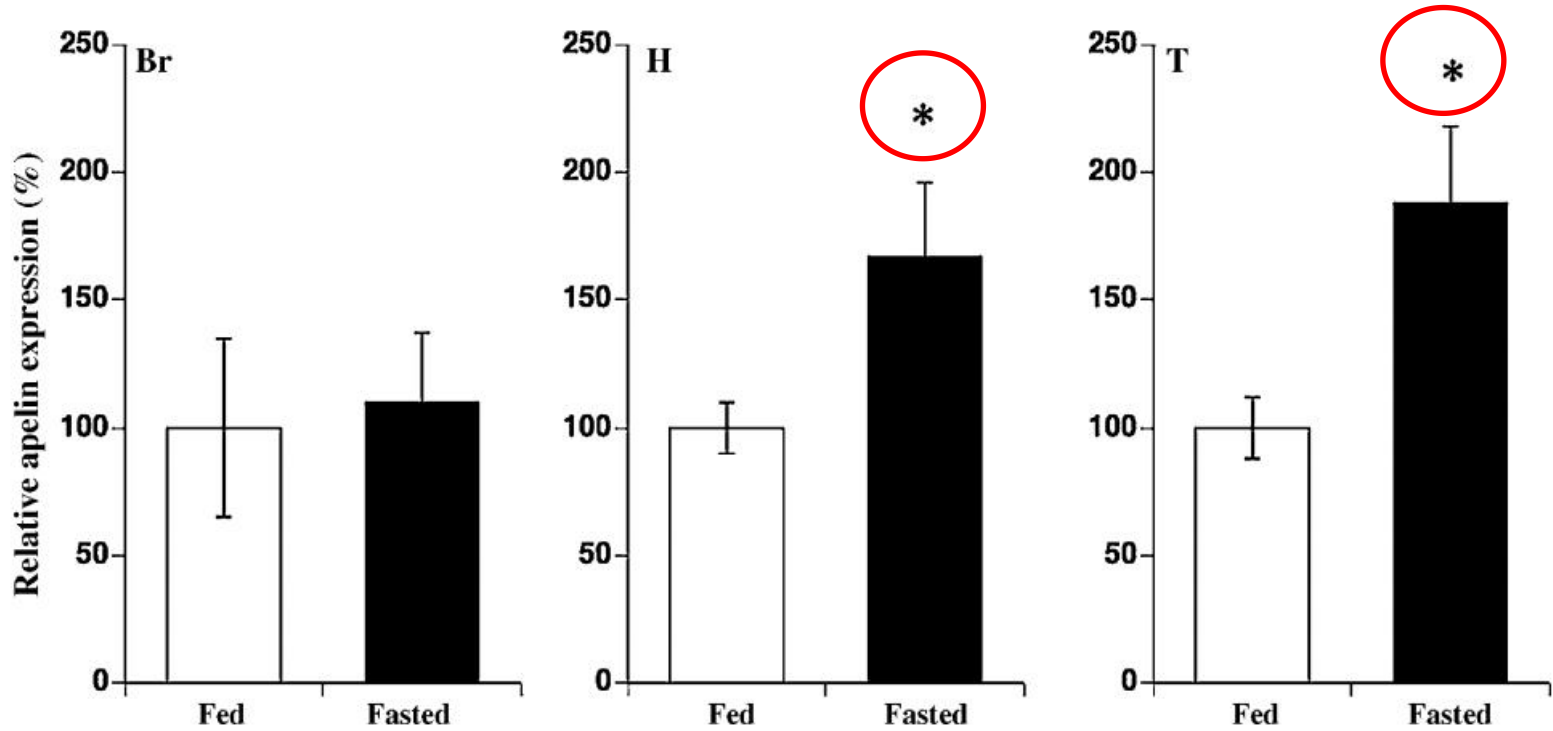
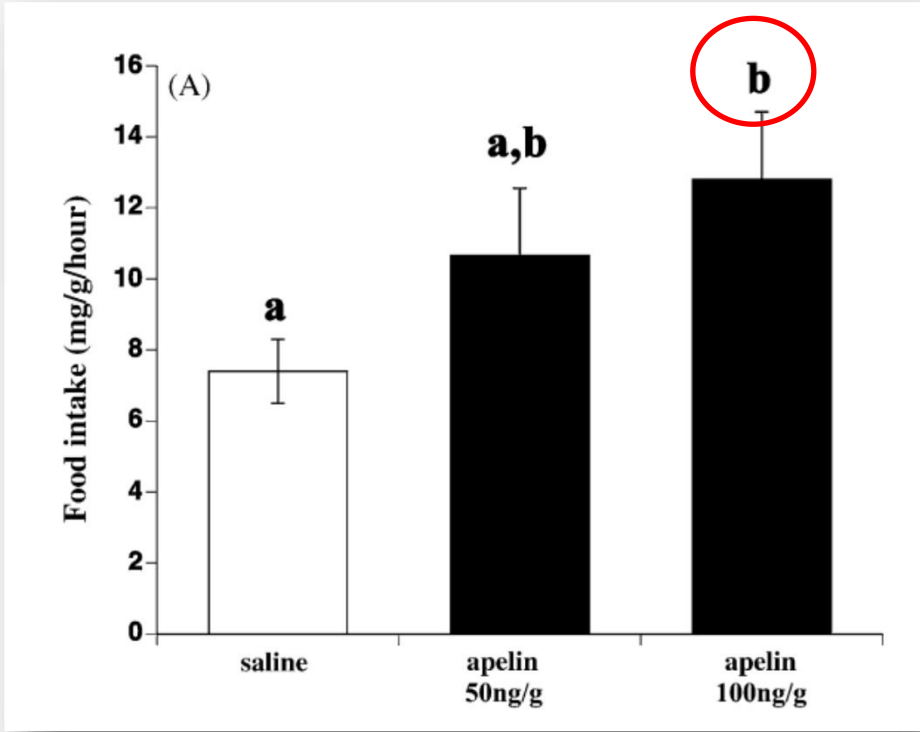


Fig. 3. Apelin mRNA abundance in whole brain (Br), hypothalamus (H) and telencephalon (T) of fed and fasted goldfish ($n = 5-8$ fish per group). Expression levels in the fed group were normalized to 100%. Data is presented as mean \pm SEM. Stars indicate significant differences between the fed and fasted groups.

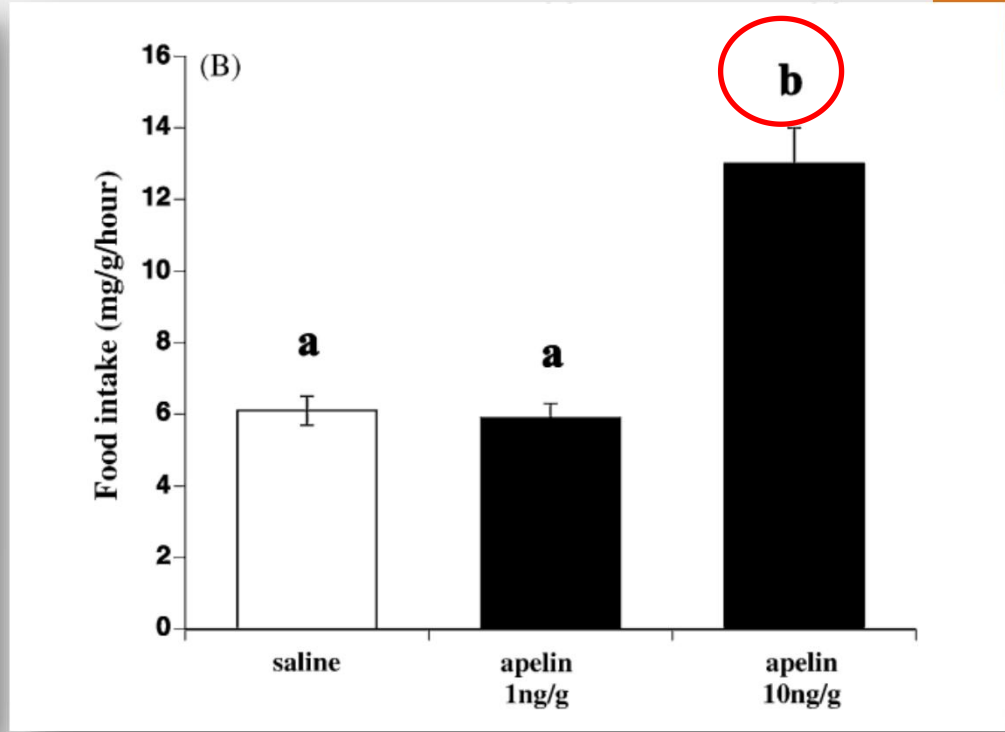
全脑 (BR)

下丘脑 (H)

和端脑 (T)



腹腔注射(ip)对摄食的影响



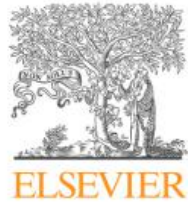
侧脑室注射(icv)对摄食的影响

结果总结：

- Apelin mRNA在整个大脑以及一些外围组织，包括脑，脾，心脏和脂肪均有表达；
- 饥饿组Apelin mRNA在下丘脑和端脑表达显著高于饲喂组，在全脑中没有显著性差异；
- 侧脑室和腹腔注射apelin-13显著增加摄食量；

Apelin在金鱼中担当促进食欲的因子。

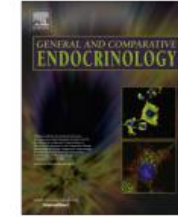
它在大脑和周围的广泛分布也表明Apelin在鱼类中可能具有多种生理调节作用。



Contents lists available at ScienceDirect

General and Comparative Endocrinology

普通与比较内分泌学 IF: 2.667



穴鱼

Peripheral injections of cholecystokinin, apelin, ghrelin and orexin in cavefish (*Astyanax fasciatus mexicanus*): Effects on feeding and on the brain expression levels of tyrosine hydroxylase, mechanistic target of rapamycin and appetite-related hormones



Carla C. Per...

Departments of Bio...

ARTICLE

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Feeding
Injection
Hormone
mRNA expression



...), apelin, ghrelin, and orexin on food intake in *A. mexicanus*. CCK (50 ng/g) induced a decrease in food intake (50 ng/g), and ghrelin (100 ng/g) induced an increase in food intake in control fish. In order to better understand the effects of injections on the brain expression levels of tyrosine hydroxylase (TH), and mechanistic target of rapamycin (mTOR), we performed peripheral injections of apelin and cocaine and amphetamine related transcript (CART). After brain apelin injections, apelin injections increased brain TH expression. In contrast, orexin treatment increased brain TH expression. CART and orexin brain expressions. Our results suggest that the enzymes mTOR and orexin all regulate food intake in cavefish.

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胆囊收缩素 (CCK) 是由小肠粘膜I细胞释放的一种肽类激素。

作用：

(一) 促进胰腺腺泡分泌各种消化酶，促胆囊收缩，排出胆汁。

(二) 作用于迷走神经传入纤维，通过迷走反射刺激胰酶分泌。

(三) 通过特效食物和身体内的各种酶的复合作用，帮助分解出蛋白质分解产物、脂肪酸盐、HCl等刺激CCK (胆囊收缩素) 分泌的分解因子，刺激下丘脑内侧，产生大量的饱腹信号。



胃饥饿素是胃内产生的一种肽，它能够调节食欲、进食和身体构成。
是热量限制与神经保护的代谢纽带。



Apelin 是一种多肽，前原蛋白由77个氨基酸组成，经内质网剪切成不同长度的活性多肽。其内生受体APJ是一种G蛋白偶联受体，在体内多种器官细胞上表达，主要包括中枢神经系统跟心血管系统。这种特性暗示了Apelin/APJ系统参与人体内多种生理过程。



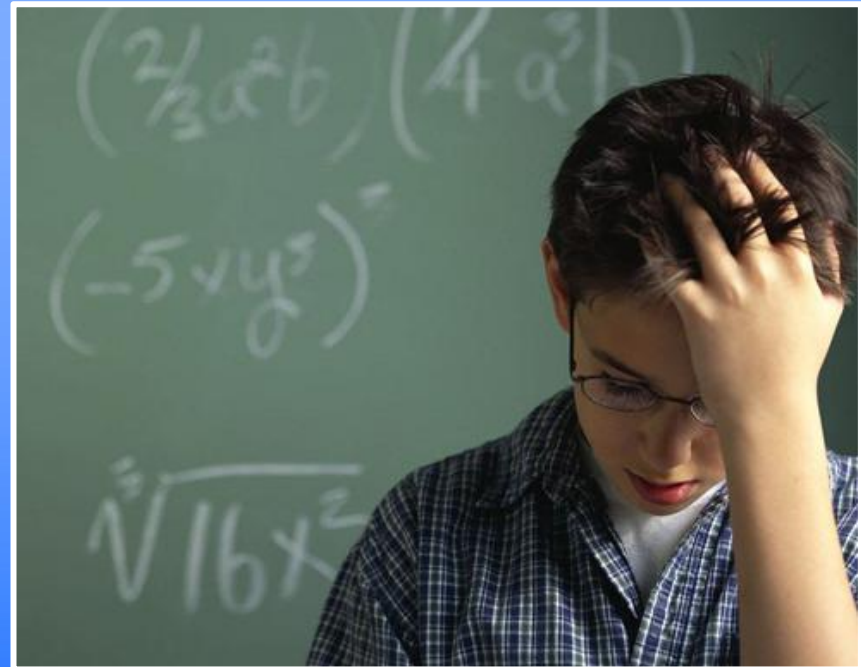
食欲肽(orexin)是由下丘脑特异性分泌的一种能调节睡眠、摄食及能量平衡的神经肽。包括食欲肽A和食欲肽B。

食欲肽会高度刺激跟觉醒状态有关的脑核及其相关的神经递质系统（例如包括多巴胺、去甲肾上腺素、抗组胺药及乙酰胆碱）。血中葡萄糖的高水平会抑制食欲肽的生产。



CART (可卡因和安非他命调节转录肽)

- 广泛分布于中枢及周围神经系统提示具有许多重要的生理功能，包括可能应激反应、摄食行为、免疫功能、自主调节、体液平衡、代谢过程、性功能和内分泌调节等。
- 对调节摄食及能量代谢的研究表明，在大鼠脑室内灌注重组的CART可抑制摄食，而给予抗体则可使摄食增加；正常大鼠禁食1-2天后，下丘脑CART的mRNA水平急剧下降，脑室内灌注CART可抑制饥饿引起的进食反应。因此，CART是一种食欲抑制因子。



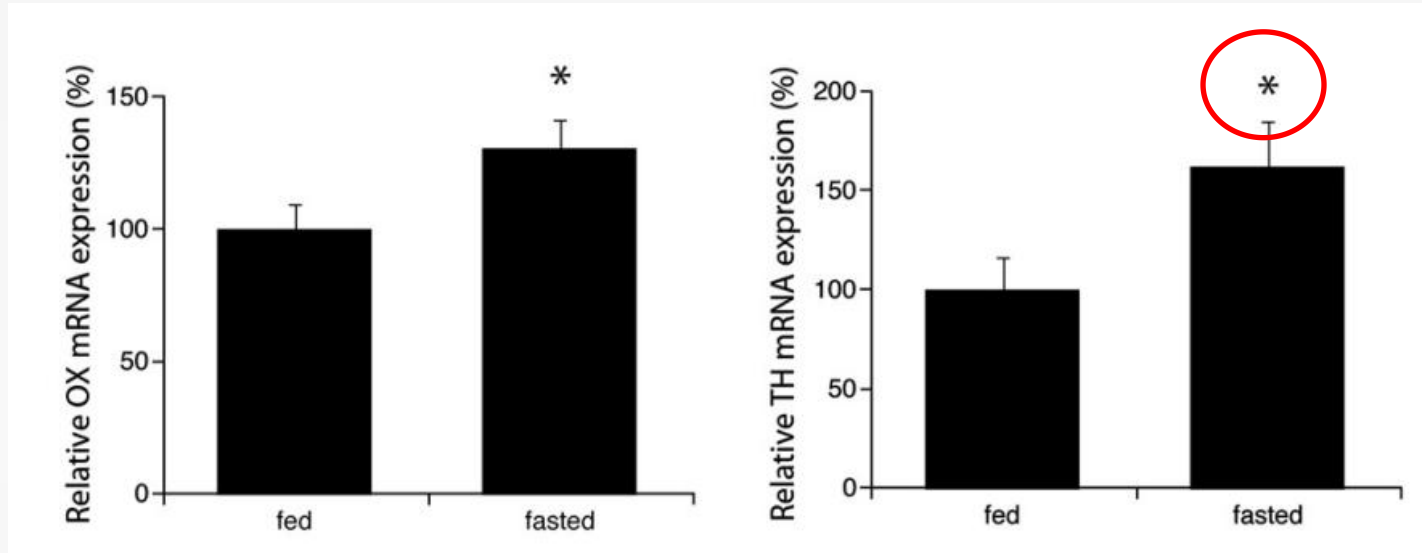
1) 酪氨酸羟化酶-TH

酪氨酸羟化酶（英语：Tyrosine hydroxylase）是负责催化氨基酸L-酪氨酸转变为二羟基苯丙氨酸（多巴）的酶。多巴是多巴胺的一个前体，相应地，后者亦是去甲肾上腺素与肾上腺素的前体。在人体中，酪氨酸羟化酶由TH基因编码出来。

此加氧酶被发现与所有含儿茶酚胺的细胞溶质中。此起始步骤是产生儿茶酚胺的限速步骤。

饥饿会使鱼类TH表达水平升高，说明TH起到调节脊椎动物摄食的作用

(Wall and Volkoff, 2013).



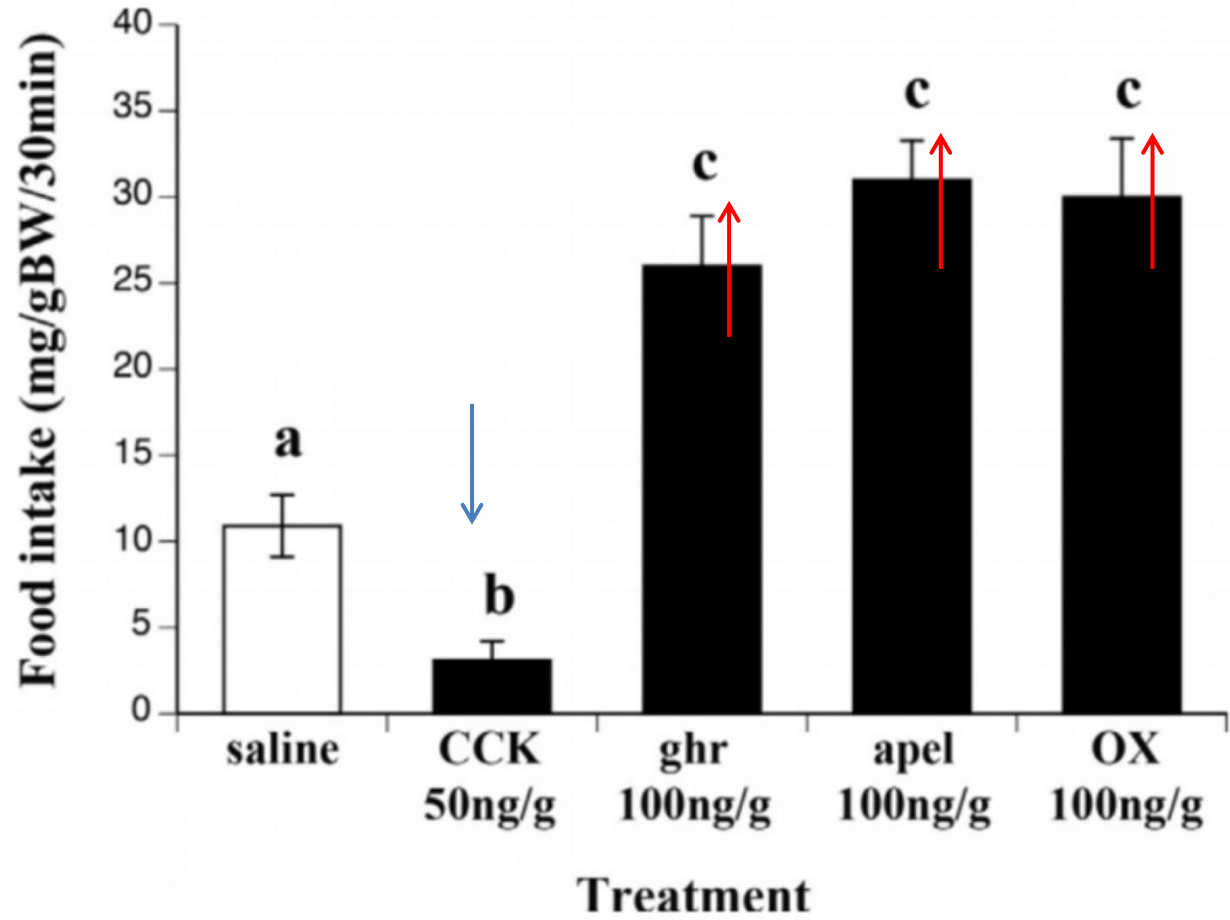
2) 雷帕霉素靶蛋白-mTOR

雷帕霉素靶蛋白 (mTOR) 是一种丝氨酸-苏氨酸蛋白激酶，是PI3K/Akt信号通路的重要组成部分，在感知细胞营养和能量状态中发挥关键作用。

在啮齿类动物中，禁食状态下弓状核 (ARC) 神经元的mTOR通路活性降低，而在喂食后活性升高(Woods et al., 2008; Xu et al., 2009)。

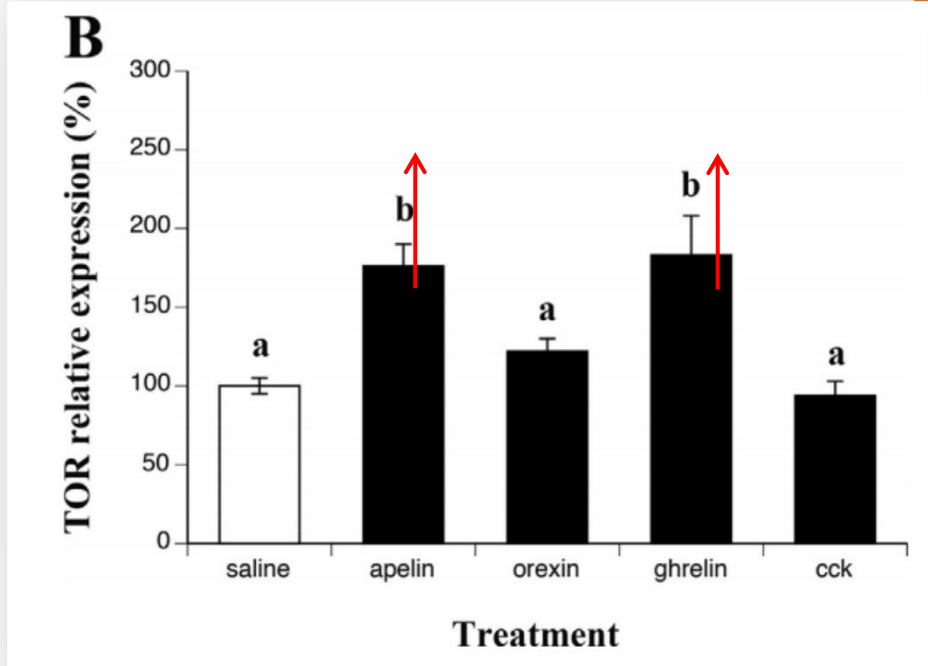
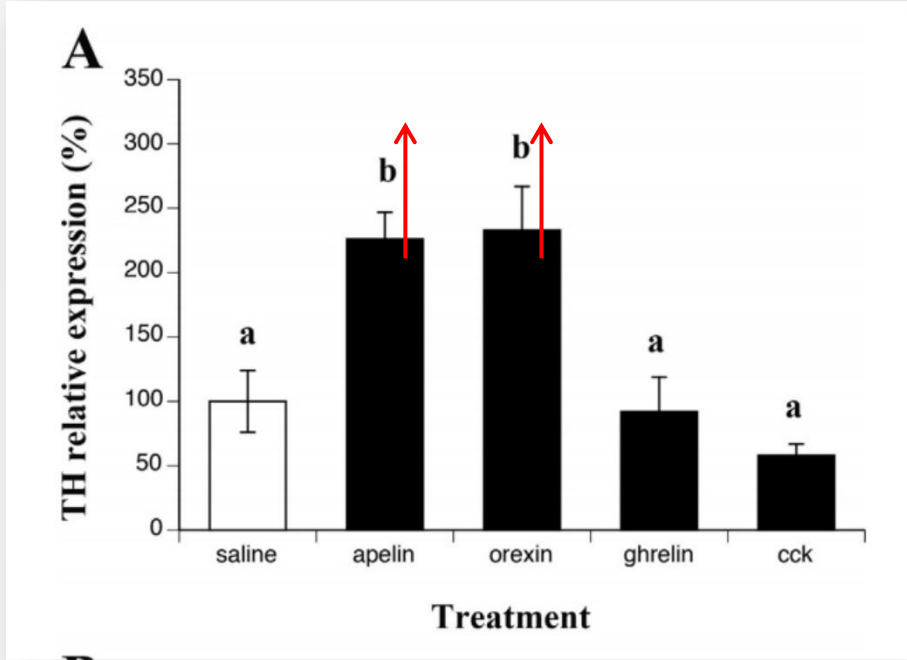
在斑马鱼中，禁食肝脏mTOR的表达降低(Craig and Moon, 2011)。

最近的哺乳动物中的证据显示mTOR通路和几个喂养相关的激素有关，包括NPY和CART (inhoff et al. , 2010)、胆囊收缩素 (lembke et al. , 2011)、生长素(Martins et al., 2012; Xu et al., 2010)，和瘦素 (Cota et al. , 2006)。



盐水 缩胆囊素 胃饥饿素 apelin 食欲肽

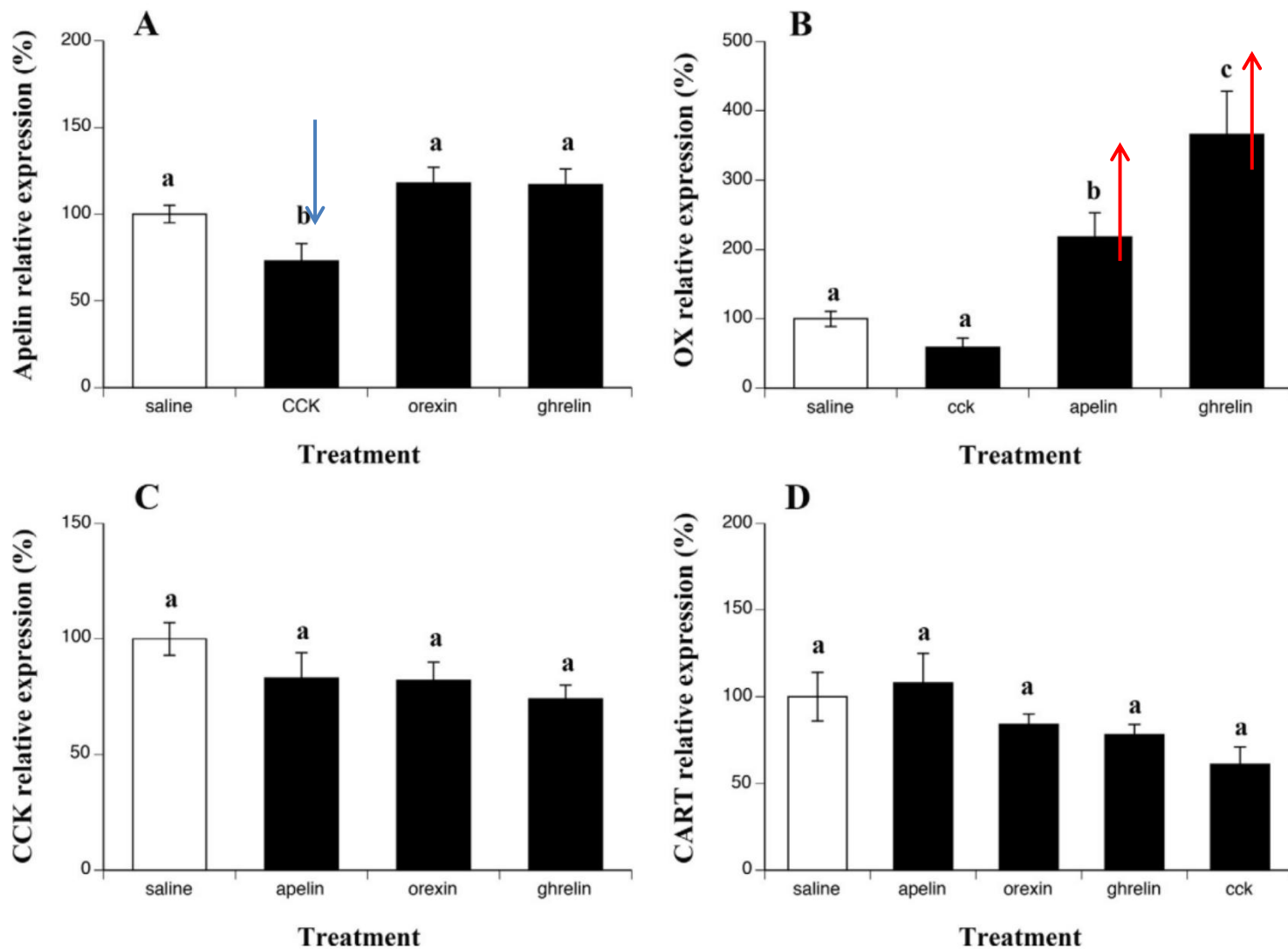
对摄食的影响



TH : 酪氨酸羟化酶

TOR : 雷帕霉素靶蛋白

Apelin-13对大脑两种代谢酶的影响



腹腔注射saline, CCK, ghrelin, apelin, 和orexin 后
全脑CCK, ghrelin, apelin, 和orexin的表达丰度

结果总结：

- 📍 CCK (50 ng/g) 降低了摄食，而apelin (100 ng/g) ， orexin (100 ng/g) 和Ghrelin (100 ng/g) 促进了穴鱼摄食；
- 📍 CCK降低了大脑apelin的表达；
- 📍 Apelin促进了大脑TH、 mTOR和orexin的表达上调；
- 📍 orexin促进了大脑TH的表达上调；
- 📍 Ghrelin促进了mTOR和orexin的表达上调；

CCK降低了摄食和大脑apelin的表达，但是不改变CART（一种食欲抑制因子），提示CCK可能是通过抑制apelin来抑制食欲。

注射Apelin和orexin促进了TH的表达，可能表明TH部分介导这两者的行为。事实上有研究表明，中枢儿茶酚胺与apelin (Xu et al., 2011) ， orexin (Eriksson et al., 2010) 相互作用。

Apelin和ghrelin促进mTOR的表达，说明这两者涉及细胞内PI3K/AKT/mTOR信号通路。

酶TH和mTOR以及激素CCK， apelin ， orexin 和Ghrelin通过一个复杂的相互作用网络来调控穴鱼的摄食。



ORIGINAL ARTICLE

Comparatively examining of the apelin-13 levels in the *Capoeta trutta* (Heckel, 1843) and *Cyprinus carpio* (Linnaeus, 1758)S. Köprücü¹ and S. Algül²

1 Fisheries Faculty, Firat University, 23119 Elazig, Turkey, and

2 Medicine Faculty, Firat University, 23119 Elazig, Turkey

Summary

Apelin is a recently discovered peptide produced by several tissues in the various vertebrates and fish. Apelin has been suggested to have role in regulation of many diverse physiological functions including food intake, energy homeostasis, immunity, osmoregulation and reproduction. In this study, apelin-13 levels in the blood serum of *Cyprinus carpio* and *Capoetta trutta* were determined. Then the results were compared between two species and sexes of each species. Apelin-13 level was analysed using the enzyme-linked immunoassay (ELISA) kit (Rat apelin-13 ELISA kit, catalog no: CSB-E14367r). Apelin-13 level in the blood serum of *C. trutta* was significantly higher than those of the *C. carpio* ($p < 0.05$). However, its levels were observed to be no significant difference ($p \geq 0.05$) that compared to between sexes of each species. There was a significant negative correlation ($r = -0.829$, $p = 0.0001$) between the apelin-13 level and body weight of *C. carpio*. However, no significant correlation ($r = -0.022$, $p = 0.924$) between the apelin-13 level and weight of *C. trutta* observed.

Keywords Apelin, *Cyprinus carpio*, *Capoetta trutta*, ELISA**Correspondance** S. Köprücü, Fisheries Faculty, Firat University, 23119 Elazig, Turkey. Tel: +90-424-2370000-4517; Fax: +90-424-2386287;

E-mail: skoprucu@firat.edu.tr

Received: 27 June 2014; accepted: 15 July 2014

鼠apelin-13
ELISA检测试剂盒

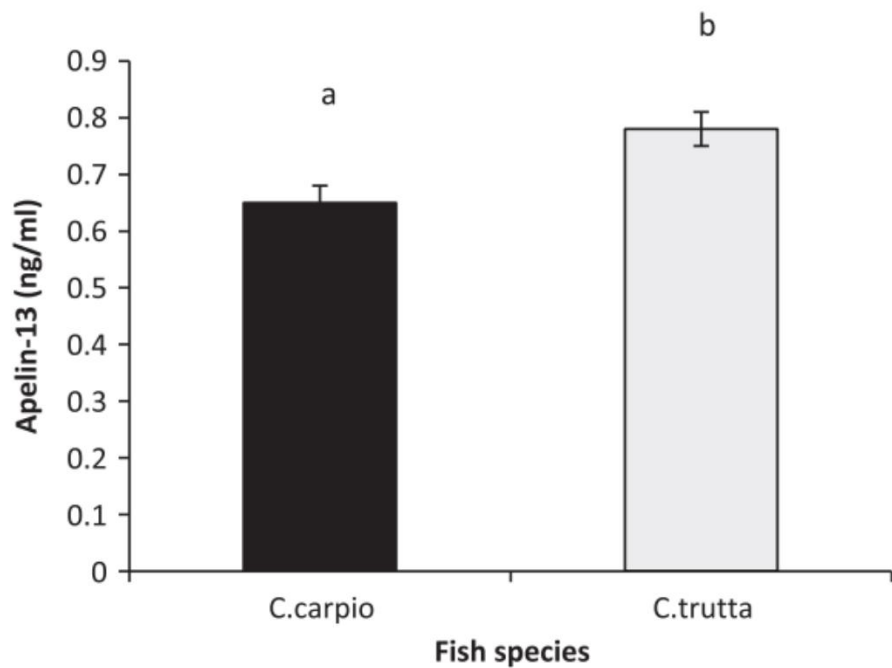


Fig. 1 Apelin-13 levels in the blood serum of *Capoeta trutta* (n = 21) and *Cyprinus carpio* (n = 23) (mean \pm SEM, $p < 0.05$).

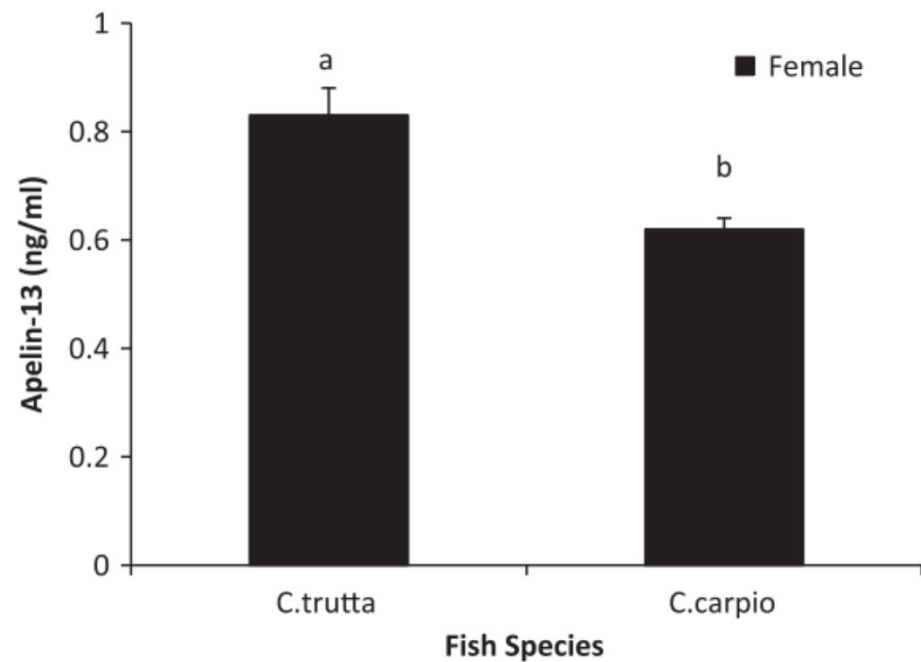


Fig. 4 Apelin-13 levels in the blood serum of female *Capoeta trutta* (n = 13) and *Cyprinus carpio* (n = 9) (mean \pm SEM, $p < 0.05$).

褐鲟apelin表达量高于鲤鱼

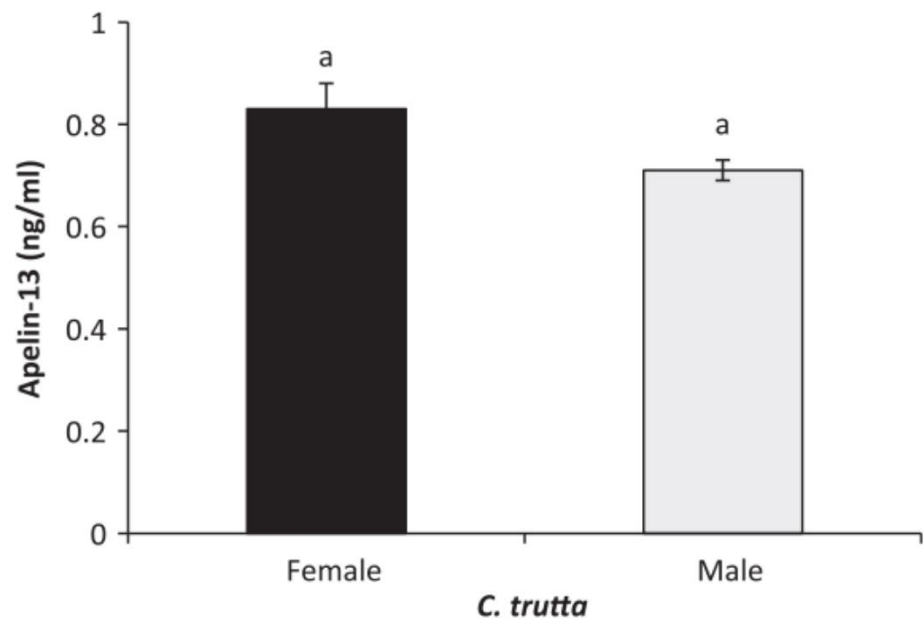


Fig. 2 Apelin-13 levels in the blood serum of male (n = 8) and female (n = 13) of *Capoeta trutta* (mean \pm SEM, p > 0.05).

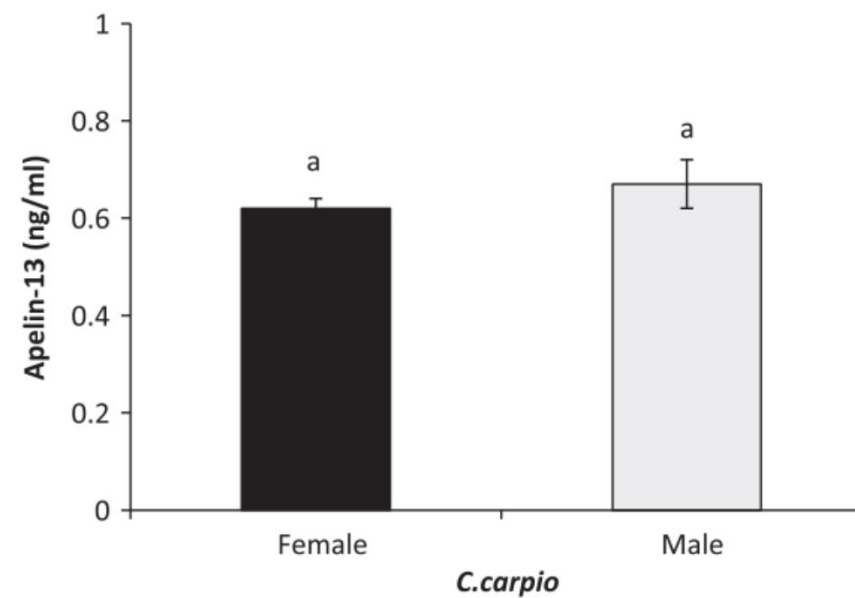
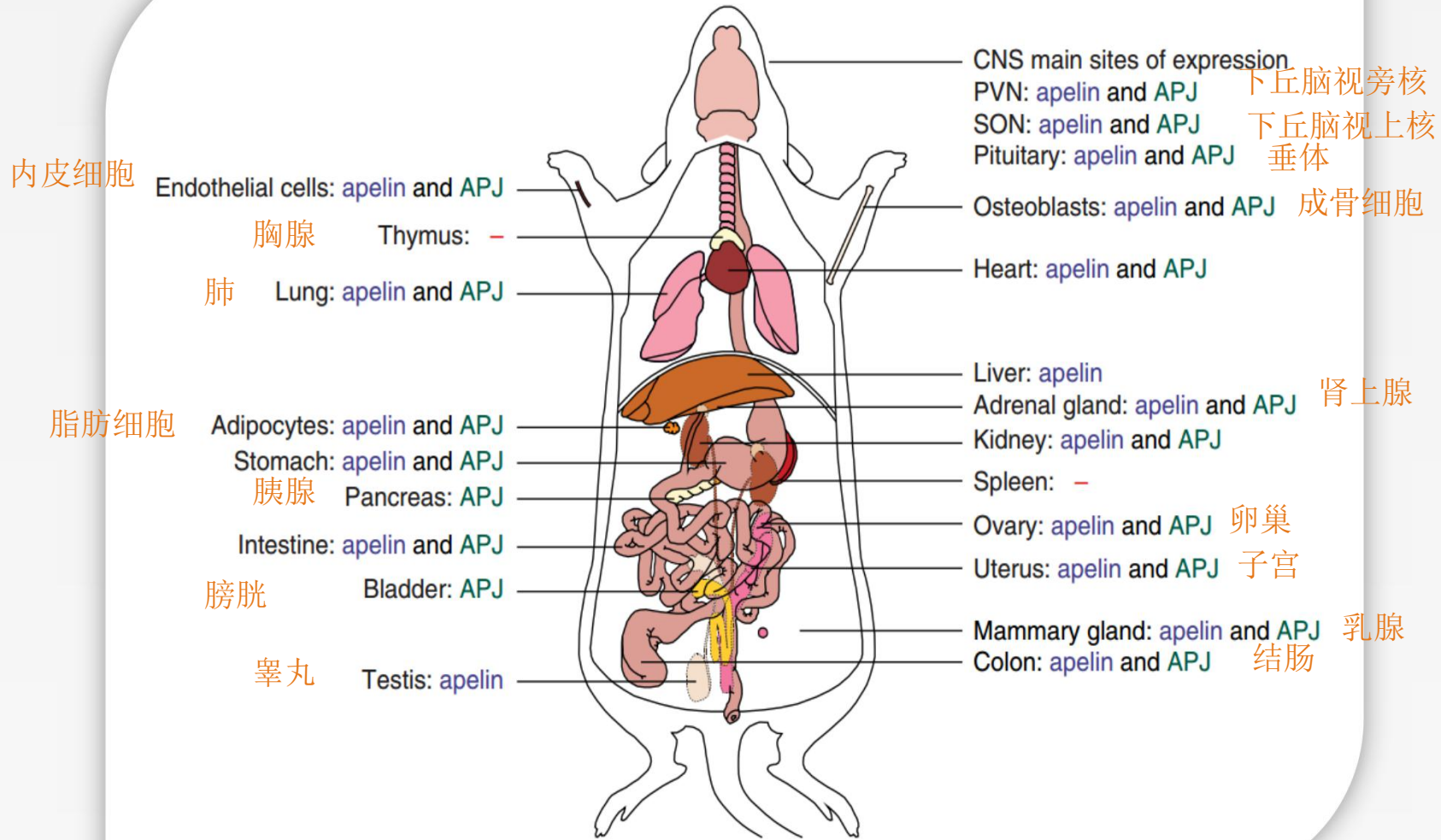


Fig. 3 Apelin-13 levels in the blood serum of male (n = 14) and female (n = 9) of *Cyprinus carpio* (mean \pm SEM, p > 0.05).

性别间没有显著性差异

总结讨论

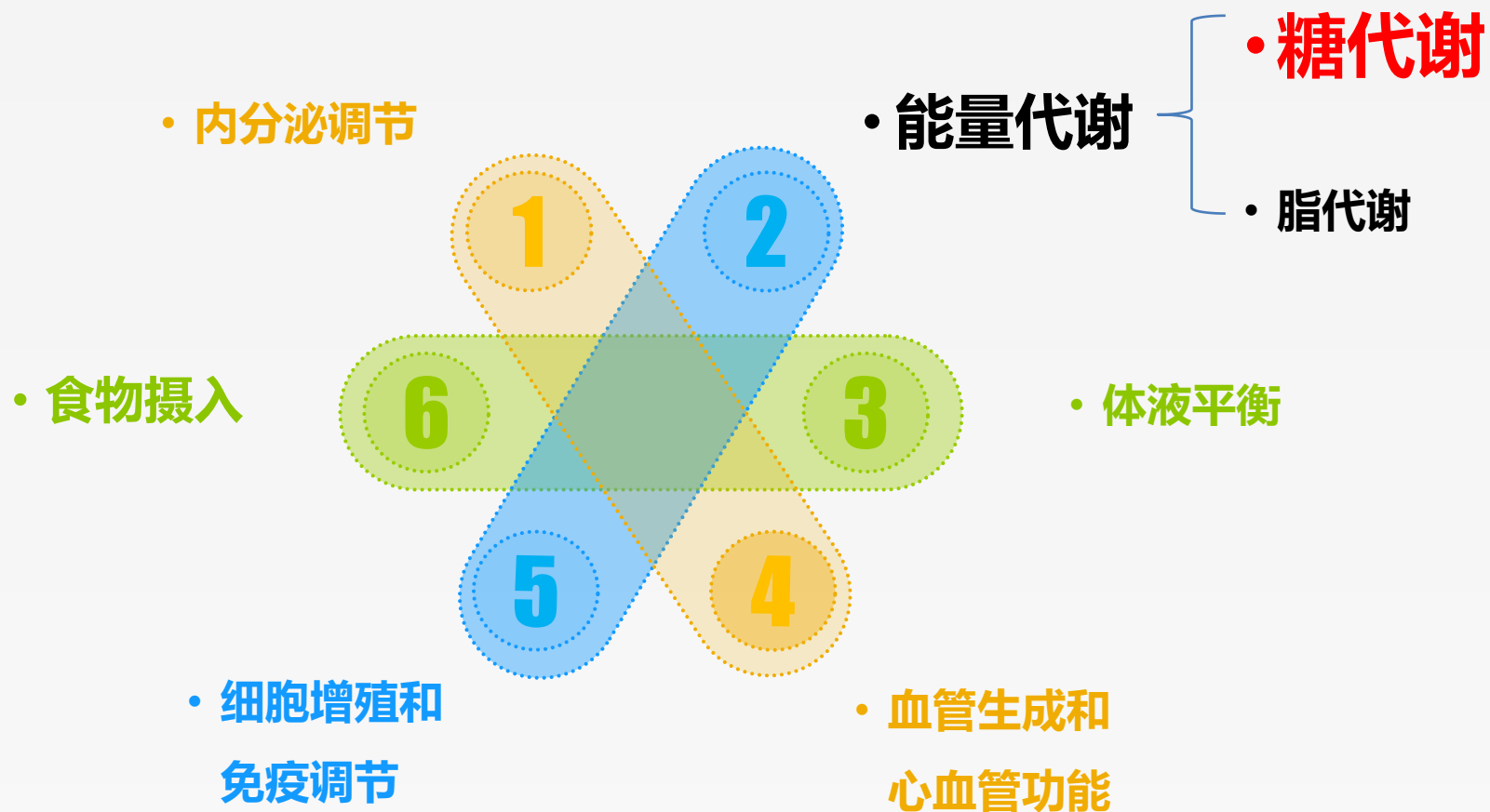


大鼠APJ和apelin组织分布

(O'Carroll et al., 2013, Journal of Endocrinology)

Apelin的生物学作用

总结讨论



Apelin组织分布及其功能分析

①脑（下丘脑和前脑）

下丘脑调节**体温、摄食、水平衡、血糖和内分泌腺活动**等重要的生理功能。

②心脏

在斑马鱼中，apelin缺乏或过量都会损害心脏的形成，具有调节心血管功能

③脾脏

Apelin抑制脾脏细胞因子的产生，参与免疫反应。

④肾脏

调节肾功能和体液平衡。

⑤性腺

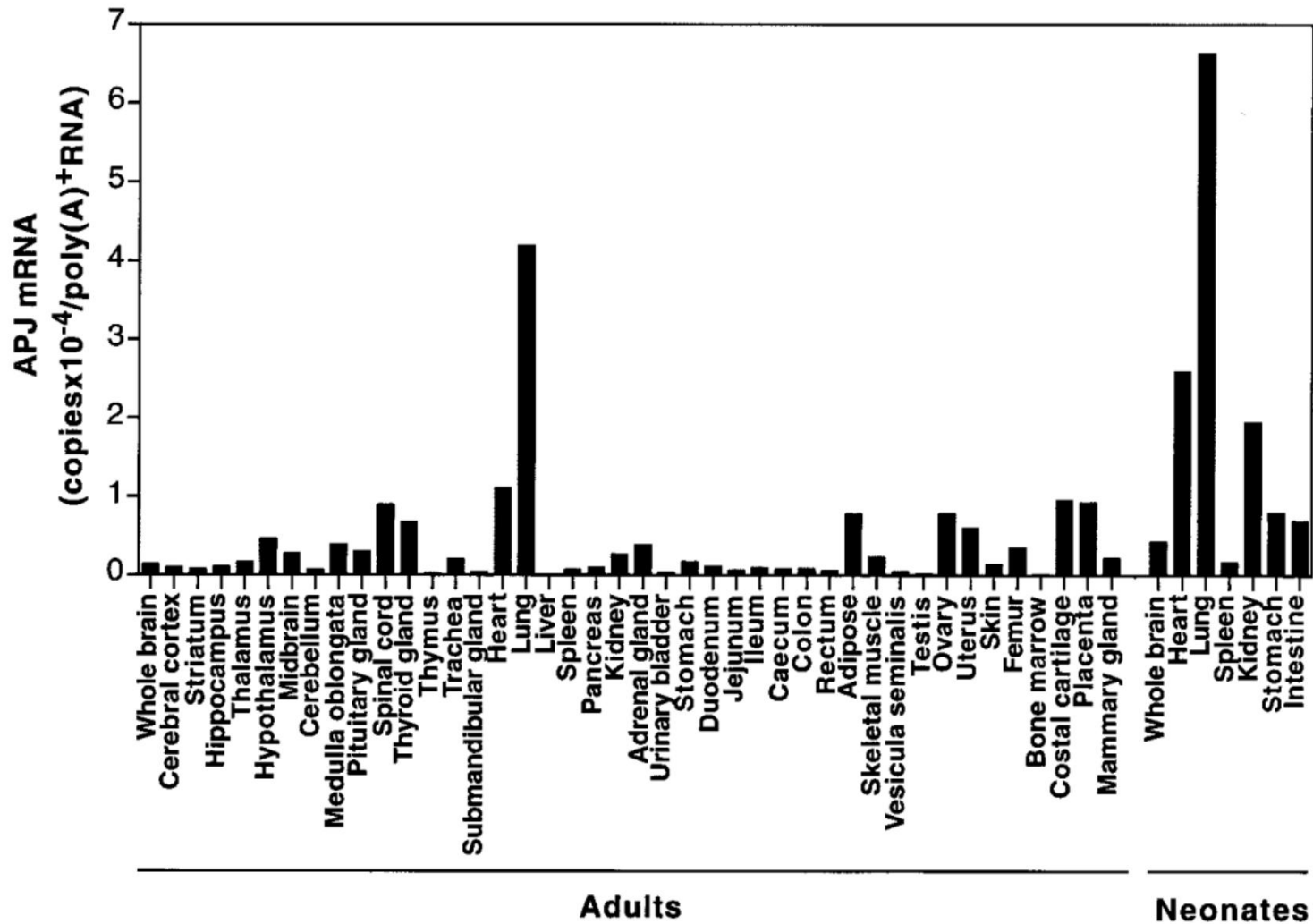
调节发育。

⑦肝脏

参与糖脂代谢。

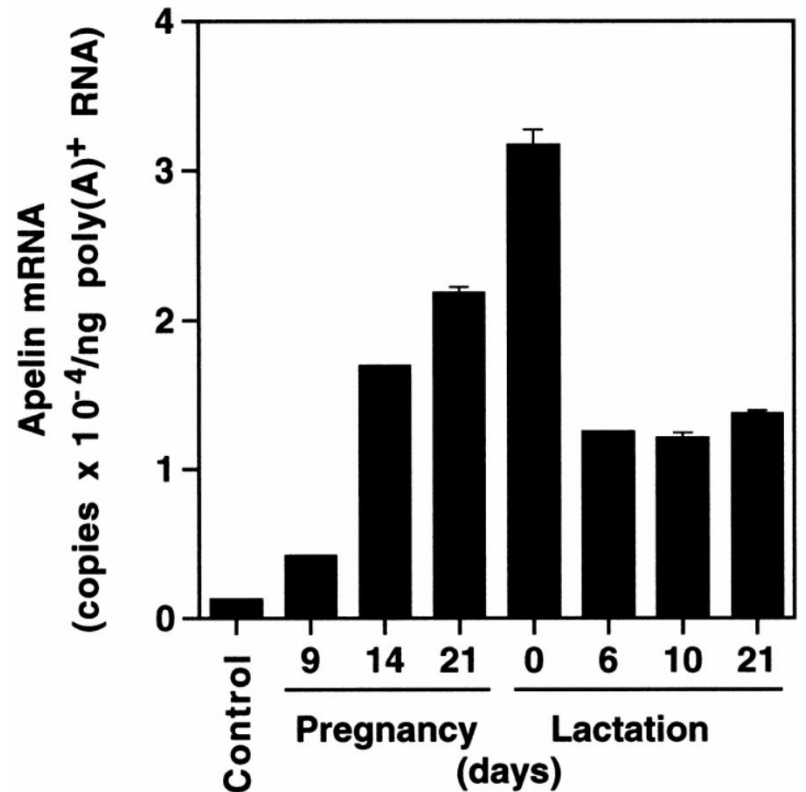
⑧肠道

参与糖脂代谢。



新生儿外周组织中APJ mRNA表达量高于成人。

说明Apelin-APJ系统调节发育。 (Hosoya, 2000)



大鼠乳腺apelin mRNA在妊娠期和哺乳期表达增加，并在分泌时达到最大值。

(Habata, 1999)

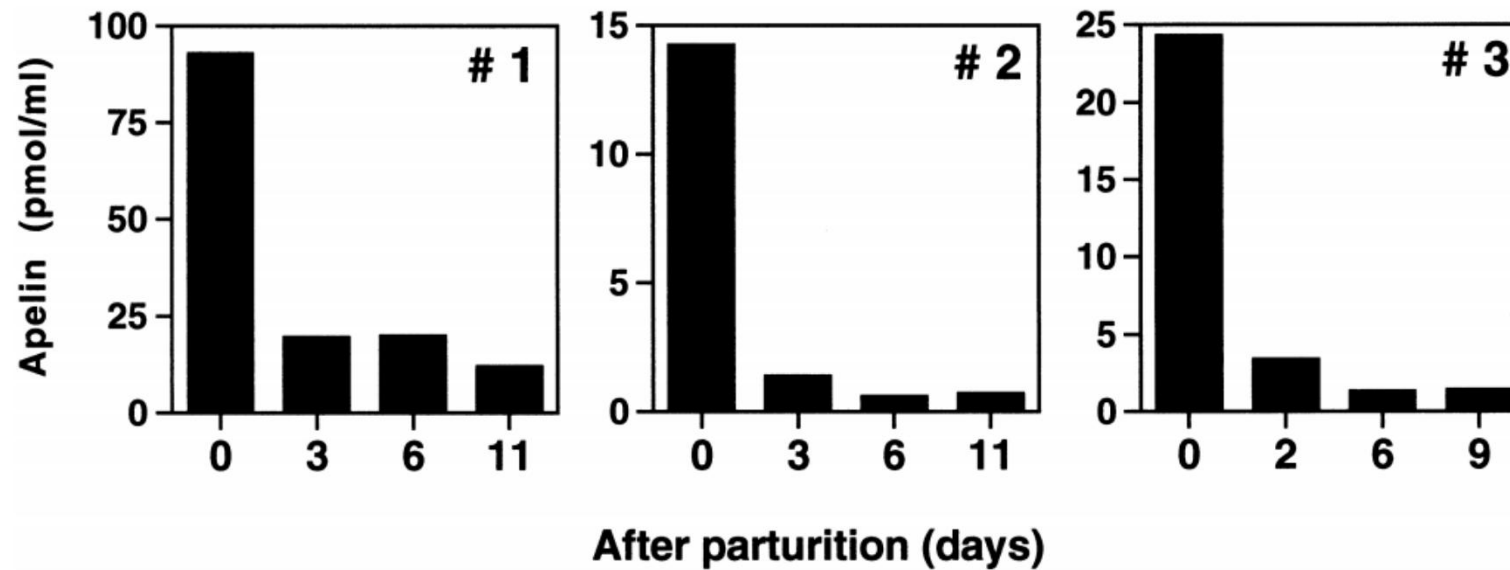


Fig. 5. Contents of apelin in bovine colostrum and milk. The contents of apelin were quantified on the basis of the cAMP-production-inhibitory activities in a manner similar to that described in Fig. 4. Bovine colostrum and milk were obtained from three Holstein cows (No. 1–3) on the indicated days after parturition.

Apelin 存在于牛初乳和牛奶中。

(Habata, 1999)

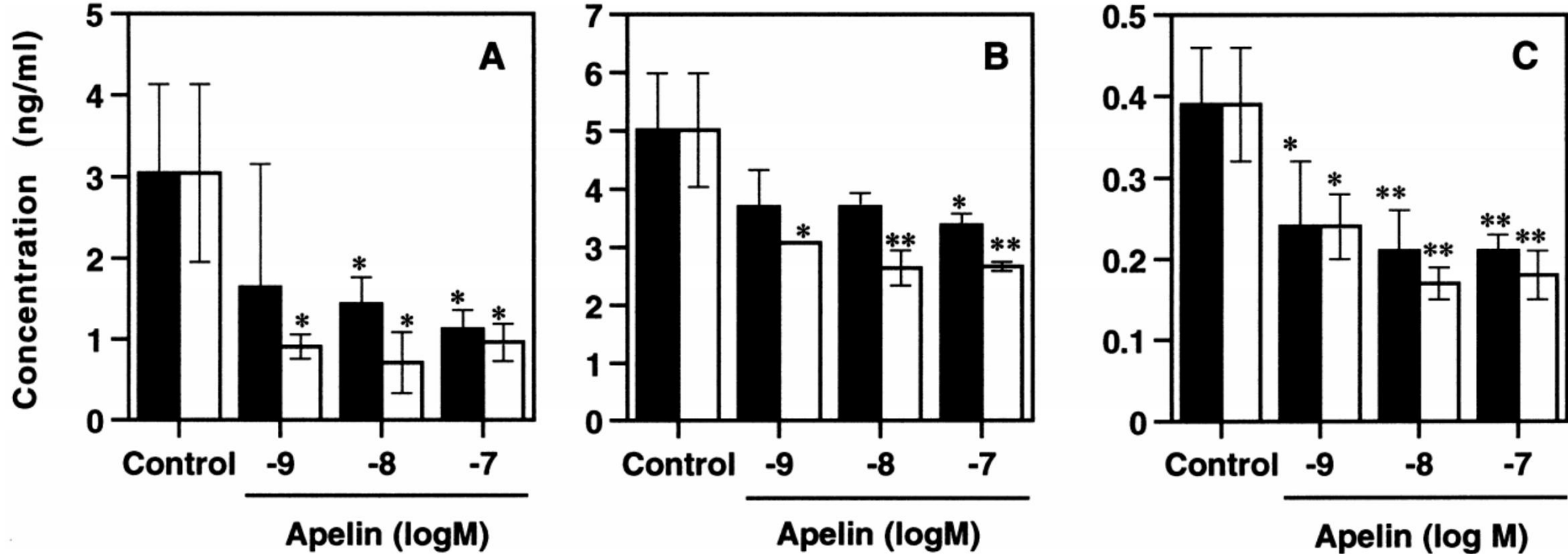


Fig. 6. Suppression of cytokine production in mouse spleen cells by apelin. Spleen cells obtained from BALB/c were cultured in microplates coated with anti-CD3 mAbs in the presence or absence (control) of apelin-36 (closed columns) and [pGlu]apelin-13 (open columns) at the indicated concentrations. The amounts of IFN- γ (panel A), IL-2 (panel B), and IL-4 (panel C) in culture supernatants were determined by EIA. Values represent mean \pm S.D. in triplicate assays. ** $P < 0.01$; * $P < 0.05$, as compared with a control in Student's t -test. Apelin抑制小鼠脾细胞产生细胞因子

1 细化设计，分析原因

组织分布和饥饿实验中，全脑检测和不同的脑组织间显示出差异性，这是因为脑的不同部位具有不同的分工，如下丘脑和端脑被证明是能量平衡和摄食调节的重要领域。

这提示我们在设计实验中应注意细化，并对不同的实验结果进行对比和分析。找出合理的解释和发现问题的关键。

2 大胆假设，小心求证

以组织分布为例，存在即合理，存在的原因和表达丰度的原因可以进行相应的推测和分析。在哺乳动物实验中，有些地区，如嗅区、犁区、皮质和齿状回有APJ的分布，却没有apelin的产生。这可能意味着在这里合成APJ,然后运输到其他地区，在那里有配体的产生，这个地方APJ才具有功能性；也有可能是APJ表达量低于检测值；也有可能是抗体敏锐性低；亦或许该地区存在其他位置相关受体。同时实时定量与免疫组化、原位杂交的结果也不尽相同。

这提示我们应**广泛查阅相应文献，推测应有理有据。**

下丘脑

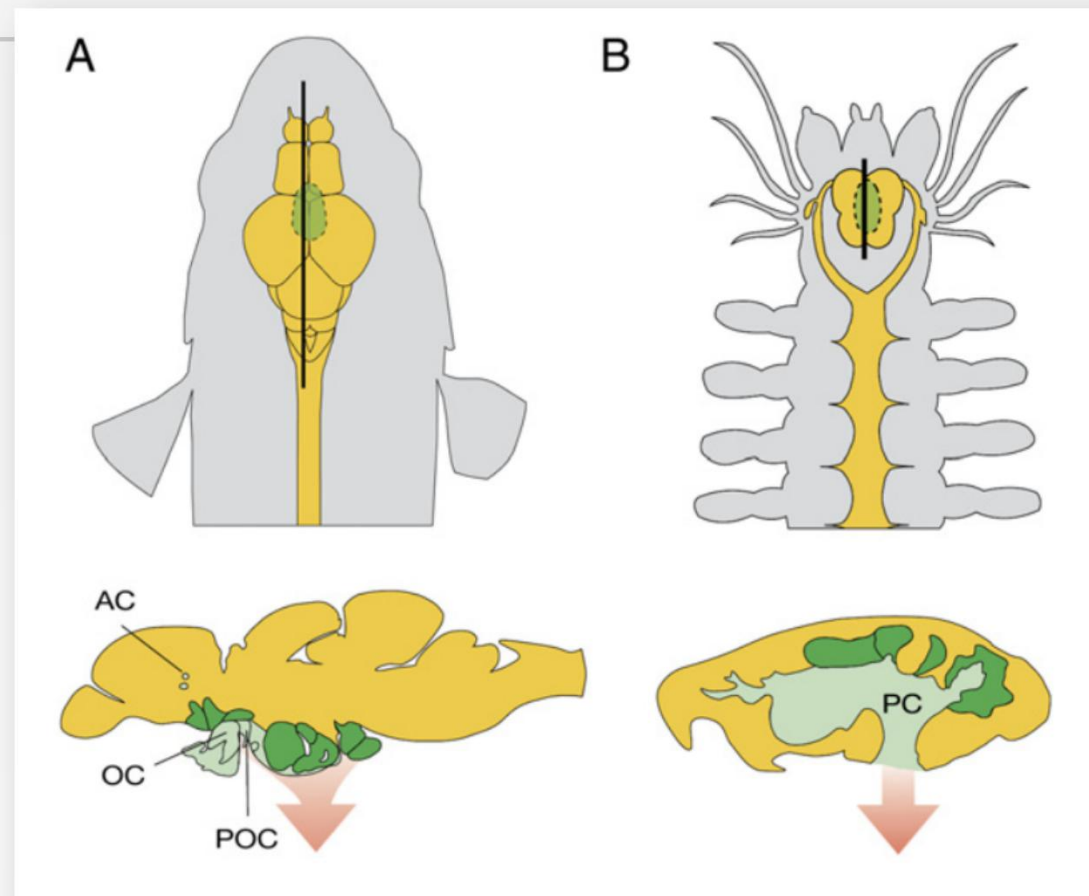


Figure 1. Neuroendocrine Centers in the Fish and Nereidid Forebrain

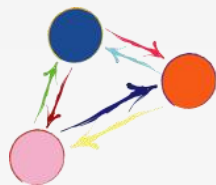
(Top) Schematic dorsal views of adult zebrafish (A) and nereidid (B) heads, indicating the CNS (yellow) and the main medial neurosecretory brain centers (green). Black lines: positions of sections. (Bottom) Respective parasagittal sections, showing main neurosecretory brain nuclei (green) and main sites of neurosecretory release (red arrow). Characteristic commissures and neuropil, light green; AC, anterior commissure; OC, optic chiasm; POC, postoptic commissure; PC, preoral commissure. Data integrated from Matsumoto and Ishii, 1992; Thorndyke and Goldsworthy, 1988; Wullimann et al., 1996.

(Tessmar-Raible, 2017, Cell)

3 细化设计，分析原因

APJ和apelin饥饿实验中的差异，可能是由于另一受体（APJa）介导apelin对喂养的远期影响。**这提示我们在接下来的实验中，应分别检测两个受体。**

4 A → B, or



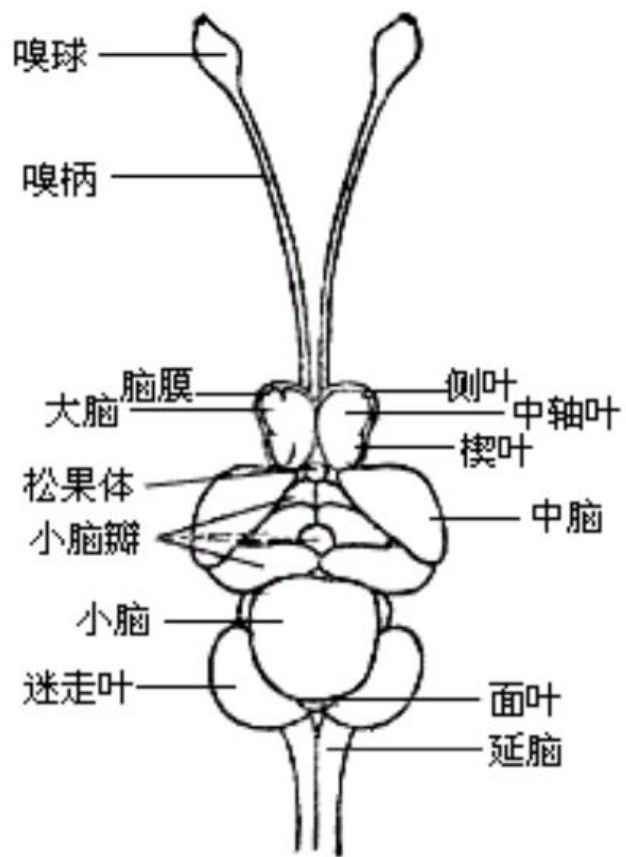
CCK, apelin, orexin 和 Ghrelin 均为食欲相关因子，其相互作用调节摄食，另外如 AgRP/NUCB2 和 PYY 均参与食欲调节。**这提示我们应在实验中检测相关因子和通路之间的互动，这将为揭示机理（如代谢）提供更多的思路和更好的解释。**



Thanks

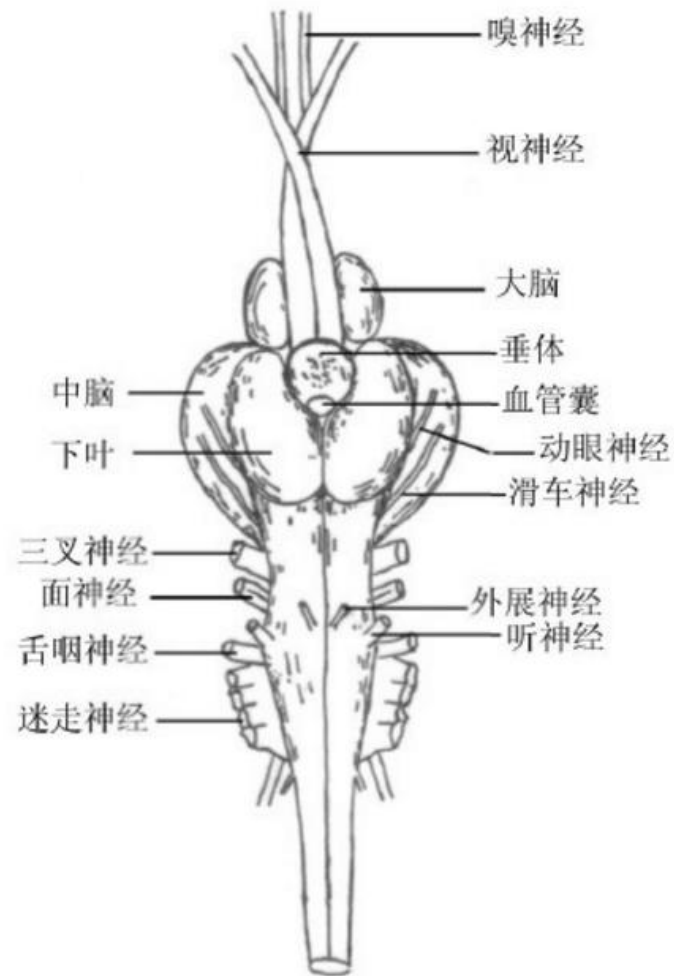
感谢您的聆听

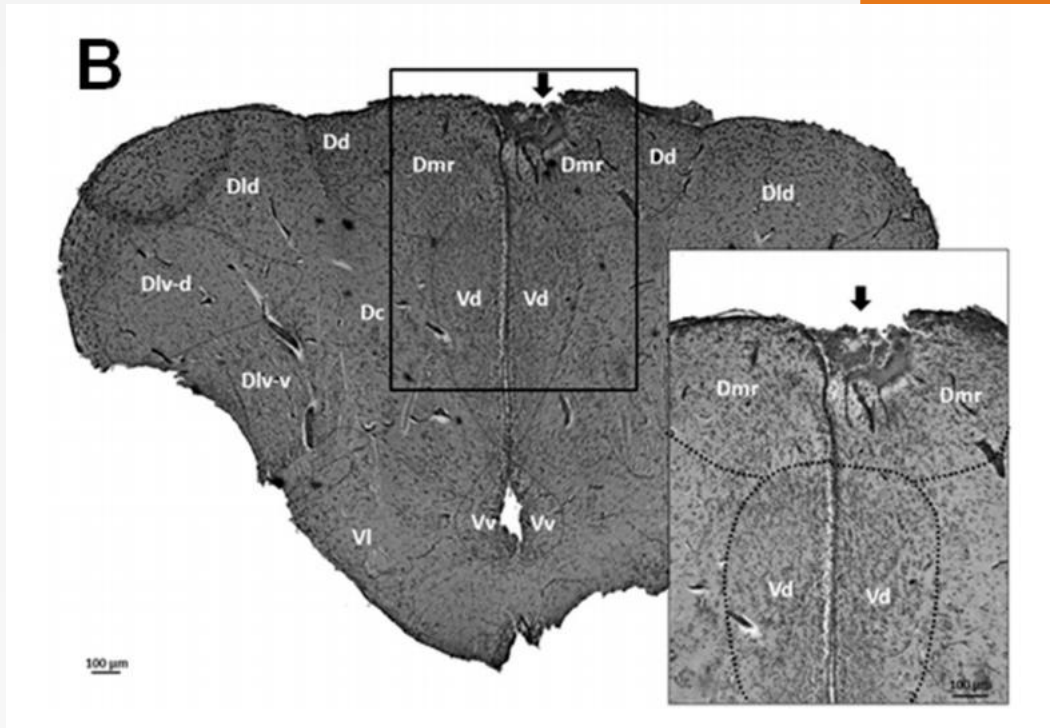
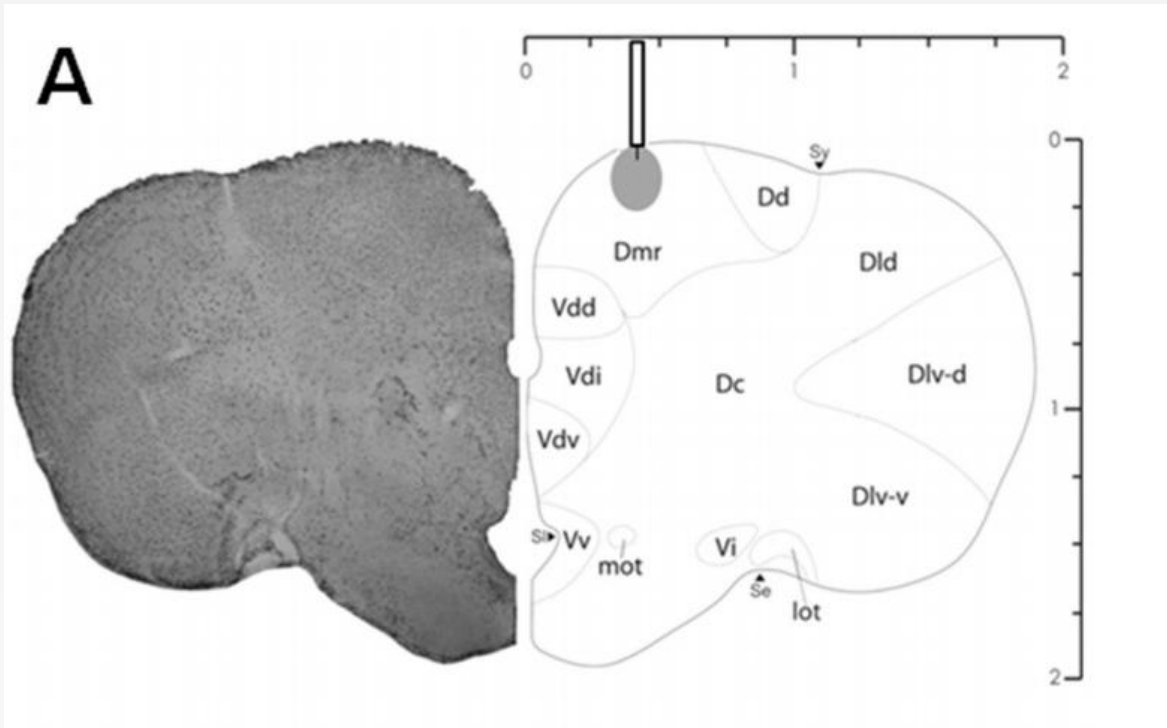
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鲤鱼的脑（背侧面）





1) 酪氨酸羟化酶-TH

酪氨酸羟化酶（英语：Tyrosine hydroxylase）是负责催化氨基酸L-酪氨酸转变为二羟基苯丙氨酸（多巴）的酶。多巴是多巴胺的一个前体，相应地，后者亦是去甲肾上腺素与肾上腺素的前体。在人体中，酪氨酸羟化酶由TH基因编码出来。

此加氧酶被发现与所有含儿茶酚胺的细胞溶质中。此起始步骤是产生儿茶酚胺的限速步骤。

儿茶酚胺是一种含有儿茶酚和胺基的神经类物质。儿茶酚胺（CA）包括去甲肾上腺素（NA或NE）、肾上腺素(Ad或E)和多巴胺（DA）。

儿茶酚胺的作用

- 1、对代谢的作用：儿茶酚胺参与生热作用的调节，通过 β 受体增加氧耗量而产热。并可促进机体内储备能量物质的分解。
- 2、儿茶酚胺对细胞外液容量和构成及水、电解质的代谢有重要的调节作用。
- 3、儿茶酚胺可引起肾素、胰岛素和胰高血糖素、甲状腺激素、降钙素等多种激素分泌的变化。