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NUCB2/nesfatin-1 对小鼠摄食行为的调控及机制*

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摘要 目的:探讨 NUCB2/nesfatin-1 对小鼠摄食行为的调控及机制。**方法:**利用侧脑室埋管,免疫组化染色等方法,探讨侧脑室和外周注射 nesfatin-1 对小鼠摄食行为的影响。**结果:**侧脑室注射不同剂量 nesfatin-1 (0.3 μ g, 1 μ g, 3 μ g), 注药后 4 h 夜间进食量明显减少, 且呈显著剂量依赖关系($t=2.61\sim4.78$, $P<0.05\sim0.01$), 侧脑室注射 3 μ g nesfatin-1, 小鼠前 3 小时累积摄食量明显降低($t=8.69\sim10.73$, $P<0.01$), 且持续降低 12 小时($t=2.64$, $P<0.05$), 同时餐间间隔时间明显延长($t=2.66$, $P<0.05$), 每分钟/1-4 h 进食量明显降低 ($t=2.63$, $P<0.05$), 且进食每克食物所用时间明显增加 ($t=3.02$, $P<0.05$)。在下丘脑弓状核, 外侧区和背内侧核均有 NUCB2/nesfatin-1 免疫阳性神经元表达。皮下或腹腔注射 nesfatin-1, 小鼠进食量和进食行为均无显著改变($P>0.05$)。**结论:**中枢 nesfatin-1 可抑制小鼠摄食行为。

关键词:进食; 下丘脑; 小鼠; nesfatin-1; 饱足

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Regulatory Effect of NUCB2/nesfatin-1 on Feeding Behavior in Mice and the Potential Mechanism *

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ABSTRACT Objective: To investigate the regulatory effect of NUCB2/nesfatin-1 on feeding behavior in mice and the potential mechanism. **Methods:** Nesfatin-1 was injected intracerebroventricularly (icv) by lateral ventricle buried pipe and peripherally (intraperitoneal, i.p, subcutaneous, s.c) on mice. Nesfatin-1 immunostaining was performed. **Results:** Nesfatin-1 (0.3, 1 or 3 μ g/mouse, icv) caused a dose-dependent reduction of 4-h dark phase food intake ($t=2.61\sim4.78$, $P<0.05\sim0.01$). Nesfatin-1 (3 μ g/mouse, icv) led to the reduction of 3-h cumulative food intake ($t=8.69\sim10.73$, $P<0.01$) lasting for 12 h ($t=2.64$, $P<0.05$), significantly increased inter-meal intervals ($t=2.66$, $P<0.05$) and time spent in meals ($t=3.02$, $P<0.05$) and significantly decreased meal size ($t=2.63$, $P<0.05$). NUCB2/nesfatin-1-immunopositive neurons were found in the hypothalamic arcuate nucleus, lateral and dorsomedial hypothalamic nuclei. When injected peripherally, neither food intake nor any feeding microstructure parameters were altered. **Conclusion:** The central nesfatin-1 leads to a dose-dependent reduction of food intake in mice.

Key words: Food intake; Hypothalamus; Mouse; Nesfatin-1; Satiation

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前言

Nesfatin-1 是 Oh-I 等从大鼠下丘脑 NUCB2 基因中发现的^[1]。禁食 24 h 的大鼠, PVN(室旁核)NUCB2/nesfatin-1 mRNA 表达显著降低^[1]。侧脑室、第三或第四脑室注射 nesfatin-1, 均可引起大鼠夜间进食量的减少^[2,3]。但 nesfatin-1 对进食行为的改变机制仍不清楚。本研究拟采用脑室注射, 探讨 nesfatin-1 对小鼠摄食行为的影响及机制。有研究表明, 大鼠禁食 24 h 后胃 NUCB2 mRNA 和血浆 NUCB2/nesfatin-1 水平均显著降低^[4],

提示外周 nesfatin-1 可能有潜在调控进食作用。目前仅有一项研究显示, 腹腔注射 nesfatin-1 后可减少小鼠夜间进食^[5], 而大鼠无此作用^[4]。因此, 本研究我们还比较给自由进食小鼠侧脑室、腹腔或皮下注射 nesfatin-1, 观察小鼠夜间摄食及摄食行为的影响。

1 材料与方法

1.1 动物分组

成年雄性昆明小鼠(6-8 周龄)48 只, 随机分为 NS 对照组

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(16只)和实验组(32只)。小鼠在特定温度(25 ± 2) $^{\circ}\text{C}$, 24小时昼夜循环光照条件下生活,自由进食和水。

1.2 侧脑室埋管

经腹腔注射硫酸仲丁巴比妥(100~150 mg/kg)麻醉小鼠,参照小鼠脑立体定位图谱,将自制同芯套管植入右侧脑室,并固定。术后5天进行实验。

1.3 进食实验

32只小鼠(每组8只)侧脑室分别注射1 μL nesfatin-1(0.3 μg , 1 μg , 3 μg)或1 μL NS,随之观察24 h进食量。另取小鼠16只,分别腹腔注射100 μL nesfatin-1(70 μg)或100 μL NS,随之监测24 h进食量。进食量=实验前食物量-实验后食物量。

1.4 免疫组化染色

小鼠经腹腔注射硫酸仲丁巴比妥(100~150 mg/kg)麻醉,4%多聚甲醛经心灌注,取脑、后固定。将脑置于10%蔗糖24 h,冰冻切片机切片。切片经含 nesfatin-1 抗体(1:200,美国Phoenix公司)的孵育液中孵育过夜(4 $^{\circ}\text{C}$),PBS缓冲液洗涤后,

切片用生物素羊抗兔 IgG(1:500,美国 Phoenix 公司)室温孵育1 h, DAB 显色。切片经梯度乙醇和二甲苯脱水,封片。光镜下观察结果。对照组以正常兔血清代替一抗,其他步骤同上。

1.5 统计学分析

所有数据用均数 $\pm$ 标准差($\bar{x} \pm s$)表示,用 GraphPad Prism 3.0 软件分析,两组间比较采用 t 检验, $P < 0.05$ 有统计学意义。

2 结果

2.1 侧脑室注射 nesfatin-1 对小鼠摄食的影响

与 NS 对照组相比,侧脑室注射不同剂量 nesfatin-1 (0.3 μg , 1 μg , 3 μg), 小鼠注药后前4 h的夜间进食量明显减少,且呈显著剂量依赖关系;与 NS 对照组比较,注射1 μg 和3 μg 组差异有统计学意义($t=2.61\sim 4.78$, $P < 0.05\sim 0.01$,图1A)。与注射 NS 对照组相比,侧脑室注射3 μg nesfatin-1,抑制小鼠进食主要发生在注射后的2-3 h ($t=17.0$, $P < 0.01$,图1B)和3-4 h ($t=11.6$, $P < 0.01$ 图1B)。

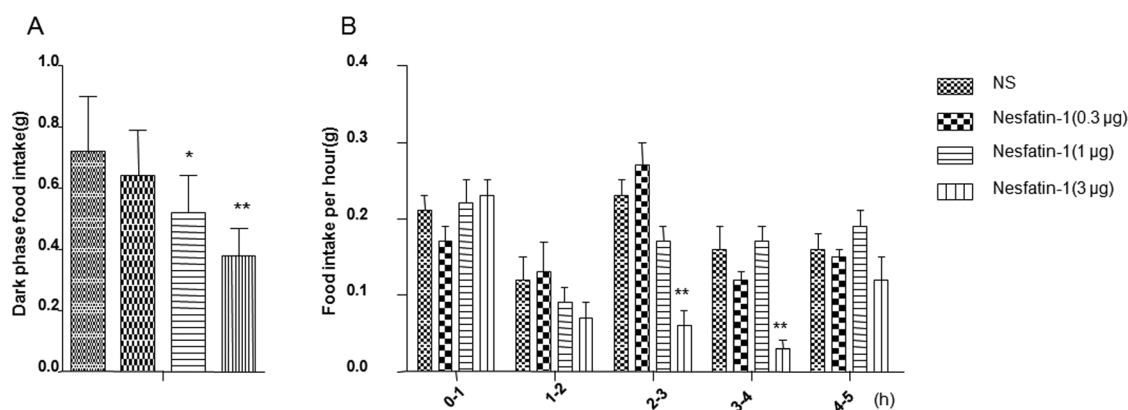


图1 侧脑室注射 nesfatin-1 对小鼠摄食的影响

Fig.1 The effect of different dose of nesfatin-1 on food intake in ad libitum fed mice

注:(A)侧脑室注射前后4h夜间进食量;(B)侧脑室注射后每小时进食量,

* $P < 0.05$, ** $P < 0.01$,与 NS 对照组相比。

Notes: (A) Dark food intake was assessed for the first 4 h post icv injection; (B) food intake per hour post icv injection,

* $P < 0.05$, ** $P < 0.01$ vs. NS control group.

因为侧脑室注射3 μg nesfatin-1可引起小鼠摄食量改变,因此之后我们仅对该剂量进行了累积摄食量研究。与 NS 对照组相比,侧脑室注射3 μg nesfatin-1,小鼠前3小时累积摄食量开始明显降低($t=4.69\sim 5.73$, $P < 0.01$; Fig. 2A),一直持续到12小时($t=2.64$, $P < 0.05$; Fig. 2B)。但如果阶段性观察小鼠摄食量,与 NS 对照组比较,侧脑室注射3 μg nesfatin-1,只见0-4小时区间的摄食量明显减少($t=5.74$, $P < 0.01$; Fig. 2C),而其他区间无显著改变($P > 0.05$; Fig. 2C)。

上述结果显示,侧脑室注射3 μg nesfatin-1后1-4h的小鼠摄食量明显减少,因此,我们又进一步分析了小鼠进食行为变化。结果显示,侧脑室注射3 μg nesfatin-1后,小鼠进食次数明显降低($t=3.43$, $P < 0.01$; 表1),进食量也明显减少($t=2.84$, $P < 0.05$);但小鼠每次进餐持续时间($t=1.45$, $P > 0.05$),1-4 h的总进食时间($t=1.74$, $P > 0.05$)以及每次进食时间的百分率($t=1.74$, $P > 0.05$)均无显著变化。与侧脑室注射 NS 组相比,侧脑室注射3 μg nesfatin-1,小鼠餐间间隔时间明显延长($t=2.66$, $P < 0.05$),每

分钟/1-4 h的进食量明显降低($t=2.63$, $P < 0.05$),且进食每克食物所用时间明显增加($t=3.02$, $P < 0.05$)。但侧脑室注射 nesfatin-1 后的反应不是立即发生的,我们发现,小鼠第一餐延迟时间无显著改变($t=1.45$, $P > 0.05$; 表1),第一餐持续时间也无显著变化($t=1.20$, $P > 0.05$),第一餐进食速度也变化不明显($t=1.44$, $P > 0.05$)。

2.2 小鼠下丘脑 nesfatin-1 的表达

免疫组化显示,弓状核(Arc, Fig. 3A)的腹内侧和背外侧区、下丘脑外侧区(LHA, Fig. 3B)和下丘脑背内侧核(DMH, Fig. 3C)均有 NUCB2/nesfatin-1 免疫阳性神经元表达。

2.3 腹腔注射或皮下注射 nesfatin-1 对小鼠进食的影响

表2显示,与侧脑室注射不同,与 NS 对照组相比,给小鼠腹腔注射70 μg /100 μL nesfatin-1,小鼠24 h内进食量无显著变化($t=1.09$, $P > 0.05$,表2)。同样,皮下注射 nesfatin-1(70 μg /100 μL),小鼠进食量也无显著改变($t=1.02$, $P > 0.05$,表2)。在此期间,小鼠每小时食物摄入量也无显著变化($t=0.72$, $P >$

0.05,表 2),且腹腔或皮下注射 nesfatin-1 与 NS 对照组相比,小鼠进食行为也无明显变化($t=1.12, P>0.05$,表 2)。

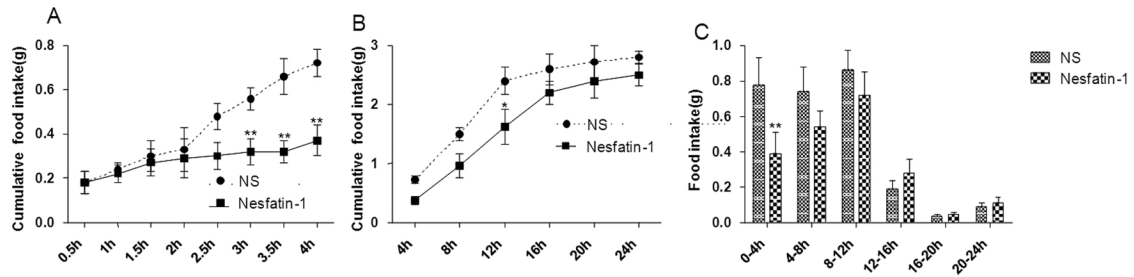


图 2 侧脑室注射 nesfatin-1 对小鼠累积进食量的影响

Fig.2 The effect of nesfatin-1 injected intracerebroventricularly on cumulative food intake in ad libitum fed mice

注:(A、B)侧脑室注射 nesfatin-1 对小鼠累积进食量的影响;(C)侧脑室注射 nesfatin-1 对小鼠每 4 小时进食量的影响 * $P<0.05$, ** $P<0.01$,与 NS 对照组相比。

Note: (A, B) The effect of nesfatin-1 injected intracerebroventricularly on cumulative food intake in ad libitum fed mice; (C) The effect of nesfatin-1 injected intracerebroventricularly on food intake/4-h periods in ad libitum fed mice.* $P<0.05$, ** $P<0.01$ vs. NS control group.

表 1 侧脑室注射 nesfatin-1 对小鼠摄食行为的影响($\bar{x} \pm s$)

Table 1 The effect of nesfatin-1 injected intracerebroventricularly on feeding behavior in ad libitum fed mice ($\bar{x} \pm s$)

Group	NS	Nesfatin-1
Meal frequency (number/4 h)	3.68 $\pm$ 1.02	1.89 $\pm$ 0.12 **
Bout frequency (number/4h)	13.8 $\pm$ 3.2	12.1 $\pm$ 1.6
Meal size (g/meal)	0.14 $\pm$ 0.02	0.08 $\pm$ 0.02 *
Meal duration(min/meal)	12.5 $\pm$ 2.64	13.2 $\pm$ 1.78 *
Total meal time(min/4 h)	41.5 $\pm$ 9.23	28.5 $\pm$ 7.69
Time spent in meals (min/4 h)	24.6 $\pm$ 6.54	14.8 $\pm$ 3.35**
Inter-meal interval (min)	24.8 $\pm$ 7.64	46.9 $\pm$ 9.64 *
Eating rate(mg/min)	2.21 $\pm$ 0.12	1.12 $\pm$ 0.09
Satiety ratio (%)	220 $\pm$ 63	860 $\pm$ 98
Latency to 1 st meal(min)	19.1 $\pm$ 1.5	22.7 $\pm$ 1.8
Duration 1 st meal(min)	23.5 $\pm$ 5.1	24.0 $\pm$ 4.7
Eating rate1 st meal(mg/min)	5.8 $\pm$ 0.9	4.2 $\pm$ 1.0

注:* $P<0.05$; ** $P<0.01$,与 NS 对照组相比。

Notes: * $P<0.05$; ** $P<0.01$ vs. NS control group.

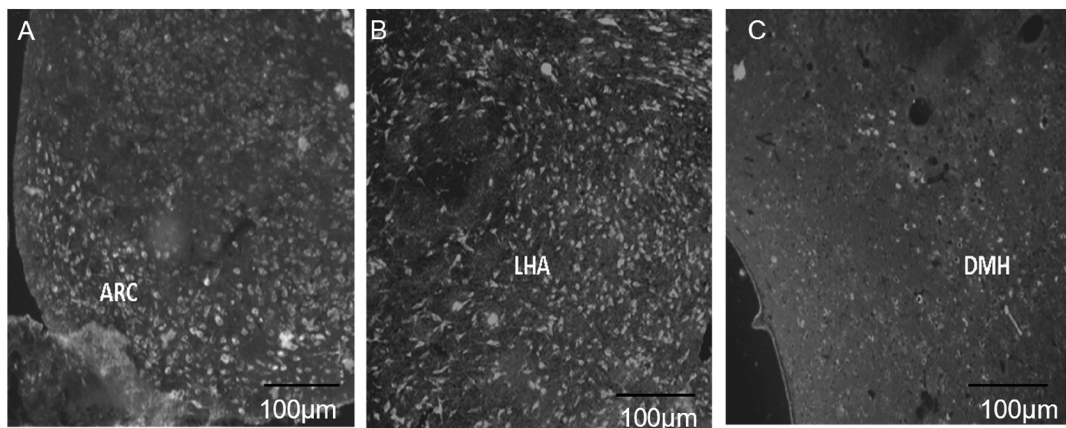


图 3 下丘脑 NUCB2/nesfatin-1 免疫阳性细胞表达

Fig.3 Representative microphotographs of NUCB2/nesfatin-1-immunoreactive cells in hypothalamic sections of naïve mice.

注:(A)弓状核,(B)下丘脑外侧区,(C)下丘脑背内侧核 bar=100 μ m。

Notes: (A) ARC, (B) LHA, (C) DMH Bar=100 μ m.

表 2 腹腔注射或皮下注射 nesfatin-1 对小鼠摄食的影响($\mu\text{g}, \bar{x} \pm s$)Table 2 The effect of nesfatin-1 injected ip or sc on food intake in ad libitum fed mice ($\mu\text{g}, \bar{x} \pm s$)

Group	Intraperitoneal injection		Subcutaneous injection	
	NS	Nesfatin-1	NS	Nesfatin-1
0-1h(g)	0.35 $\pm$ 0.18	0.37 $\pm$ 0.09	0.24 $\pm$ 0.04	0.19 $\pm$ 0.06
1-2h(g)	0.17 $\pm$ 0.10	0.24 $\pm$ 0.08	0.28 $\pm$ 0.05	0.29 $\pm$ 0.05
2-3h(g)	0.18 $\pm$ 0.05	0.24 $\pm$ 0.06	0.30 $\pm$ 0.07	0.20 $\pm$ 0.04
3-4h(g)	0.15 $\pm$ 0.02	0.19 $\pm$ 0.09	0.22 $\pm$ 0.03	0.16 $\pm$ 0.05
Meal frequency (number/4h)	5.5 $\pm$ 0.6	5.7 $\pm$ 1.0	6.1 $\pm$ 0.5	0.2 $\pm$ 0.0
Bout frequency (number/4h)	31.8 $\pm$ 8.7	39.2 $\pm$ 5.8	6.3 $\pm$ 0.5	0.2 $\pm$ 0.0
Meal size (g/meal)	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	20.9 $\pm$ 3.1	18.5 $\pm$ 2.9
Meal duration (min/meal)	26.9 $\pm$ 8.2	27.2 $\pm$ 2.8	28.6 $\pm$ 4.4	22.8 $\pm$ 5.6
Total meal time (min/4h)	116.0 $\pm$ 26.5	140.0 $\pm$ 12.7	107.4 $\pm$ 11.9	24.6 $\pm$ 2.9
Time spent in meals (min/4h)	48.3 $\pm$ 11.0	58.3 $\pm$ 5.3	111.3 $\pm$ 6.5	21.1 $\pm$ 2.5
Inter-meal interval (min)	22.1 $\pm$ 4.9	19.7 $\pm$ 3.7	44.8 $\pm$ 5.0	5.5 $\pm$ 1.1
Latency to 1st meal (min)	23.1 $\pm$ 10.7	15.1 $\pm$ 6.4	53.1 $\pm$ 7.1	4.5 $\pm$ 0.4
Duration 1st meal (min)	14.9 $\pm$ 4.5	15.1 $\pm$ 4.8	9.3 $\pm$ 3.3	10.6 $\pm$ 1.9
Eating rate 1st meal (mg/min)	4.3 $\pm$ 1.7	4.1 $\pm$ 0.6	4.5 $\pm$ 1.1	2.9 $\pm$ 0.5
Eating rate/4h (mg/min)	3.9 $\pm$ 0.4	4.5 $\pm$ 0.5	4.1 $\pm$ 0.2	4.3 $\pm$ 0.3

3 讨论

本研究显示,自由进食小鼠侧脑室注射 nesfatin-1 可显著减少夜间摄食量,且呈现剂量依赖性。注射后第一个 2 h, nesfatin-1 厌食作用不明显,注射后第二个 2 h,小鼠摄食量明显减少,之后摄食又恢复到正常水平。故侧脑室注射后 2-4 h nesfatin-1 作用达到高峰。且小鼠 12 h 夜间累积摄食量显著减少,可能是侧脑室注射 nesfatin-1 后厌食作用所致^[2]。有研究报道侧脑室注射 nesfatin-1 2-3 h 后,长期插管大鼠夜间进食减少可达 87%,且作用可持续 6 h^[4]。有研究^[4]应用小鼠 nesfatin-1 通过质谱分析,证实 nesfatin-1 质量为 9.61 kDa。Nesfatin-1 作用与其种属差异无关,应激刺激可能会影响 nesfatin-1 的活性和稳定性。

本研究发现小鼠侧脑室注射 nesfatin-1 后食欲减退,表现为进食量和进食次数减少,进食间隔增加,且可增加饱腹感。推测 nesfatin-1 所致饱腹感可能使小鼠摄食减少^[6]。而腹腔或皮下注射 nesfatin-1 不影响小鼠夜间摄食,提示,nesfatin-1 饱食作用主要是激活了中枢摄食环路,从而增加了肠道中的饱食肽^[7]。孤束核 nesfatin-1 表达较多^[10],可能是内源性 nesfatin-1 的主要作用部位。有研究报道侧脑室注射 nesfatin-1 导致摄食减少可能是通过 CRF2 受体信号通路介导,故选择性 CRF2 拮抗剂 astressin2-B 可阻断 nesfatin-1 厌食作用^[9],而选择性 CRF2 激动剂 urocortin 3 作用可能与 nesfatin-1 相似。Urocortin 3 和 nesfatin-1 均具有中枢延迟作用,厌食作用均发生于注射后 3-4 h^[8]。侧脑室注射 urocortin 3 后进食次数也显著减少,进食间隔增

加,高剂量可减少进食量,增加饱足感^[19]。提示,侧脑室注射 nesfatin-1 摄食调节作用可能与下游 CRF2 信号通路有关,但这种作用机制仍需进一步研究。

Nesfatin-1 抑制摄食的中枢部位在小鼠 PVN 和脑干^[2,4]。应用 125I-nesfatin-1 研究表明,nesfatin-1 主要表达于小鼠的下丘脑^[9]。后来研究发现 NUCB2/nesfatin-1 神经元在下丘脑摄食相关核团如 PVN, Arc, LH 以及脑干(背侧迷走神经复合体)等均有分布^[10]。Nesfatin-1/NUCB2 神经元的编码可能与下丘脑中 α -黑色素细胞刺激素、抗利尿激素、生长激素释放激素、CRF、生长抑素及 NPY 等类似^[3,11,17]。小鼠 NUCB2/nesfatin-1 的特异性可通过抗 nesfatin-1 抗体和 NUCB2/nesfatin-1 免疫标志来表现。通过 Western Blot 中 10 kDa 环和对应 NUCB2^[4]的 50 kDa 环可识别 nesfatin-1。

外周也有 NUCB2/nesfatin-1 表达,包括大鼠^[11]和小鼠^[11,18]肠道、胃和胰腺的内分泌细胞及大鼠的脂肪组织^[14]。大鼠进食状态也可调控循环 NUCB2/nesfatin-1 水平^[15]。静脉注射 nesfatin-1 可通透血脑屏障^[14],且具有不饱和性。有研究表明大鼠腹腔注射 nesfatin-1 不影响进食量^[4],而另一项研究则称自由进食小鼠腹腔注射 nesfatin-1^[15],3 h 夜间累积进食量明显减少。本研究证实自由进食小鼠腹腔或皮下注射高剂量 nesfatin-1,小鼠夜间进食量无明显改变。提示,nesfatin-1 外周作用不明显。

总之,侧脑室注射 nesfatin-1 可剂量依赖性的减少自由进食小鼠摄食,与 nesfatin-1 增加饱腹感相关,可能通过下游 CRF2 信号通路介导。外周注射 nesfatin-1 则不影响小鼠夜间摄食行为,这种差异有待进一步研究证实。

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