Impact of environmental enrichment on individual food intake and hypothalamic genes related to energy balance

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Background

An enriched environment (EE) may protect against obesity by influencing hypothalamic genes related to energy balance. The rodent EE incorporates both social and inanimate (toys) components and sometimes physical exercise. We hypothesize that an EE protects against obesity by reducing individual food intake and affecting hypothalamic signaling factors related to energy balance.

Methods

Animals: Sprague Dawley rats (n=32) weighing ~300 g were randomized into 2 groups of equal body composition, and placed into either EE or control housing (C). All animals were maintained on RD D12450B chow.
Housing: EE cages (17x17x12 inch, plexiglass) included toys and crinkle bedding and housed 4 animals per cage. Rats in the C group were individually housed in standard shoebox cages with a single nylabone. Toys were removed during scheduled meal times, and order was rotated upon replacement. Once a week cages were cleaned and toys were switched between cages, such that each group of animals interacted with each set of toys during two separate weeks.
Experimental Protocol: For both EE and C, feeding took place during scheduled meal times for one hour at the onset of the light cycle and for 4 hours during the initial phase of the dark cycle. EE cages were cleared of toys and bedding during meal times and transparent partitions were placed in the cages so that individual food intake could be monitored. Body weights were recorded every 48 hours and body composition was measured every 2 weeks. After 8 weeks animals were sacrificed at the end of the dark cycle, and hypothalamic nuclei were dissected and stored at -80°C.
qRT-PCR: Total mRNA from cultured cells was isolated using a commercial kit (Qiagen). Primers for the following genes were designed using MacVector 12: OX1R (NM_198959), OX2R (NM_198962), BDNF (NM_012513), OXA (NM_013179), OXT (NM_012996.3), OXTR (NM_012871.2), TrkB (NM_001163168.2) and GAPDH (NM_017008). PCR reactions were performed in a Roche LightCycler. Relative mRNA levels were normalized to GAPDH using the Δ-ΔCT method.

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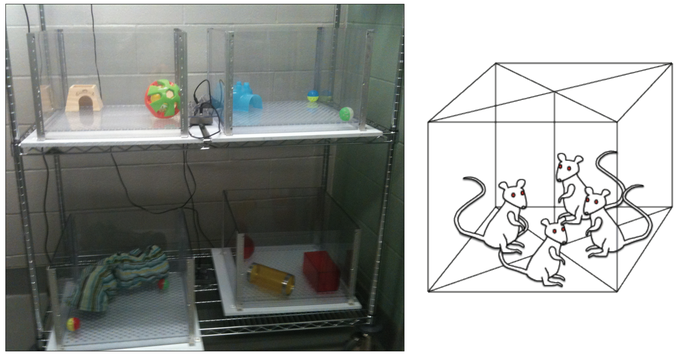

Figure 1: A novel enriched environment social housing paradigm to enable individual measurments of food intake. Cages were 17x17 inches and included toys, crinkle bedding (not pictured), and social housing (4 rats/cage). Diagram of how animals were partitioned during meal times using clear plexiglass (above right).

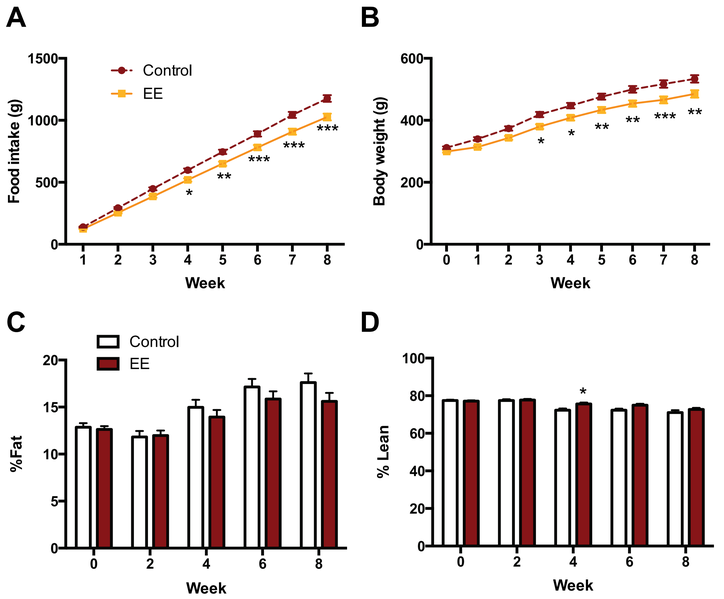

Figure 2: Animals housed in an EE had reduced cumulative food intake (A) and gained less weight (B) compared with animas in standard housing. Body fat % (C) and lean mass % (D) over the 8 week period.
There was a significant interaction between the effects of time and housing on cumulative food intake $F_{(7, 210)}$ =12.87; p<0.0001 (A). There was a main effect of both time and housing. Post hoc analysis showed that animals housed in an EE ate less than C from weeks 4-8 (p<0.05). There was an interaction between time and housing on body weight $F_{(8,240)}$ =6.125; p<0.0001 (B). There was a main effect of time and housing. Animals in EE had reduced body weights from weeks 3-8 (p<0.05). There was a significant interaction between time and housing in % body fat $F_{(4,120)}$ =2.87; p<0.05 (C) and % lean mass $F_{(4,120)}$ =4.85; p<0.01 (D). Simple main effects analysis revealed a non-significant trend toward reduced % body fat and elevated % lean mass in animals in EE housed animals. *Two-way ANOVA (treatment x time) with Sidak's multiple comparisons test, n=16/group; *p<0.05, **p<0.01, ***p<0.001.*

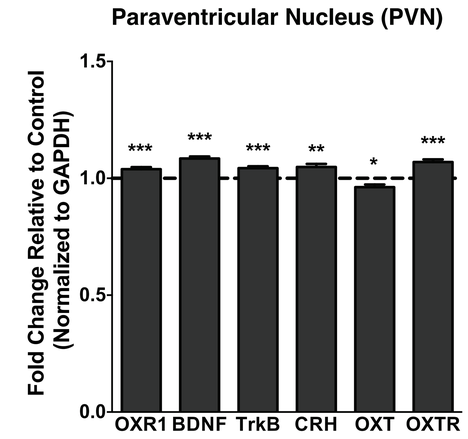

Figure 3: Environmental enrichment elevates expression of orexin 1 receptor (OXR1), brain derived neurotrophic factor (BDNF), tropomyosin-related kinase receptor B (TrkB), corticotropin-releasing hormone (CRH) and oxytocin receptor (OXTR) and decreases expression of oxytocin (OXT) in the hypothalamic paraventricular nucleus. *Two-tailed unpaired t-tests; n=16/group *p<0.05, **p<0.01, ***p<0.001*

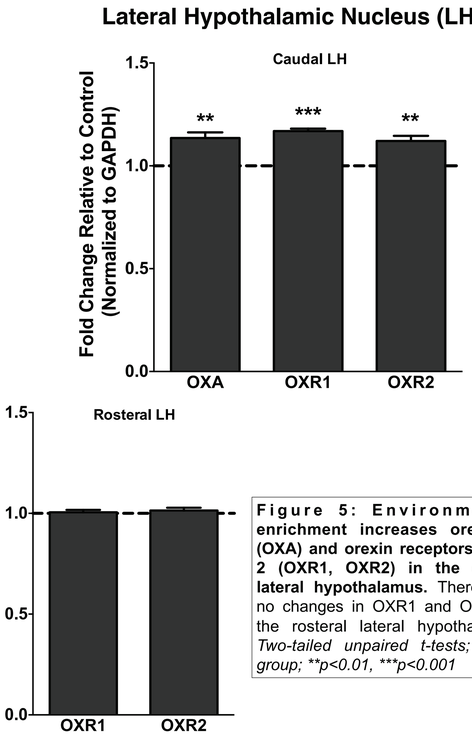

Figure 5: Environmental enrichment increases orexin A (OXA) and orexin receptors 1 and 2 (OXR1, OXR2) in the caudal lateral hypothalamus. There were no changes in OXR1 and OXR2 in the rosteral lateral hypothalamus. *Two-tailed unpaired t-tests; n=16/group; **p<0.01, ***p<0.001*

Discussion

1) EE including social housing reduces individual food intake and protects against weight gain.

2) Anorexigenic signaling pathway genes in the PVN, (BDNF, trkB, CRH) were elevated.

- BDNF injections in PVN decrease food intake, body weight, and elevate energy expenditure [1].
- BDNF regulates expression of CRH in the PVN [2]; activation of CRH receptor slows gastric emptying and reduces feeding [3, 4].
- Blocking CRH receptors abolishes BDNF effects on food intake and body weight [5].
- ***These data suggest existence of a common pathway whereby EE elevates PVN BDNF, increasing expression of CRH and reducing food intake.***

3) EE elevates OXA expression in the LH and increased OXR1 in the PVN as well as increased levels of CRH.

- Activation of OXR1 by OXA in PVN elevates CRH-mediated pathways [6].
- ***BDNF/trkB receptor signaling may mediate this effect, as BDNF/trkB have known plasticity functions and regulate expression of CRH.***

4) OXT was reduced in the PVN, but its receptor was elevated.

- In VMN, OXT [7] and BDNF [8, 9] both reduce feeding and elevate energy expenditure.
- ***We did not observe differences in expression of these peptides between EE and C housed animals in the VMN (data not shown).***

5) Animals housed in an EE had elevated levels of OXA in the caudal LH as well as increased receptors OXR1 and OXR2 suggesting enhanced orexinergic tone.

- Lateral hypothalamic increases in orexin expression and signaling promote resistance to obesity via elevating spontaneous physical activity [10].

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