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Food hoarding and associated neuronal activation in brain reward circuitry in Mongolian gerbils

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ABSTRACT

Mongolian gerbils (*Meriones unguiculatus*) display food hoarding and thus provide an opportunity to study the neuromechanisms underlying this behavior. In the present study, male gerbils exhibited a bimodal expression of food hoarding behavior—some displayed high levels of food hoarding whereas others virtually lacked this behavior under normal laboratory conditions with free access to food. Food hoarding was found to be associated with an increase in neuronal activation, indicated by Fos immunoreactive (ir) staining, in several brain areas including the nucleus accumbens, ventral tegmental area (VTA), and lateral hypothalamus. Food hoarding was also associated with increases in the number of cells labeled for tyrosine hydroxylase (TH-ir), the rate limiting enzyme for dopamine conversion, and the number of cells co-labeled for TH-ir/Fos-ir in the VTA, suggesting that dopamine in the brain reward circuitry may be involved in food hoarding. Further, we found that 22 h of food deprivation induced food hoarding in some, but not all, males that naturally did not display food hoarding. In these males, however, food hoarding did not increase TH-ir or TH-ir/Fos-ir expression in the VTA. Together, these data indicate that male Mongolian gerbils display diverse phenotypes of food hoarding behavior and that dopamine in the brain reward circuitry may be involved in the control of naturally occurring, but not food deprivation-induced, food hoarding.

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1. Introduction

Food hoarding is an important adaptive strategy for coping with seasonal fluctuations in food availability [1]. Hoarding food can improve an animal's chances of survival in a period of food scarcity, allows individuals to optimize foraging and feeding, enhances its competitive status for limited resources, and ensures a continuous even flow of food/nutrition to offspring during reproduction [2]. Food hoarding is exhibited by a variety of rodent species, including rats, white-footed mice (*Peromyscus leucopus*), Syrian hamsters *(Mesocricetus auratus)*, Siberian hamsters *(Phodopus sungorus)*, red squirrels (*Sciurus vulgaris*), and Mongolian gerbils *(Meriones unguiculatus)* [3–7]. Although the propensity for an animal to hoard food may be influenced by a variety of environmental factors, such as photoperiod, ambient temperature, illumination, and physical characteristics of food items [4,8,9], food deprivation – a naturally occurring energy challenge faced by wild animals [10] – is a vital factor to induce food hoarding in almost all species examined [3,4,6,11,12].

Scientists have made efforts to examine the mechanisms underlying food hoarding that occurs naturally or that is induced by food deprivation. For example, electrical stimulation of the lateral hypothalamus (LH) has been found to facilitate stimulus-induced feeding and to elicit intense hoarding activity in satiated rats [13,14]. Lesions of dopamine neurons in the ventral tegmental area (VTA) markedly attenuate food hoarding in rats [15]. Lesions of the hippocampus result in species- and/or experiment-dependent effects, as hippocampal lesions inhibit food hoarding in mice [16] while lesions more restricted to the dorsal hippocampus significantly increase food hoarding under deprivation conditions in rats [17]. Furthermore, food deprivation has been shown to facilitate food hoarding in a variety of rodent species examined, including rats [18], hamsters [3,11,12] and gerbils [6,19]. Food deprivation decreases circulating levels of leptin [11,20–22], whereas leptin treatment inhibits food hoarding induced by food deprivation [11,20,21]. Although these studies have indicated potential roles of certain brain areas, neurotransmitter systems, and hormones in food hoarding, we still know very little about the neuromechanisms involved in the control of food hoarding behavior. In addition, many studies have been conducted in traditional laboratory rodents, such as rats, that usually do not display food hoarding. As animals show remarkable species differences in behavior and their underlying neuromechanisms, and food hoarding is critical for the survival and

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reproductive success of wild animals [2,12], it is important to examine the neural as well as hormonal mechanisms underlying food hoarding in species naturally displaying this behavior.

Mongolian gerbils are widely distributed in semi-arid and arid grasslands and farmlands southeast of the Baikal area in Russia, Mongolia, and northern China. These animals are cooperative breeders and live in groups year-round without hibernation [23]. They mainly select seeds of annual dicot and some foliage as food. They are seasonal food hoarders with no cheek pouches, and most members of the social group take part in food hoarding when the standing crop is near the annual high [24–26]. Previous studies have shown that Mongolian gerbils display individual, but not sex, differences in food hoarding [19,24] (but see [6,19]). In addition, androgen treatment inhibits [6,19] whereas food deprivation facilitates food hoarding [19]. However, the neural mechanisms underlying food hoarding have not been examined in this species. In the present study, we characterized food hoarding, examined the associated changes of serum leptin and neuronal activation – particularly the activation of dopamine (DA) neurons in the brain reward circuitry – and evaluated the effects of food deprivation on the induction of food hoarding in male gerbils.

2. Materials and methods

2.1. Subjects

Subjects were adult male Mongolian gerbils that were offspring of our laboratory breeding colony. After weaning, subjects were housed in same-sex groups, consisting of 3–4 individuals, in plastic cages ($300\times150\times200$ mm) which contained wood shaving bedding, and were under a 16L:8D photoperiod (lights on at 0400 h). The temperature was maintained at 23 ± 1 °C. All animals had *ad libitum* access to water and commercial standard rat pellets (Beijing KeAo Feed Co.). At about 10 months of age, subjects were housed individually for 1 month, followed by 2 weeks of acclimation in the food hoarding apparatus (see below). All experimental procedures complied with the guidelines for animal care and use as stipulated by the Institute of Zoology at the Chinese Academy of Sciences.

2.2. Food hoarding apparatus

The food hoarding apparatus was constructed similarly to the design described by Bartness [3] and Cabanac [27]. Briefly, the apparatus consisted of a plastic home cage ($320\times210\times160$ mm) connected to a food cage ($300\times200\times150$ mm) by a 900 mm long plastic tube (50 mm internal diameter). At the beginning of the acclimation period, the subject was given a water bottle, wood shaving bedding, and four cotton balls in the home cage. Food pellets were provided on the lid of the food cage. Subjects were free to move around within the apparatus to have access to food and water.

2.3. Food hoarding measurements

The food hoarding experiment started on the day immediately following acclimation. At 0900 h, food pellets were put directly into the food cage. During the next 2 h, subjects were allowed to eat and carry food pellets from the food cage to the home cage. At 1100 h, any remaining food pellets were taken out from both the food and the home cages and were weighed. Food hoarded was determined by the amount of food pellets in the subject's home cage. Food intake was determined by subtracting the food hoarded and the food remaining in the food cage from the total amount placed in the food cage.

2.4. Body weight and fat

Subjects were weighed at 0900 h each morning before the food hoarding experiment. After completion of each experiment, subjects were sacrificed by an overdose of CO_2. Trunk blood was collected for leptin radioimmunoassay and brains were rapidly removed, frozen on dry ice, and stored at −80 °C for immunohistochemical staining. Stomach, small intestine, and cecum were also removed. The remaining carcass was dried in an oven at 60 °C to constant mass and then weighed to obtain the dry mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus, as described previously [28].

2.5. Serum leptin radioimmunoassay

Serum leptin levels were determined by radioimmunoassay (RIA) with the ^{125}I Multi-species Kit (Cat. No. XL-85K, Linco Research Inc.), which was validated previously in Mongolian gerbils [22]. The detection limit was 1.0 $ng\cdot ml^{-1}$ when using a 100-μl sample and the intra-assay variability was about 4.9% [29].

2.6. c-Fos and TH immunohistochemistry

Coronal brain sections of 20 μm thickness were cut on a cryostat. Sections were mounted directly onto poly-lysine-coated slides and stored at −80 °C. Brain sections at 80 μm intervals were processed for c-Fos and TH immunohistochemistry using previously established methods [30]. Briefly, brain sections were air-dried at room temperature, fixed by using 4% paraformaldehyde in 0.01 M phosphate buffered saline (PBS, pH 7.4) for 1 h, and washed three times in 0.01 M PBS (pH 7.4) for 5 min. Sections were incubated in 1% sodium borohydride for 20 min, 3% H_2O_2-Methanol for 10 min, 1% Triton X-100 for 10 min, and 10% normal goat serum for 1 h in 0.01 M PBS. For c-Fos immunoreactive (Fos-ir) staining, sections were incubated with rabbit anti-c-fos antibody (c-Fos [4]-G: *sc*-52; 1:8000; Santa Cruz Biotechnology, Santa Cruz, CA) in 0.01 M PBS with 0.1% Triton X-100 and 2% normal goat serum for 48 h at 4 °C. Thereafter, sections were incubated in biotinylated goat anti-rabbit IgG (BA-1000; 1:300; Vector, Burlingame, CA) for 2 h at room temperature and avidin-biotin complex (Vectastain Elite, Vector, Burlingame, CA) in 0.01 M PBS for 90 min. Staining was detected using 3′-diaminobenzidine (DAB/H_2O_2 tablet; Sigma, St. Louis, MO) with NiCl powder and rinsed in 0.01 M PBS for 20 min. For TH immunoreactive (TH-ir) staining, sections were subsequently incubated with rabbit anti-TH (AB152; 1:8000; Chemicon, Temecula, CA) in 0.01 M PBS with 0.1% Triton X-100 and 2% normal goat serum overnight at room temperature, biotinylated goat anti-rabbit IgG (BA-1000; 1:300) for 2 h, and avidin–biotin complex for 90 min. Sections were then stained using a Sigma DAB Kit and rinsed in 0.01 M PBS. Finally, sections were dehydrated in alcohol, transcended by Xylene, and cover-slipped with Permount.

2.7. Data quantification and analysis

Photomicrographs were captured by using a Nikon Eclipse 80i microscope with a SPOT RT_{KE} 7.4 Slider (Diagnostic Instruments) camera. Black punctate nuclear staining for c-Fos (Fos-ir) and brown cytoplasmic staining for TH (TH-ir) were found in various brain areas. Profile counting for Fos-ir cells was conducted bilaterally in selected brain areas, including the nucleus accumbens (NAcc); paraventricular (PVN), ventromedial (VMH), and lateral (LH) nuclei of the hypothalamus; hippocampus; and ventral tegmental area (VTA). In addition, TH-ir single-labeled and TH-ir/Fos-ir double-labeled cells were counted in the PVN and VTA. Brain areas were defined according to a rat brain atlas [31]. For each brain area, data were quantified from 3 representative sections that were anatomically matched between subjects, and the mean from each subject was used for data analysis.

Data were analyzed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Prior to statistical analysis, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests. Body weight, food intake, and food hoarded over the course of the food hoarding experiment were analyzed by one way

analysis of variance (ANOVA) with repeated measures, and significant differences were further evaluated by *t* test. Group differences in serum leptin concentration, body fat, and the number of Fos-ir, TH-ir, as well as TH-ir/Fos-ir cells in each brain area were analyzed by *t* test. Pearson correlation analysis was also performed to determine correlations between serum leptin concentration and the amount of food hoarded and between the number of TH-ir/Fos-ir cells in the VTA and the amount of food hoarded in the groups of males that displayed food hoarding behavior. All results are presented as means ± SEM, and $p<0.05$ was considered to be statistically significant.

3. Experimental design

3.1. Experiment 1: Does food hoarding behavior activate the brain reward circuitry?

This experiment was conducted in male gerbils to characterize their food hoarding and to examine neuronal activation in the brain associated with food hoarding. We screened subjects to distinguish those males that displayed food hoarding from the ones that did not display food hoarding, and then examined neuronal activation associated with food hoarding in the brain reward circuitry. Subjects were individually acclimated to the food hoarding apparatus for 2 weeks. Thereafter, the food hoarding experiment began and the subject's body weight, food intake, and the amount of food hoarded were monitored daily for 12 days. Each day, a food hoarding measurement was conducted, as described above, during 0900–1100 h. Thereafter, food pellets were placed on the lid of the food cage, so that the subject had free access to food but could not display food hoarding behavior for the next 22 h. Food intake during the 22 h was calculated by subtracting the remaining food on the lid from the amount offered. Consequently, the subject's daily food intake included the intake during the 2 h food hoarding experiment and the intake during the remaining 22 h each day. Immediately after food hoarding measurements on day 12, subjects were sacrificed. Their trunk blood was taken for leptin measurement, brain tissues were processed for Fos-ir and TH-ir staining, and carcasses were dried to obtain the dry mass for fat measurement. Based on the amount of food hoarded, subjects were assigned into two groups—a "fed hoarding" group (FH, n = 9), in which subjects hoarded an average of more than 20 g of food pellets per day and a "fed non-hoarding" group (FNH, n = 8), in which subjects hoarded less than 2 g of food pellets per day.

3.2. Experiment 2: Is the brain reward circuitry involved in food hoarding induced by food deprivation?

Because some male gerbils did not naturally display food hoarding behavior, we focused on those animals to examine whether food deprivation can induce food hoarding, and if so, whether the brain reward circuitry is involved. Food hoarding measurements, as described in Experiment 1, were conducted for 7 days in a group of male gerbils to obtain baseline data of body weight, food intake, and food hoarded. Thereafter, 11 subjects that displayed no food hoarding behavior (FNH, as described in Experiment 1) were identified and then went through experimental food deprivation. Fasting began at 1100 h on day 7 and lasted until 0900 h of the following day (22 h fast), followed by 2 h during which food was freely available and food hoarding measurements were obtained. This procedure was repeated for 15 consecutive days, and the subject's body weight, food intake, and food hoarded were recorded daily. Subjects had no opportunities to eat except during their daily 2 h of food hoarding period. At the end of the experiment, subjects were sacrificed, their blood was taken for leptin measurement, brain tissues were processed for Fos-ir and TH-ir staining, and carcasses were dried for fat measurement. Subjects that hoarded more than 20 g of food pellets per day during the food deprivation experiment were referred as "fast hoarding" (FAH, n = 5), while subjects that hoarded less than 2 g of food pellets per day were designated as "fast non-hoarding" (FANH, n = 6). There were no intermediates. Because the amount of food hoarded, food intake, and body weight did not differ across 7 days of baseline measurement, the data were collapsed within each group to generate baseline means for further data analyses, as described above.

4. Results

4.1. Experiment 1: Does food hoarding behavior activate the brain reward circuitry?

4.1.1. The amount of food hoarded and food intake

Male gerbils showed discrete patterns of food hoarding behavior. Some displayed consistent, high levels of food hoarding behavior

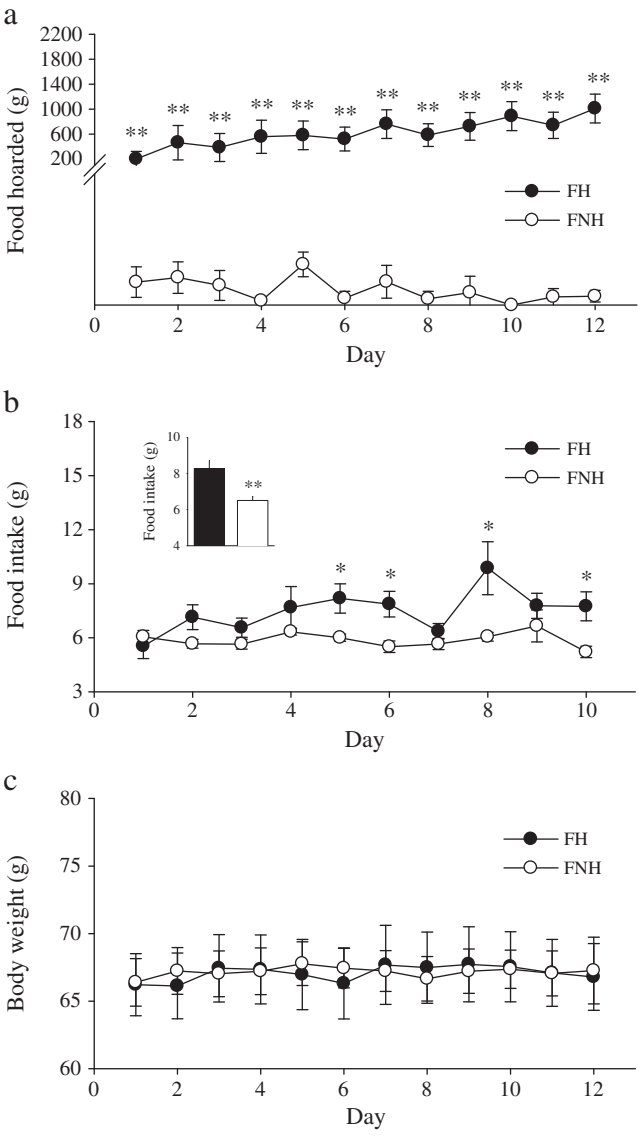

Fig. 1. Differences in the amount of food hoarded, food intake, and body weight of male Mongolian gerbils. (a) Male gerbils displayed discrete patterns of food hoarding behavior indicated by the amount of food hoarded. Some displayed consistent, high levels of food hoarding (FH), whereas others exhibited minimal or no food hoarding (FNH) during the daily 2 h test. FH males hoarded more food than FNH males across 12 days of the entire food hoarding test. (b) Overall, FH males had more daily food intake than FNH males (inset). In particular, FH males ate more than FNH males on days 5, 6, 8, and 10 during the food hoarding experiment. (c) FH and FNH males did not differ in their body weight. Data are presented as mean ± SEM. *: $p<0.05$, **: $p<0.01$.

(FH); others virtually lacked food hoarding behavior (FNH). There were no intermediates. FH males hoarded more food than FNH males, and such differences existed on the first day and throughout the entire food hoarding experiment ($F_{(1,9)} = 13.61$, $p<0.01$; Fig. 1a). Group differences were also found in food intake. Overall, daily food intake for FH males was higher than for FNH males ($F_{(1,15)} = 11.77$, $p<0.01$; Fig. 1b inset), and such differences fluctuated over the course of the food hoarding experiment (Fig. 1b). On days 5, 6, 8, and 10, during the food hoarding experiment, FH males ate more than FNH males.

4.1.2. Body weight, body fat, and serum leptin concentration

Body weight did not differ between FH and FNH males (Fig. 1c). The two groups also did not differ in the amount of body fat (9.24 ± 1.77 g for FH *vs* 8.96 ± 1.48 g for FNH males) and in the serum leptin concentration (26.70 ± 3.13 ng·ml^{-1} for FH *vs* 30.48 ± 3.02 ng·ml^{-1} for FNH males). However, the serum leptin concentration was negatively correlated with the amount of food hoarded in male gerbils ($r = -0.73$, $p<0.05$; Fig. 2a).

4.1.3. Fos-ir and TH-ir staining in the brain

Group differences were found in the number of Fos-ir cells in selected brain areas. FH males had more Fos-ir cells than did FNH males (Figs 3a and 4) in the NAcc ($t_{(15)} = 3.53$, $p<0.01$), LH ($t_{(15)} = 3.65$, $p<0.01$), and VTA ($t_{(15)} = 6.05$, $p<0.01$). The two groups did not differ in the number of Fos-ir cells in the PVN, VMH, and hippocampus (Figs. 3a and 4).

TH-ir cells were found in the PVN and VTA. While no group differences were detected in the number of TH-ir cells in the PVN, FH males had more TH-ir cells in the VTA than did FNH males ($t_{(15)} = 3.47$, $p<0.01$, Figs. 3b and 4). Some of the TH-ir labeled cells in the PVN and VTA also contained Fos-ir labeling. In the VTA, FH males had more TH-ir/Fos-ir double-labeled cells compared to FNH males ($t_{(15)} = 2.49$, $p<0.05$, Figs. 3c and 4). No group differences were found in TH-ir/Fos-ir labeling in the PVN (Figs. 3c and 4). Finally, the number of TH-ir/Fos-ir cells in the VTA was found to be positively correlated with the amount of food hoarded ($r = 0.90$, $p<0.01$; Fig. 2b).

4.2. Experiment: Is the brain reward circuitry involved in food hoarding induced by food deprivation?

4.2.1. The amount of food hoarded and food intake

Although no differences were found in the basal levels of the amount of food hoarded, male gerbils showed discrete patterns of food hoarding behavior in response to food deprivation. Some males displayed a significant increase in the amount of food hoarded following fasting (FAH) while others continued to display no or minimal food hoarding (FANH), and such differences persisted throughout the entire course of the food deprivation manipulation ($F_{(1,9)} = 13.61$, $p<0.001$, Fig. 5a). Furthermore, no differences were found in the basal levels of food intake between FAH and FANH males (Fig. 5b). One day immediately following fasting, a significant decrease in food intake was found in both groups ($F_{(20,180)} = 4.90$, $p<0.001$; Fig. 5b). Thereafter, the amount of food intake bounced back to basal levels in both groups.

4.2.2. Body weight, body fat, and serum leptin concentration

FAH and FANH males did not differ in basal levels of body weights. There was a significant decrease in body weights in both FAH and FANH males ($F_{(12,108)} = 5.11$, $p<0.001$) during the first 8 days following the onset of food deprivation (Fig. 5c). FAH males had a lower level of body fat (5.68 ± 1.45 g) than FANH males (9.94 ± 1.08 g) ($t_{(9)} = 3.61$, $p<0.05$ for one-tail test). The two groups did not differ in serum leptin concentrations (19.56 ± 6.34 ng·ml^{-1} for FAH *vs* 27.05 ± 4.98 ng·ml^{-1}

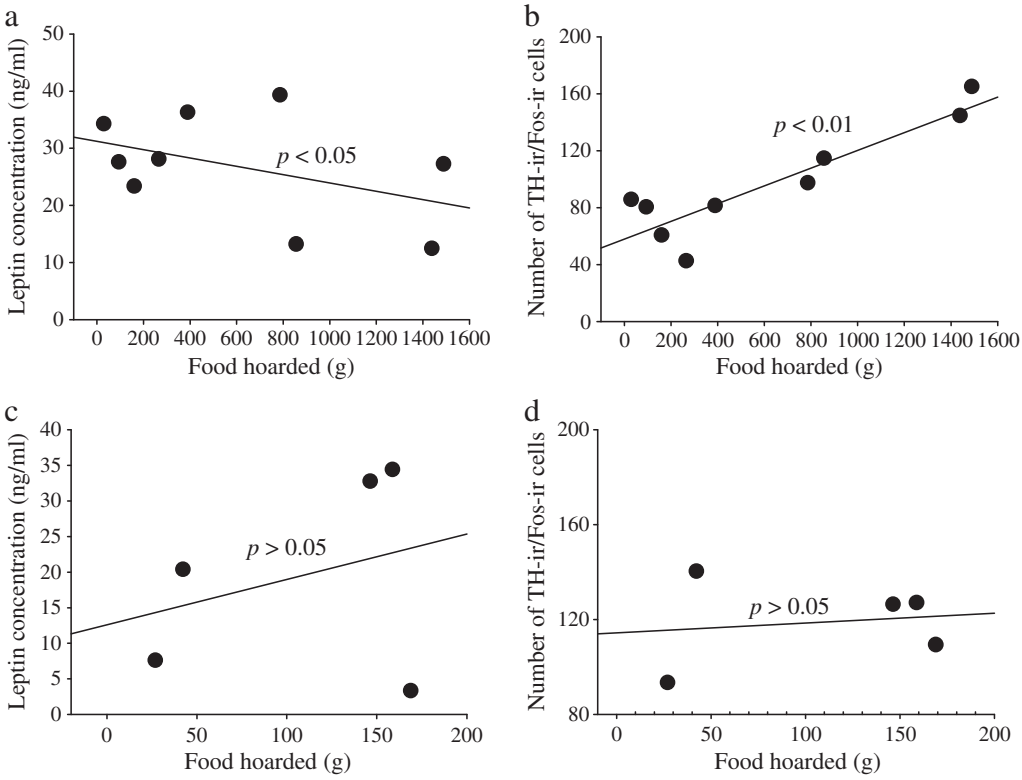

Fig. 2. Correlations between serum leptin concentrations and the amount of food hoarded (a and c) and between the number of TH-ir/Fos-ir cells in the VTA and the amount of food hoarded (b and d). For males with free access to food, the serum leptin concentration was negatively correlated with the amount of food hoarded (a) whereas the number of TH-ir/Fos-ir cells in the VTA was positively correlated with the amount of food hoarded (b). Such correlations were not found in males that were food deprived (c and d).

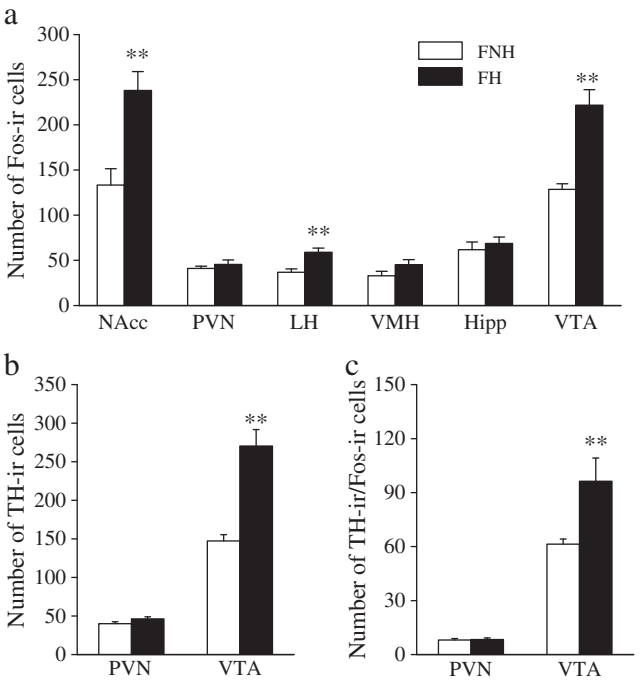

Fig. 3. Differences in the number of cells labeled for Fos-ir, TH-ir, or TH-ir/Fos-ir in selected brain areas in male Mongolian gerbils with free access to food. (a) Males that displayed food hoarding (FH) had more cells labeled for Fos-ir in the nucleus accumbens (NAcc), lateral hypothalamus (LH), and ventral tegmental area (VTA) than males that displayed no food hoarding (FNH). Such differences were not found in Fos-ir labeling in the paraventricular (PVN) and ventromedial (VMH) nuclei of the hypothalamus and hippocampus (Hipp). (b) FH males had more cells labeled for TH-ir in the VTA, but not PVN, than FNH males. (c) FH males also had more cells double-labeled for TH-ir/Fos-ir in the VTA, but not PVN, than FNH males. Data are presented as mean ± SEM. **: $p<0.01$.

for FANH males). No correlation was found between serum leptin concentration and the amount of food hoarded (Fig. 2c).

4.2.3. Fos-ir and TH-ir staining in the brain

Fos-ir cells were found in selected brain areas, as described in Experiment 1. FAH males had more Fos-ir cells in the VTA ($t_{(9)}=3.17$, $p<0.01$) but less Fos-ir cells in the hippocampus ($t_{(9)}=4.40$, $p<0.01$) than FANH males. No group differences were found in any other brain areas examined (Table 1). Finally, the two groups also did not differ in the number of TH-ir cells or TH-r/Fos-ir cells in the PVN or VTA (Table 1). No correlation was found between the number of TH-ir/Fos-ir cells in the VTA and the amount of food hoarded (Fig. 2d).

5. Discussion

Food hoarding behavior plays an important role in the survival and reproductive success of wild animals [2,12]. In the present study, we found that male Mongolian gerbils showed remarkable individual differences in food hoarding – some displayed high levels of food hoarding whereas others virtually lacked this behavior – either with free access to food or during food deprivation manipulation. Food hoarding was found to be associated with increased neuronal activation in selected brain areas, such as the NAcc and VTA, in the brain reward circuitry. In addition, dopamine neurons in the VTA were activated during food hoarding under normal laboratory conditions but not during food deprivation. Together, these data suggest that male Mongolian gerbils display diverse phenotypes of food hoarding behavior, and that dopamine in the brain reward circuitry may be involved in the control of naturally occurring, but not food deprivation-induced, food hoarding.

5.1. Bimodal expression of food hoarding behavior

Animals show individual differences in their behaviors including food hoarding [7,32]. Such differences were also reported in food hoarding behavior in a field population of Mongolian gerbils [6]. In the present study, male gerbils displayed different levels of food hoarding. Interestingly, such behavioral differences showed a bimodal expression – some individuals displayed high levels of food hoarding whereas others showed virtually no food hoarding – as previously reported in Syrian hamsters [11]. It is interesting to note that such bimodal expression of food hoarding existed under normal laboratory conditions with free access to food and during food deprivation in male gerbils. The mechanisms underlying such diverse behavioral phenotypes remain to be examined. Nevertheless, some speculations can be made based on our behavioral data. First, individual gerbils may differ fundamentally in the central mechanisms controlling food hoarding behavior, such that gerbils showed a bimodal pattern of behavior (i.e., either displayed or did not display food hoarding). Second, this regulating mechanism can be affected or modulated by environmental factors, leading to behavioral plasticity [2]. This is supported by our data showing that some gerbils that initially did not display food hoarding began to display this behavior under food deprivation, as reported in other rodent species [3,4]. It has been suggested that an animal's social status (e.g., dominant *vs* subordinate) may function as a social factor to affect its food hoarding behavior [11]. This, however, might not be the case as our subjects were singly housed for a month prior to the experiment. Third, it is intriguing to see that even under food deprivation, some animals still did not display food hoarding persistently over the course of our experimental manipulation. These data suggest that these no food hoarding animals (FANH) may have alternate strategies, other than an increase in food hoarding, to compensate for the increased energy challenge during food deprivation. This notion is supported by our data showing that FANH males had a higher level of body fat than FAH males and that the two groups did not differ significantly in their body weight during food deprivation. Of course, we cannot exclude the possibility that a further increase in energy challenge, such as prolonged food deprivation, may cause more animals to display high levels of food hoarding, as suggested by studies in other rodent species [4,33].

5.2. Brain reward circuitry is involved in naturally occurring food hoarding

Previous studies in rats have shown that electrical stimulation of the LH facilitates food hoarding [13,34] whereas lesions of the NAcc or VTA impair food hoarding [35,36], implicating these brain areas in the control of food hoarding behavior. In the present study, FH males showed higher levels of neuronal activation, indicated by Fos-ir expression, in the LH, NAcc, and VTA than FNH males, indicating involvement of these brain areas in food hoarding in male gerbils. Most interestingly, compared to FNH males, FH males had more cells in the VTA that were labeled for TH-ir or for TH-ir/Fos-ir, indicating enhanced dopamine expression and increased activation of dopamine neurons associated with the display of food hoarding behavior. This effect was also area-specific as no group differences in TH-ir and TH-ir/Fos-ir labeling were found in the PVN. Dopamine neurons in the VTA project into the NAcc [37] and this brain reward circuitry has been implicated in a variety of naturally occurring motivated behaviors including food intake [38], mating [39], and drinking behavior [40]. Our data provide further evidence to support the notion that this dopamine pathway is involved in motivated behaviors [37], including food hoarding [36]. Indeed, mesolimbic dopamine has previously been implicated in food hoarding in rats, as lesions of dopamine neurons in the VTA attenuate food hoarding [35,36] and such behavior could be reinstated to control levels by L-dopa administration [36]. In hamsters, treatment with apomorphine, a dopamine receptor agonist, also enhances hoarding behavior [41]. A positive correlation between the number of TH-ir/Fos-ir cells in the VTA and the amount of food hoarded in the present study

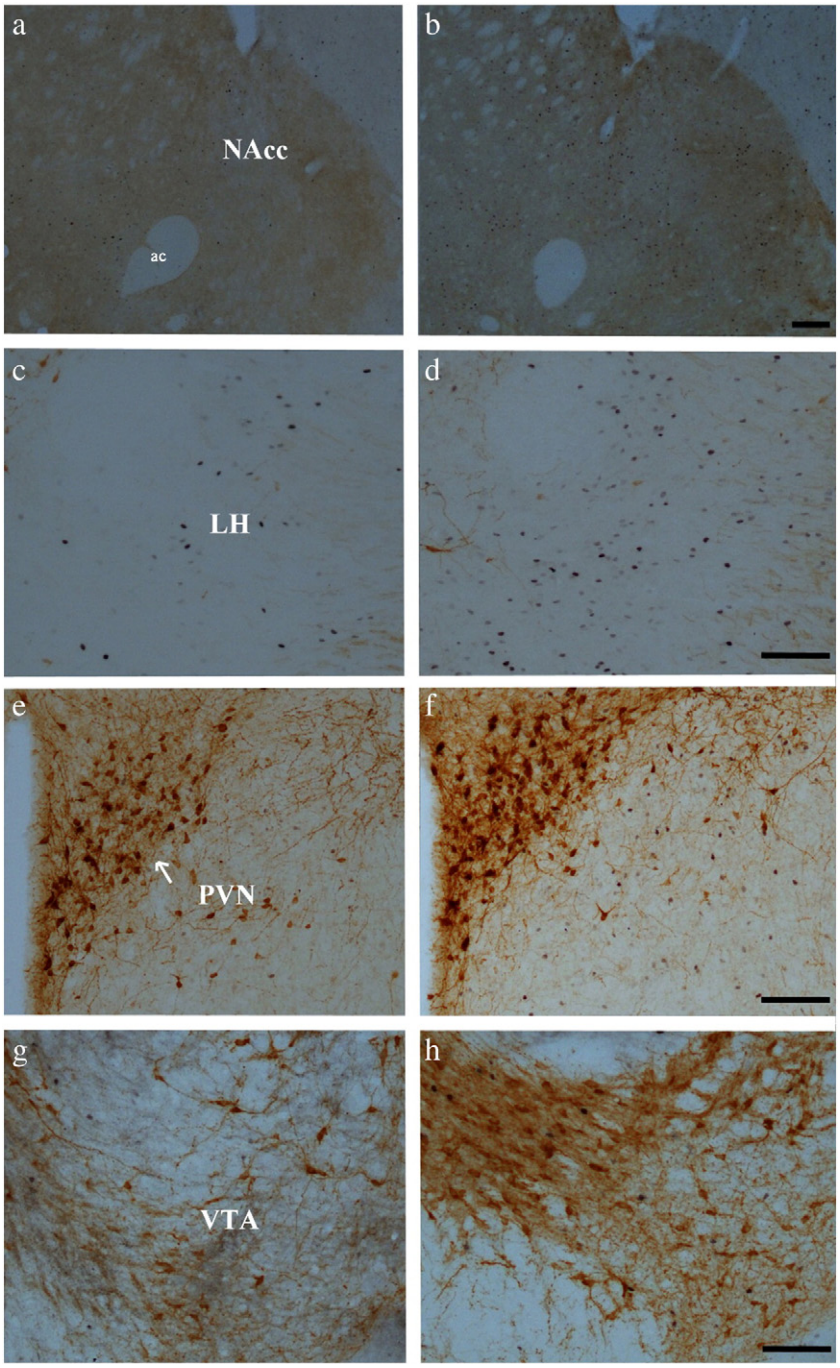

Fig. 4. Photomicrographs displaying cells labeled for Fos-ir (black punctate nuclear staining) and TH-ir (brown cytoplasmic staining) in the nucleus accumbens (NAcc; a and b), lateral hypothalamus (LH; c and d), paraventricular nucleus of the hypothalamus (PVN; e and f), and ventral tegmental area (VTA; g and h) in FNH (left column) and FH (right column) male Mongolian gerbils. ac: anterior commissure, scale bar = 100 μm.

further supported the role of mesolimbic dopamine in food hoarding in male gerbils. It has been suggested that central dopamine is involved in the activational aspects of food motivation [42]. It will be interesting to examine, in further studies, whether activation of mesolimbic dopamine in the gerbil brain is associated with arousal, rewarding aspects of food stimulation, or activation and/or maintenance of food hoarding behavior.

5.3. Food deprivation induced food hoarding

Food deprivation has been found to induce food hoarding behavior in several rodent species such as hamsters [3,11] and rats [4,18]. It has been suggested that increased energy challenges associated with food deprivation play a major role in facilitating food hoarding [2]. Therefore, it is not surprising that food deprivation also induced food hoarding in some of the male gerbils in the present study. It should be noted, however, that these gerbils (FAH) did not show higher Fos-ir staining in the NAcc, or TH-ir and TH-ir/Fos-ir staining in the VTA, than those gerbils that did not display food hoarding (FANH). Further, the number of TH-ir/Fos-ir cells in the VTA did not correlate with the amount of food hoarded. Therefore, it is likely that although the dopamine pathway from the VTA to NAcc is involved in food hoarding displayed under normal laboratory conditions with free access to food, fasting-induced food hoarding may be controlled by neural mechanisms other than dopamine. For example,

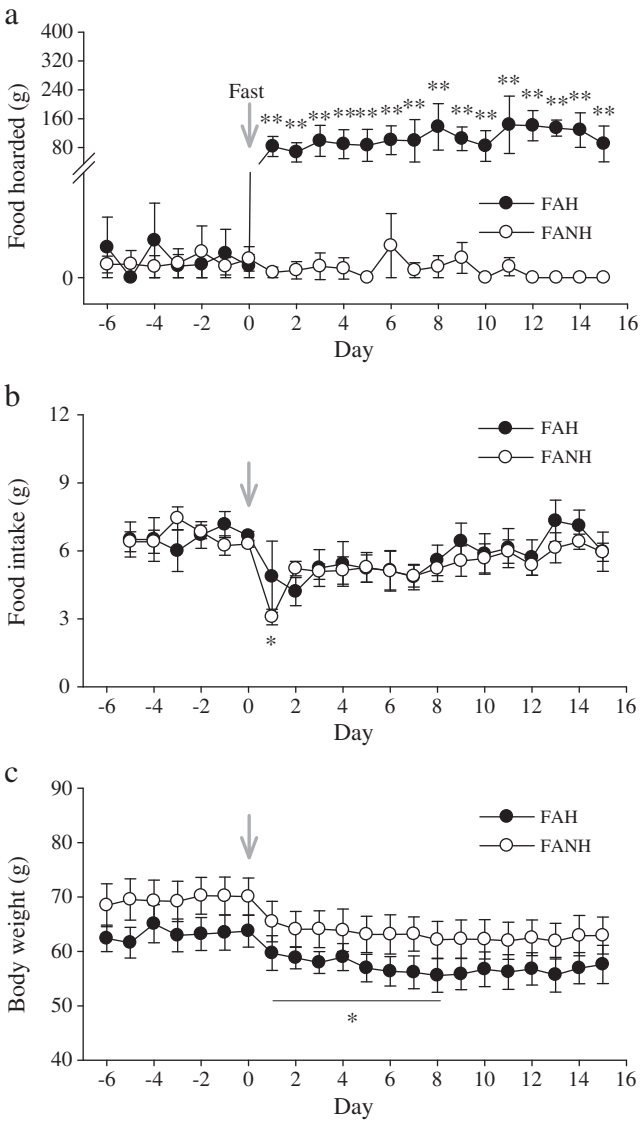

Fig. 5. Differences in the amount of food hoarded, food intake, and body weight in male Mongolian gerbils that were undergoing 15 days of food deprivation. (a) No differences were found in basal levels of food hoarded. However, male gerbils showed discrete patterns of food hoarding in response to fasting (indicted by the arrow). Some males displayed a significant increase in food hoarding in response to fasting (FAH), others continued to display basal levels of food hoarding (FANH), and such differences persisted throughout the entire course of food deprivation. **: $p<0.01$. (b) A significant, transient decrease in food intake was found in both FAH and FANH males one day following fasting. *: $p<0.05$ compared to the baseline. (c) FAH and FANH males did not differ in basal levels of body weight. However, food deprivation significantly decreased body weight in both FAH and FANH males during the first 8 days of fasting. **: $p<0.01$ compared to the baseline. Data are presented as mean ± SEM.

food deprivation is stressful [43] and thus neuromechanisms mediating stress responses may be involved in the control of food hoarding induced by food deprivation [44]. It was noted that the amount of food hoarded by food-deprived males (FAH) was substantially lower than that by males with free access to food (FH), further indicating potential differences in the two types of food hoarding behavior and the underlying neuromechanisms.

Our data also indicate that FAH males had a lower level of Fos-ir expression in the hippocampus than did FANH males, indicating a decreased activity in the hippocampus associated with fasting-induced food hoarding behavior. These data are consistent with data from a previous study showing that hippocampal lesions facilitate food hoarding behavior in food-deprived rats [17]. Interestingly, in the present study, Fos-ir expression in the hippocampus did not differ between FH and FNH males with free access to food. In a previous study, hippocampal lesions were reported to significantly decrease or to have no effects on food hoarding in rats with free access to food [45]. These data, together, support the notion that differential effects of the hippocampus on food hoarding depend on the animal's physiological status and/or environmental conditions.

It will be difficult to study the effects of food deprivation on food hoarding behavior in animals that naturally display high levels of food hoarding, mainly because one may likely see a ceiling effect and thus will not be able to distinguish food hoarding induced by food deprivation from that occurring naturally. Nevertheless, such effects on food hoarding behavior and its underlying neuromechanisms of those animals should not be ignored and need to be investigated in further studies.

Table 1
Group differences in Fos-ir, TH-ir, and TH-ir/Fos-ir labeling in the brain of male Mongolian gerbils that were undergone food deprivation.

Marker	Brain area	FANH	FAH	*t* test
Fos-ir	NAcc	168.00 ± 29.04	266.00 ± 99.40	ns
	LH	40.11 ± 7.01	33.00 ± 2.78	ns
	VMH	40.27 ± 3.63	33.13 ± 5.35	ns
	Hippocampus	108.17 ± 8.94	58.13 ± 8.26	$p<0.01$
	PVN	44.72 ± 3.07	37.87 ± 3.07	ns
	VTA	138.33 ± 5.88	182.13 ± 13.52	$p<0.01$
TH-ir	PVN	35.00 ± 2.11	31.20 ± 1.79	ns
	VTA	206.22 ± 17.49	232.13 ± 15.18	ns
TH-ir/Fos-ir	PVN	5.17 ± 0.48	5.73 ± 0.71	ns
	VTA	105.17 ± 8.14	118.93 ± 8.14	ns

5.4. Serum leptin concentration and food hoarding

Serum leptin has been implicated in food intake and food hoarding [11,20,21]. In particular, food deprivation decreases circulating levels of leptin [11,20–22] whereas leptin treatment inhibits food hoarding induced by food deprivation [11,20,21]. In the present study, however, circulating levels of leptin did not differ between males with free access to food and males under food deprivation or between food hoarding and no food hoarding males within each condition. One possibility is that leptin has species-specific effects on food hoarding. Alternatively, small sample sizes may have prevented us from detecting group differences. Nevertheless, a negative correlation was found between serum leptin concentrations and the amount of food hoarded, indicating a potential role of leptin in food hoarding in gerbils with free access to food, which is consistent with findings in hamsters [21]. The lack of such correlations between serum leptin and the amount of food hoarded in food-deprived gerbils further supports the notion that food hoarding induced by food deprivation is controlled by different mechanisms.

In summary, data from the present study indicate that male gerbils displayed bimodal patterns of food hoarding behavior under normal laboratory conditions, and that the dopamine reward pathway from the VTA to NAcc may be involved in the control of food hoarding behavior. In addition, food deprivation also induced bimodal patterns of food hoarding behavior in male gerbils that naturally do not display food hoarding, but this behavior was unlikely to be controlled by central dopamine. Together, these data characterize food hoarding behavior and illustrate a potential role of central dopamine in food hoarding in male Mongolian gerbils.

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