Behavioural Processes 79 (2008) 195–198

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Short communication

Experimentally induced sickness decreases food intake, but not hoarding, in Siberian hamsters (*Phodopus sungorus*)

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ARTICLE INFO

Article history:
Received 23 May 2008
Received in revised form 11 July 2008
Accepted 31 July 2008

Keywords:
Food intake
Cytokines
Lipopolysaccharide

ABSTRACT

A wide range of physiological and behavioral alterations occur in response to sickness. Sickness behaviors, rather than incidental by-products or side-effects of acute illness, serve as adaptive functional responses that allow animals to cope with a pathogenic challenge. Among the more salient sickness behaviors is a reduction in food intake; virtually all sick animals display marked decreases in this behavior. Food intake, however, is only one component of the food-related behavioral repertoire. For many mammalian species, food hoarding represents a substantial portion of the total energetic budget. Here we tested the effects of experimental sickness on food hoarding and food intake in a naturally food hoarding species, Siberian hamsters (*Phodopus sungorus*). Adult male and female hamsters received injections of lipopolysaccharide (LPS) to induce sickness or control injections. LPS-induced sickness resulted in a marked decrease in food intake in both males and females, but did not decrease hoarding in either sex. These results support previous findings suggesting that food hoarding and food intake appear to be differentially regulated at the physiological level.

1. Introduction

Animals acutely sick from systemic infections commonly display a constellation of non-specific symptoms including changes in core body temperature (i.e., fever), increased sleep, reduced general activity (i.e., lethargy) and social behavior, as well as decreased food intake (reviewed in Aubert, 1999; Hart, 1988; Konsman et al., 2002). These symptoms have traditionally been interpreted as the inability to achieve normal homeostatic functioning due to an impaired or debilitated state. More recently, however, it has been suggested that the behavioral features of illness, rather than being viewed as maladaptive responses or side-effects of disease, serve as part of a coordinated suite of behavioral adaptations (i.e., "sickness behaviors") (Aubert, 1999; Dantzer, 2001; Hart, 1988). These behavioral responses have important adaptive utility as part of the acute phase response which involves a range of metabolic and immunological changes required to successfully fend off pathogenic infection (Berczi, 1993; Hart, 1988).

Because of the robust decreases in food intake seen after infection across several species, research has focused primarily on sickness-induced changes in food intake and feeding behavior (Kent et al., 1996). Treatment with the endotoxin lipopolysaccharide (LPS), a key component of the outer membrane of Gram-negative bacteria, induces sickness and decreases food intake in a wide range of rodents and has been directly linked to increases in circulating levels of pro-inflammatory cytokines (e.g., interleukin-1β) (Aubert, 1999; Dantzer, 2001; Kelley et al., 2003; Kent et al., 1996). Furthermore, intracerebroventricular administration of LPS induces anorexia in animals allowed *ad libitum* access to food and decreases operant responding for a food reward (reviewed in Kent et al., 1996). Lastly, recent evidence suggests that LPS not only reduces food intake, but also affects the qualitative experience of food (Aubert and Danzer, 2005).

Unlike studies examining the effects of sickness on food intake, considerably less is known regarding the consequences of sickness on other food-related behaviors including foraging and food hoarding. One initial report examined the effects of experimentally induced sickness on food intake and food hoarding in laboratory rats (*Rattus norvegicus*) (Aubert et al., 1997). Specifically, rats were allowed a daily 30-min feeding session during which they were given access to food from a second cage located at the end of a short alley. Treatment with LPS reduced food hoarding, but to a lesser extent than food intake (Aubert et al., 1997). These findings, while supporting the notion of sickness-induced decreases in appetite, also point to a secondary, anticipatory component of food-related behavior (e.g., hoarding) that may be less influenced by sickness.

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doi:10.1016/j.beproc.2008.07.009

The present study assessed the effects of sickness on food intake and hoarding in Siberian hamsters (*Phodopus sungorus*). Siberian hamsters are an ideal species with which to address the effects of experimentally induced sickness on food intake and hoarding. Siberian hamsters, unlike rats and house mice, are a naturally prodigious food-hoarding species that possess morphological adaptations (e.g., sublingual cheek pouches) that allow significant quantities of food to be collected during a given foraging bout. Thus, Siberian hamsters are able to partition available food into internally consumed food, as well as external food stores (Day and Bartness, 2003). The goal of the present study was to examine the effects of experimentally induced sickness on food intake and hoarding in male and female Siberian hamsters.

2. Materials and methods

2.1. Animals and housing

Twenty-seven adult male ($n = 14$) and female ($n = 13$) Siberian hamsters (*P. sungorus*) (>60 days of age) were obtained from our laboratory breeding colony. Hamsters were individually housed in an S-shaped foraging apparatus as described previously (Bartness and Clein, 1994; Wood and Bartness, 1996). Briefly, each apparatus consisted of two polypropylene cages, a lower, “home” cage containing bedding (Sani-Chips, P.J. Murphy Forest Products, Montville, NJ) and cotton nesting material, and an upper cage containing food and water. The two cages were connected by a tract of PVC tubing approximately 1.78 m in length and 3.8 cm in diameter. The two vertical tracts of inner tubing were covered with aluminum screening material to allow the animals to perform vertical ascents between cages. Home cages were covered with aluminum foil pans to simulate the relatively dark subterranean burrows occurring in natural living conditions. The upper cages were stocked with approximately 50 g of 97-mg food pellets contained in 9.5-cm Petri dishes (Formula P, Noyes Precision Pellets, New Brunswick, NJ) and tap water *ad libitum*. All animals were kept in a colony room illuminated with a LD16:8 light cycle; ambient temperature of the rooms was $20 \pm 2\,^{\circ}$C and relative humidity was $50 \pm 5\%$. All procedures were approved by the Bloomington University Animal Care and Use Committee (BIACUC).

2.2. Experimental procedures

Animals were placed in their respective hoarding apparatuses and allowed to forage for a 2-week baseline period during which initial daily body masses, food intake and food hoarding were measured. The amount of food pellets was consistently maintained ~50 g (i.e., well above mean daily intake/hoard), ensuring the animals had *ad libitum* access to food at all times. Food intake and food hoarding were measured daily, while body mass was assessed bi-daily. Food hoards (consisting of food found in the cage bedding and any pouched food), were removed from the cages of all animals each day. On Day 9 of the baseline period, blood samples were obtained via the retro-orbital sinus after light anesthesia (diethyl ether, Sigma Chemical, St. Louis, MO).

2.3. Lipopolysaccharide treatment

On Day 13, animals from each sex were randomly selected and assigned to one of two groups, a vehicle-injected control group and an LPS-injected group. The LPS-injected group received 50 μg of LPS (from *Salmonella typhimurium*, L-6511, Sigma, St. Louis, MO) dissolved in 0.1 ml of 0.9% saline solution. Control animals received 0.1 ml of saline solution. All injections occurred at 1400 EST. Following injections, animals were immediately returned to their respective cages and food intake, food hoarding, and body mass values were recorded as described above. Blood samples were drawn on Day 8 post-immunization. On Day 14 post-immunization animals were killed by an overdose of ketamine cocktail, terminal body masses were obtained and animals received necropsies. Serum anti-LPS immunoglobulin G (IgG) concentrations were assayed using an enzyme-linked immunosorbent assay (ELISA) (modified from Demas and Sakaria, 2002) to estimate the magnitude of the immune response to LPS.

3. Results

Food intake and hoarding were each analyzed using separate three-way (injection × sex × day) mixed model analyses of variance (ANOVA) with repeated measures (SPSS, Chicago, IL) with all 24 days comprising the within-subjects factor (i.e., day). A significant main effect of injection on food intake was observed ($F_{1,23} = 26.58$; $p < 0.001$). In addition, there was a significant injection × day interaction ($F_{1,23} = 16.29$; $p < 0.001$) Specifically, decreased food intake was observed immediately after hamsters were injected with LPS on Day 13 (Fig. 1). Food intake levels remained significantly low for three to four days post-injection and gradually increased back to baseline levels on each of these days. The decrease in food intake occurred with a similar magnitude in both sexes and no such decrease was seen in the saline injected groups. In contrast to food intake, there was no significant main effect of LPS on food hoarding (Fig. 2). LPS-treated males appeared to show decreased food hoarding compared with control animals, although this trend was not statistically significant. There was a significant main effect of sex on food hoard size ($F_{1,23} = 7.12$; $p = 0.014$; specifically, male hamsters hoarded significantly more food than did females. There were no significant interactions between sex and injection type in either food hoarding or food intake.

Differences in serum anti-LPS IgG in LPS-treated hamsters were determined via independent *t*-test. There was no sex difference in serum anti-LPS IgG concentrations in LPS-treated hamsters (percent plate positive values of $88.26 \pm 9.86\%$ in males and $69.04 \pm 2.06\%$ in females. Antibody responses were not determined in control animals, as these animals lack anti-LPS immunoglobulins. Body mass was analyzed using a three-way (injection × sex × day) mixed model ANOVA. There was a significant main effect of sex on body mass ($F_{1,23} = 25.28$; $p < 0.001$); male hamsters weighed significantly more than female hamsters. There was no effect of LPS on body mass and none of the interactions were statistically significant. Lastly, there were no significant main effects of sex or treatment on splenic, reproductive or white adipose tissues masses.

4. Discussion

The primary finding of the present study is that LPS-induced sickness decreased food intake, but not food hoarding in male and female hamsters. There was no apparent sex difference in sickness-induced food intake or hoarding, although males displayed a moderate, albeit non-significant, decrease in food hoarding compared with female LPS-treated hamsters. LPS-injected animals displayed measurable anti-LPS IgG responses, although there was no sex difference in serum IgG. Collectively, these results are consistent with the hypothesis that experimentally induced sickness differentially affects food intake versus food hoarding, in support of previous findings suggesting that different physiological mechanisms may underlie these specific behaviors (Day and Bartness, 2003, 2004). Physiological responses that sustain hoarding behavior, even during periods of sickness, are likely adaptive because

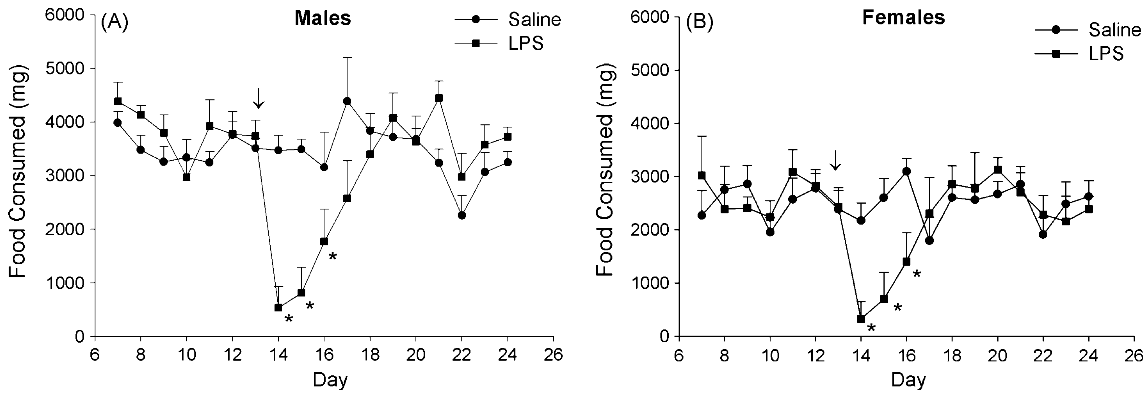

Fig. 1. Mean (±S.E.M.) food intake in (A) male and (B) female Siberian hamsters injected with lipopolysaccharide or saline control across days. All animals were injected on Day 13 (as indicated by the arrow). Significant differences in pair-wise means with each day are indicated by an asterisk (*) if $p < 0.05$.

they allow individuals to establish readily available food caches that could be consumed upon overcoming sickness. This would be particularly important in Siberian hamsters, a naturally food hoarding species, where external food caches play an important role in the "energetic equation."

Previous research has shown that defense against pathogens through activation of the immune system confers obvious benefits, but can also be energetically costly (Sheldon and Verhulst, 1996; Demas, 2004). Therefore an optimal level of immune function is expected to depend on the balance between the obtained benefits and the incurred energetic costs (Bonneaud et al., 2003). Despite these findings, which suggest the need for *increased* food (energy) intake in order to sustain heightened immune activation, it is also well-established that decreased food intake lowers circulating concentrations of specific nutrients (e.g., iron, zinc) that are vital to pathogen replication (e.g., Weinberg, 1972). Given this apparent paradox, animals that are capable of maintaining energy input in the form of food hoarding and establishment of an external food cache while reducing energy input in terms of *consumed* food, would likely be able to maximize both immune responses and pathogen resistance. In addition, by maintaining a food cache, animals would have ready access to food for consumption immediately following the acute phase response, potentially aiding in the recovery of health.

Although neither sex significantly decreased food hoarding in response to experimentally induced sickness, there was an observed trend towards decreased food hoarding in males compared with females. Although the precise mechanisms for this trend are not known and were not directly tested in the present experiment, it is possible that females are less sensitive, either physiologically (e.g., cytokine production) or behaviorally (e.g., response to cytokines), to sickness than males. At an ultimate level of analysis, a pregnant or lactating female challenged with illness would still need to provision her offspring with sufficient nourishment, and thus, the costs of decreasing energy input may be greater in females compared with males. An alternative, less intriguing explanation for the failure for hamsters, especially females, to decrease food hoarding in response to sickness is the potential of a "floor effect" in which levels of hoarded food were sufficiently low that a significant decrease was not possible. This possibility is not likely given that previous studies have demonstrated the complete cessation of food hoarding in response to physiological manipulations in food-hoarding rodents (Demas and Bartness, 1999). Previous studies, however, have demonstrated that food hoarding can be reduced in Siberian hamsters when given 24 h *ad libitum* access to food (Bartness and Clein, 1994). Interestingly, restricting access to food to a limited period of time during the dark phase (when hamsters consume the majority of their total daily food intake) increases food hoarding in this species (Bartness and Clein, 1994). Future studies examining the effects of sickness on food hoarding using a restricted feeding paradigm will help clarify this issue.

Collectively, the results of the present study demonstrate that experimentally induced sickness markedly decreases food intake but not food hoarding in male and female Siberian hamsters. These results suggest that the physiological mechanisms underlying food intake versus food hoarding are independently regulated, at least in response to sickness. The results support previous research

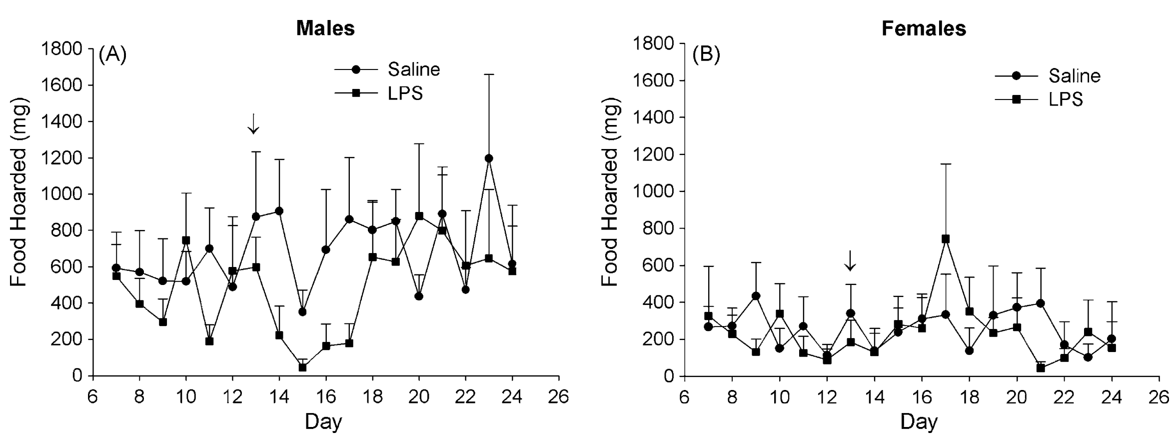

Fig. 2. Mean (±S.E.M.) food hoarding in (A) male and (B) female Siberian hamsters injected with lipopolysaccharide or saline control across days. All animals were injected on Day 13 (as indicated by the arrow).

suggesting independent neural and neuroendocrine circuits regulating food hoarding and food intake in this species (e.g., Day et al., 2005; Keen-Rhinehart and Bartness, 2007). One possibility is that inflammatory responses differ within neural circuits controlling feeding versus hoarding, which may lead to differential behavioral responses to immunological challenges such as LPS. Although this idea is intriguing, there are currently insufficient data to test it. Regardless of specific mechanisms, the lack of sickness-induced changes in hoarding appears to be an adaptive mechanism by which hamsters can continue to maintain sufficient energy stores while fending off potential infection. This is particularly relevant for Siberian hamsters, given the importance of external food hoards to the total energetic budget in this species relative to other non-food-hoarding species. More broadly, the present findings support the emerging idea that sickness behaviors, rather than being non-specific, debilitating responses to illness, are specific adaptive responses that allow an individual to cope with pathogenic infection.

Acknowledgements

The authors acknowledge Kelly Myer Polacek for technical assistance and Molly Wright for expert animal care. This research was supported in part by a Microbiology Undergraduate Summer Research Program award to A.D. and an Undergraduate Research and Creative Activity Partnership grant from Indiana University to A.D. and K.P.

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