Taste as a marker for behavioral energy regulation:Replication and extension of meal pattern evidence from selectively bred rats

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ABSTRACT

A key feature of energy regulation among species that eat discrete meals is *meal patterning* – meal frequency, size, and duration. Such animals can adjust to internal states and external circumstances with changes in one or more of those meal parameters, with or without altering total food intake. Relatively little is known about individual differences in meal patterning. We previously reported meal patterning differences between rat lines selectively bred for differential saccharin solution intake, lines that also differ in sensitivity to metabolic challenges: Relative to high-saccharin-consuming counterparts (HiS), male low-saccharin-consuming rats (LoS) ate smaller, more frequent meals. Those findings provided evidence of an association between taste and short term satiety. Twenty generations later, we describe systematic replication of the line difference in meal patterns in males and females using two different kinds of reinforcer pellet. The previous study was further extended by examining meal parameters (1) with bi- and multivariate analyses and (2) after acute food restriction and a moderate stressor. Results are discussed within a behavior-systems framework incorporating taste as a marker for behavioral energy regulation.

1. Introduction

Taste is an important component of the feeding behavioral system. For instance, sweetness is a reliable signal of easily utilized energy and bitterness of toxicity, and diverse species are motivated to find and consume sweet substances and to avoid bitter ones (reviewed in Dess et al., 2017). Taste also plays a role in individual differences in the organization of feeding behavior. For instance, reinforcer palatability influences the degree to which rats goal-track or sign-track in a food-motivated task (Patitucci et al., 2016). For more than 50 generations, we have selectively bred rats on the basis of voluntary saccharin intake (low, LoS line, versus high, HiS line), a phenotype in which both sweetness and bitterness play a role (Dess et al., 2000, 2017). We are interested in how selective pressure on a taste phenotype manifests in other behaviors related to energy regulation. *Energy regulation* is a broad construct that encompasses processes at levels of organization from cellular metabolism to complex behavior in ecological context. Within that broad framework, we examine how the taste phenotype is narrowly related to eating behavior and how it is linked to other behavioral systems, such as predatory defense and sociality.

In one program of research, LoS and HiS rats' responses to explicit metabolic challenges are assessed. Across experimental paradigms, LoS rats display greater reactivity to food scarcity. Relative to HiS rats, LoS

rats run more vigorously when their food supply is restricted (Dess et al., 2000), express flavor conditioning more dynamically when refeeding after food restriction (Dess et al., 2013), and display better behavioral energy regulation (i.e. less variation in eating rate as the "price" of food varies) when maintained in negative energy balance (Dess et al., 2007).

A second program of research concerns responses to indirect metabolic challenges. Using the "dietary niche" as a conceptual model (Dess, 1991), we have explored ways in which eating is linked to other behavioral systems. Succeeding in a dietary niche involves not only dealing with nutrient availability (e.g. foraging, food selection) but also balancing eating with other rewards and risks by, for example, foraging less frequently or less selectively when mating opportunities or probability of attack by a predator or dominant conspecific is high (Coleman et al., 2005; Dalesman et al., 2018; Fanselow et al., 1988; Moore et al., 2013). The prophylactic effect of glucose consumption after a traumatic stressor illustrates the relevance of nutrient intake to cellular metabolic processes related to anxiety (Conoscenti et al., 2017).

Extending this logic, individual differences in reward and risk reactivity may manifest in more than one behavioral system, including eating and predatory or conspecific defense, and in that sense are domain general. Specifically, we have proposed that greater aversion to bitter side tastes is a marker for domain-general reactivity to threats to

metabolic homeostasis. In support of this idea, LoS rats eat less chow in their homecage after an intense stressor than do HiS rats (100 tail-shocks; Dess and Minor, 1996). LoS rats also display higher acoustic startle amplitude than do HiS rats, even though the auditory stimulus and the defensive reflex it elicits bear no obvious relationship to taste (Dess et al., 2000); that line difference is modulated by a moderate stressor (20 footshocks) and anxiolytic drug treatment (Gonzales et al., 2008). This pattern of phenotypic correlates implies linkage of appetitive and aversive motivational processes through which eating and defensive systems can interact. We have speculated elsewhere about neurohormonal candidates for such processes, including the hypothalamic-pituitary-adrenal axis and reward mechanisms involving the nucleus accumbens (see Dess et al., 2007, 2013).

A tool that we have used to study behavioral energy regulation is a simple operant closed economy (Collier and Johnson, 1997). Rats live in an operant chamber and earn all of their food by bar pressing on a continuous reinforcement schedule. In addition to total food intake, several meal parameters – meal frequency, duration, and size – are measured. Analysis of meal parameter profiles (*meal patterning*) provides a sensitive means of exploring behavioral energy regulation. As components of a system sensitive to both environmental and dispositional factors, meal parameters typically are correlated, but they are not redundant: One meal parameter can change with little to no change in others (e.g. decreased meal size with no change in meal frequency at estrus; Eckel et al., 2000). Shifting rats between higher and lower calorie diets affects both meal frequency and meal size, but meal size changes more (South et al., 2014) and is a better predictor of adiposity (Melhorn et al., 2011). Moreover, individual differences in adiposity generated by different diets are associated with distinct meal parameter profiles (Even et al., 2011). Results such as these illustrate the value of studying *how* and *when* food is consumed, and not just what or how much is consumed.

An ecological variable to which meal patterning is sensitive is *predatory imminence* (Fanselow et al., 1988). In a safe environment, rats display a *preferred activity pattern*. When environmental cues signal potential predatory attack, meal patterns depart from the preferred pattern so as to reduce predation risk. When predation is possible but not imminent, for instance, risk can be mitigated by foraging less frequently (*pre-encounter* behavior). These dynamics can be modeled in a closed economy, using footshock parameters to model predation risk and meal frequency to model foraging bouts (Helmstetter and Fanselow, 1993).

We previously described meal patterning in male LoS and HiS rats (Dess et al., 2007). To the extent that our closed economy preparation is subjectively risky, LoS rats were expected to initiate fewer, larger meals than would HiS rats by virtue of their elevated risk reactivity. Instead, the opposite was observed: LoS rats ate more, smaller meals than did HiS rats. This result compels the view that our preparation is a safe environment in which the lines display preferred activity patterns which, for reasons to be determined, features more frequent meals among LoS rats. Palatability does not alter meal frequency in a closed economy (Sunday et al., 1983), so although the palatability of the high-sucrose reinforcer pellet we used might have differed between lines, energy regulation processes for which palatability is a marker – and not palatability *per se* – generate the line difference in meal patterning (Baldo et al., 2016).

The present study replicated and extended prior work on meal patterning in the LoS and HiS lines to establish its utility – more generally, the utility of selectively bred lines – in delineating when phenotypic variation is confined to the feeding domain and when it bridges behavioral domains. We had three specific objectives. First, we sought to establish the reliability and generality of the previously observed line difference in meal patterning, an important goal in light of the "replication crisis" (Pashler and Wagenmakers, 2012). To this end, five systematic replications were conducted with males and females using two different reinforcer pellets, to determine whether sex or type of

Table 1

Number of rats with stable baseline meal patterning and initial body weight (g, M ± SEM) in Studies 1–5.

	N	Body Weight (g)
Study 1: Males, grain-based pellets		
LoS	18	399 ± 12
HiS	18	408 ± 11
Study 2: Females, grain-based pellets		
LoS	19	249 ± 6
HiS	19	249 ± 6
Study 3: Females, purified pellets		
LoS	19	252 ± 6
HiS	19	248 ± 4
Study 4: Males, purified pellets, effect of acute deprivation		
LoS	21	411 ± 12
HiS	23	398 ± 12
Study 5: Males, purified pellets, effect of moderate stressor		
LoS	17	372 ± 14
HiS	18	361 ± 7

food comprises a boundary condition for the line difference. We did not expect the line difference in meal patterning to be specific to one sex or reinforcer.

The second objective was to examine the effects on meal patterning of a direct metabolic challenge (food deprivation; Study 4) and an indirect one (a moderate footshock stressor; Study 5) in LoS and HiS rats. We expected both manipulations to affect LoS rats more than HiS rats.

The third objective was to examine meal parameter intercorrelations in LoS and HiS rats (Studies 1–5). High correlations suggest high synchrony among meal control mechanisms, and low correlations suggest relative independence of those mechanisms. Overall synchrony and differences in synchrony between LoS and HiS rats were of interest.

2. Method

2.1. Rats

Adult male and female LoS and HiS rats from the Occidental College colony (Generations 45–52) were used. Group sizes and initial body-weights for each study are shown in Table 1. Rats were housed with same-sex littermates on a 12:12 h light:dark cycle (lights on at 0700) with free access to Purina 5001 rodent chow and water before being moved to operant chambers. Care and use of rats complied with a protocol approved by the Occidental College Institutional Animal Care and Use Committee, whose policies comply with NIH policy.

2.2. Apparatus and materials

Eight functionally identical operant chambers in which aversive stimuli have never been delivered were used (details in Dess et al., 2007). The house light was on a 12:12 h timer, with lights on at 0700. LoS and HiS lines were balanced across boxes. Rats could earn pellets 23 h per day (0900–0800) on a continuous reinforcement schedule (FR1). Few uneaten pellets were found during morning maintenance, so bar pressing on this rich schedule with the opportunity to earn unlimited pellets appears to be a satisfactory proxy for pellet consumption.

Rats earned 45-mg pellets that were either grain-based (F0165) or purified (F0021) (Bio-Serv, Flemington, NJ). Both pellets are nutritionally complete but differ in many ways (flavor, caloric density, macronutrient profile). The first ingredient (by weight) of the grain-based pellets is corn; their 3.4 cal/g derive from 10.1% fat, 25.4%, protein, and 64.5% carbohydrate, some of which is sucrose. The first ingredient of the purified pellets is sucrose; their 3.6 cal/g derive from 13.9% fat, 20.8% protein, and 65.3% carbohydrate.

In Study 5, footshocks were delivered in acrylic boxes in a room