Published in final edited form as:

Peptides. 2008 January ; 29(1): 139–147.

Behavioral controls of food intake¹

Stephen C. Benoit* and **Andrea L. Tracy**

University of Cincinnati, Department of Psychiatry

Abstract

Recent conceptualizations of food intake have divided ingestive behavior into multiple distinct phases. Here, we present a temporally and operationally defined classification of ingestive behaviors. Importantly, various physiological signals including hypothalamic peptides are thought to impact these distinct behavioral phases of ingestion differently. In this review, we summarize a number of behavioral assays designed to delineate the effects of hormone and peptide signals that influence food intake on these ingestive mechanisms. Finally, we discuss two issues that we have encountered in our laboratory which may obstruct the interpretation of results from these types of studies. First, the influence of previous experience with foods used in these behavioral tests and second, the importance of the nutrient composition of the selected test foods. The important conclusion discussed here is that the behavioral analysis of ingestion is accompanied by theoretical constructs and artificial divisions of biological realities and the appreciation of this fact can only increase the opportunities of contemporary behavioral scientists to make significant and novel observations of ingestive behaviors.

1. Introduction

Ingestive behavior is a complex process controlled by multiple physiological and psychological mechanisms. Many researchers interested in the biological controls of food intake approach ingestion as a single experimental outcome measurable by total food consumed. While food intake may be the consummate event of this sequence, a rich behavioral literature has described numerous components of this intricate process. Two general categories of behaviors have been elucidated from sequence of events that ultimately lead to ingestion. On one hand, there are behaviors involved in the seeking, locating, and acquiring foods. On the other, are behaviors associated with the act of ingestion itself, including chewing and swallowing. These different behaviors can be generally classified as ‘appetitive’ and ‘consummatory’ and are related to their temporal proximity to the ingestion of food [21].

Importantly, there are numerous central nervous system structures and circuits involved in the control of food intake and energy homeostasis [61,68,82]. Peripheral signals, such as leptin and insulin and their receptors are thought to act primarily via hypothalamic circuits containing several distinct neuropeptide systems, which effect changes in ingestive behavior (e.g., [5, 67,72]. Of these, the orexins (orexin-A), neuropeptide-Y (NPY), melanin-concentrating hormone (MCH), and the melanocortin system have received a great deal of experimental attention over the last several years (e.g., [34,60,66,79,81,83,89]. Each of these peptides is

¹This work was an invited review from the recipient of the 2007 Gayle A. and Richard D. Olson Prize for meritorious behavioral research.

*Corresponding author. Address Correspondence to: Stephen C. Benoit, University of Cincinnati, Department of Psychiatry, Obesity Research Center, 2170 East Galbraith Road, Cincinnati, OH 45237, Voice: 513-558-4312, Fax: 513-558-4800, Email: Stephen.benoit@uc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

expressed in neurons in the hypothalamus, elicits changes in food intake, and is regulated by energy balance.

Because of the redundancy in systems that can influence food intake, a logical hypothesis is that distinct neuropeptide systems may contribute specifically to the control of different phases of ingestive behavior. For example, some systems might initiate appetitive, pre-ingestive behaviors while other systems elicit hyperphagia by increasing consummatory-phase behaviors. The logic for this hypothesis comes from the observations that appetitive and consummatory phases of ingestive behavior have been reported to be differentially susceptible to various experimental and pharmacological manipulations. For example, Berridge and his colleagues have demonstrated pharmacologically, that the dopaminergic and opioidergic systems are differentially involved in appetitive and consummatory behaviors, which they elegantly describe in theoretical terms of “wanting” and “liking” (see [9,11,63] for reviews).

There is a long history of research on the behavioral mechanisms that influence food intake. A full discussion of these psychological and physiological mechanisms could easily fill entire volumes. For simplicity, here, we have chosen to divide these mechanisms into three broad categories that can be defined by their temporal relationship to the ingestion of food: pre-ingestive or appetitive, consummatory and post-ingestive mechanisms.

2. Phases of ingestive behavior

2.1 Pre-ingestive mechanisms

2.1.1 Interoceptive signals—Animals engage in a number of behaviors to find and acquire food. These appetitive or foraging acts are termed “pre-ingestive” since they occur prior to the animal engaging the food directly and consuming it. One hypothesis to be assessed is that hypothalamic peptides act on these appetitive approach and food-seeking behaviors, before the beginning of the more stereotyped consummatory behavior. The initiation of food seeking and ingestion is often considered to result from homeostatic signals that accumulate over the interval since food was last consumed. Specifically, food deprivation is thought to generate homeostatic signals related to the gradual depletion of energy stores (e.g., [53]), and these signals may make animals more likely to engage in appetitive behaviors that ultimately lead to food consumption. The first requirement for changes in physiological systems related to food deprivation to be able to effect food-seeking and other pre-ingestive behaviors is that animals must be able to detect these changes. In fact, there is compelling evidence, using Pavlovian conditioning techniques, demonstrating that animals can learn to respond to the presence or absence of these internal signals.

We have developed and extensively used a paradigm for assessing this process [22–24]. In this deprivation-discrimination procedure, rats are trained such that one level of food deprivation (e.g., 24 hr) is always associated with the delivery of a specific reinforcer while another level of deprivation (e.g., 0 hr) is not. Physiological signals related to being 24-hr food deprived then become associated with the subsequent presentation of reinforcement and being 24-hr food deprived causes the animal to anticipate the reinforcement presentation. Discrimination is achieved to the extent that an animal makes more anticipatory responses (i.e., behaviors appropriate for preparing for the reinforcement) when 24-hr food deprived (reinforced condition) than when 0-hr deprived (non-reinforced condition). Importantly, in these experiments, another group of rats receives the reverse contingency between deprivation and reinforcement (i.e., reinforcer presented when 0-hr food deprived, but not when 24-hr food deprived).

Using both appetitive (peanut oil, sucrose pellets) and aversive reinforcements (foot-shock), we have found that rats readily solve this discrimination (e.g., [23,26]). There are two important

implications of this. The first is that food deprivation (or its absence) produces reliable internal signals that the animal can detect and use to influence its behavior. The second is that once rats have been trained to respond differentially to these signals, they can then be assessed for generalization to pharmacological manipulations that influence food intake.

A key question for our research is whether or not administration of exogenous compounds that change food intake will elicit patterns of responding similar to those seen after periods of food deprivation or food repletion. That is, we seek to assess whether such manipulations might play a role in the production of interoceptive sensory stimuli that underlie the psychological constructs of “hunger” and “satiety” and contribute to the altered behavioral responses to food and food-related stimuli. In this respect, we define “hunger” and “satiety” 1) *operationally* as periods of 24- and 0-hr food deprivation, respectively, and 2) *functionally* as the consequent physiological events of different degrees of food deprivation (e.g., associated changes in peripheral signals like leptin, insulin, and glucose, as well as central neuropeptides such as NPY). The reliance on behavioral techniques unrelated to food intake to answer these questions is crucial because 1) we seek to understand what physiological systems underlie specific *psychological states* such as hunger or satiety, and 2) because they help reduce the potential confounds encountered when tests rely solely on measures of food intake (see [22,27]).

Employing this type of deprivation-discrimination in a recent experiment, we observed that intracerebroventricular administration of melanocortin agonists causes trained rats to respond as if they were 0-hr food deprived and, conversely, that melanocortin antagonists elicit responding like that following 24-hr food deprivation. Additionally, we have also demonstrated that the orexigenic stomach-derived hormone ghrelin elicits conditioned responding like that observed following a period food deprivation and that the gut peptide cholecystokinin and the adipocyte hormone leptin, both of which act to reduce food intake, generalize to a state of 0-hr food deprivation [28,29,52]. On the other hand, administration of NPY does not elicit behavior similar to 24-hr food deprivation in this paradigm, suggesting that this peptide acts via mechanisms other than inducing an interoceptive state of “hunger” [51,73].

2.1.2 Appetitive behaviors—Another well-validated procedure for evaluating the effects of pharmacological manipulations on appetitive behaviors is also based in Pavlovian conditioning. In this experimental paradigm, animals are trained to associate a discrete cue with food presentation. In one version of this procedure, rats are trained to expect a small drop of peanut oil following the presentation of one stimulus (e.g., a light) and to expect a small drop of a sucrose solution following the presentation of a different stimulus (e.g., a tone). The animals must then learn to approach a food cup to obtain the oil or the sucrose when the appropriate stimulus is presented [2,7,25]. After extensive training, the animals are treated with a pharmacological manipulation that is known to alter food intake, and they are given a test trial in which the “oil” and the “sucrose” stimuli are presented, but no oil or sucrose is delivered. Whether or not the animal approaches the food cup during each specific stimulus (oil or sucrose) is the dependent variable of interest. The extinction condition (i.e., no oil or sucrose available) during the test trial is an important feature of this paradigm. This allows us to conclude that any changes in responding are indicative of pre-ingestive mechanisms affected by the peptide of interest and are not confounded by changes in palatability or energy intake.

In addition to affecting overall approach behavior toward food-paired stimuli, different peptides and hormones might be critically involved in the selection of specific macronutrients. The use of multiple cue-nutrient pairings has allowed our lab to use this paradigm to assess hypotheses about how specific hypothalamic neuropeptides alter responses to sucrose and oil-specific stimuli while simultaneously assessing the effect on general appetitive behaviors. Data from our lab using this paradigm indicate that NPY appears to have a general effect to increase appetitive behavior in response to the context in which both foods were provided, while

appetitive behavior following administration of the melanocortin antagonist agouti-related peptide (AgRP) is nutrient-specific, increasing in response to the oil-paired cue and decreasing in response to the sucrose-paired cue [2,78].

2.1.3 Motivational mechanisms—The Pavlovian paradigm described above is an ideal tool for assessing the appetitive effects of hypothalamic peptides involved in food intake when animals are faced with cues previously associated with the delivery of food. However, those studies require only minimal effort to obtain the food reinforcer and do not address possible effects of these peptides on an animal's willingness to work for food. An operant progressive ratio response schedule [14,37], allows determination of the degree of effort a rat will expend to earn a reinforcer and, as commonly employed in the drug self-administration literature, is often considered a measure of the animal's "motivation" to obtain that particular reinforcer [65]. In this paradigm, the number of responses required to obtain each morsel of food is progressively increased during a meal. As expected, food deprivation increases the number of responses rats will make to obtain food whereas having just eaten decreases that number [47]. This paradigm can therefore be used to compare the "motivational" effects of a hormone or peptide of interest to the effects of different levels of food deprivation. This use of the paradigm has demonstrated that NPY is capable of producing increases in operant responding and that response levels are similar to 36–48 hr food deprivation [49,50]. Orexin-A and peripheral insulin also increase progressive ratio breakpoints for sucrose, although not to the same extent as NPY or significant (i.e., 24-hr or greater) food deprivation [50,75]. On the other hand, direct central administration of the adiposity signals insulin and leptin, which both reduce intake of freely available food, also reduce operant responding for a sucrose reinforcer [38].

In addition, we are currently using this paradigm to assess the effects of hypothalamic neuropeptides on responding for different macronutrients (e.g., carbohydrates vs. fats). Data from our lab suggest that AgRP selectively increases operant responding for fats, but not carbohydrates [78]. Other data using this paradigm show that a cannabinoid receptor agonist increases and an antagonist decreases PR responding for a sweet mixed-nutrient reinforcer, as well as one that is 100% fat, but with far more pronounced effects on the sweet food [80].

2.2 Consummatory mechanisms

2.2.1 Taste and palatability—Another major division of the behavioral and psychological controls of food intake comes into play during the ingestive behavior itself. Palatability (defined here as an indication of food's hedonic value) influences both the amount and type of food that is ingested [8–10]. For example, it is well known that even non-caloric solutions will elicit drinking behavior in sated rats if they are made to taste sweet [18]. It is possible that one set of physiological signals might reduce ingestion by making foods less palatable. Conversely, other signals might increase the ability of a particular taste to elicit consumption. In one example of this, food deprivation has been shown to alter preferences for mineral oil relative to saccharin, both non-caloric substances, but with drastically different orosensory properties that mimic those of fats and carbohydrates (i.e., oily versus sweet) [58].

Consistent with this general idea, some hypothalamic peptides (e.g., hypothalamic melanocortins) project to CNS areas important for taste processing (nucleus of the solitary tract) and for food hedonics, including the ventral tegmental area, nucleus accumbens, and substantia nigra [54,56,88]. Further, leptin receptors are directly expressed in the VTA, leptin deficiency leads to mesolimbic dopamine dysfunction and leptin, like being sated, has been found to regulate the hedonic qualities of brain self-stimulation [39,42,43]. The implication of this collective evidence is that the effect of leptin or its effector neuropeptides on food intake may be due to the modulation of taste-related properties, or "palatability" during the behavior itself. In fact, a recent study found that centrally administered orexin-A, MCH and NPY

increased intake of saccharin, while AgRP, ghrelin and dynorphin had no effect [44]. These results suggest that the former peptides increase taste-based consummatory behaviors independent of calories, while the latter may be more dependent on post-ingestive mechanisms.

Since food must contact receptors on the tongue in order for palatability to be assessed, and this generally results in swallowing of the food, post-ingestive (i.e., gastrointestinal) factors can easily confound interpretations of experiments designed to determine the specific effects of palatability. A procedure known as “sham feeding” is one method that can be used to assess the effects of taste and orosensory stimulation independent of post-ingestive effects. The sham feeding method involves surgical implantation of a fistula in the stomach, which can be opened during testing to allow food to drain out of the stomach, eliminating post-oral stimulation [87]. Manipulations that alter intake during sham feeding, therefore, can be attributed to effects on the orosensory characteristics of the ingested solution. Central administration of NPY enhanced sham feeding of a sucrose solution to an even greater extent than real feeding, leading to the conclusion that the NPY acts primarily via consummatory mechanisms to increase food intake [77].

While eliminating confounding post-ingestive effects, the sham feeding technique still requires animals to locate and actively ingest the selected food. A clever technique to separate the consummatory from the pre-ingestive phase is the intraoral intake paradigm. This procedure allows for direct assessment of liquid (e.g., 10% sucrose) intake without the potential confounds of general activity or differences in appetitive approach behaviors. Briefly, rats receive an implanted intra-oral catheter through which small amounts of flavored solutions can be infused directly into the oral cavity. In this way, the experiment can circumvent the usual pre-ingestive behaviors such as the approach to a bottle, and instead directly assess the consummatory effects of a particular manipulation. Further, the technique can be used in combination with intake from a bottle on the homecage to dissociate the specific pre- and ingestive behavioral effects of a treatment. For example, treatments that increase bottle-intake but not intra-oral intake are thought to be involved in appetitive or pre-ingestive behaviors. In an experiment using the intraoral intake paradigm, Seeley and colleagues [74] demonstrated that centrally administered NPY increased rats' intake of sucrose from a bottle on the homecage, but had no effect on sucrose delivered directly into the oral cavity (intraoral administration). Based on these observations, Seeley et al., concluded that NPY exerted its orexigenic effects predominantly via appetitive or pre-ingestive, rather than consummatory, mechanisms.

2.2.2 Flavor Learning—Additional evidence supporting the role of taste in the control of intake comes from experiments that examine learning about flavors. Numerous experiments indicate that the acceptance or avoidance of flavors can be manipulated through conditioning procedures [17,19,35,69,71]. For example, rats will increase their intake of a less preferred flavor solution (such as quinine) if its presentation has historically been followed by another, preferred flavor solution [13]. In other kinds of experiments, delivery of a flavor that is normally preferred can support the learning of a conditioned place preference in rats [30]. In these paradigms, rats are given an equal amount of experience in two novel contexts, each of which has a drinking tube. In one context the drinking tube delivers water or a non-preferred solution. In the other, the tube delivers a highly preferred sucrose solution. At a later test, rats are given a choice between the two contexts but without access to the drinking tubes. The common finding is that they spend more time in the context where they had received the sucrose solution. These data indicate that flavor itself can be a powerful stimulus capable of supporting learning. Indeed, we have recently observed that the adipocyte hormone leptin reduces the reinforcement potency of sweet tastes to elicit a conditioned place preference. In that experiment, food-deprived rats were given access to sucrose in a conditioned place preference paradigm after receiving leptin or saline. As occurs when rats are trained just after eating and are sated, leptin attenuated subsequent preference for the chamber that contained sucrose.

Importantly, the amount of sucrose consumed during training was extremely small (1.5 kcal), strongly implicating taste rather than calories as the key aspect of the stimulus in the paradigm [40].

2.3 Post-ingestive mechanisms

After an animal has consumed food and is operationally “sated,” post-ingestive processes are thought to be engaged which influence subsequent food intake. It is well known that animals learn about these post-ingestive consequences of eating [27,33,35,57,70]. One early example of post-ingestive learning is the conditioned taste aversion. When rats experience visceral illness after ingestion of a novel flavor, the likelihood that this flavor will be consumed again is greatly decreased [45]. In terms of the present analysis, an animal in this situation has learned that the post-ingestive consequences of eating a particularly flavored food are negative. Analogously, animals can also learn about the positive post-ingestive consequences of food intake. For example, rats can learn to associate foods or tastes with specific nutrient or caloric content [33,41,59,64,76]. This kind of learning can then influence subsequent food choice decisions. Generally, flavors paired with more calories will be preferred relative to those paired with fewer calories [1]. However, particularly high levels of nutrient density will reduce intake of an associated flavor, a phenomenon known as conditioned satiety [12]. Deprivation state can also affect these flavor preferences, with preferences for flavors paired with more caloric foods enhanced by food deprivation and depressed under states of satiety [36,86].

One way in which learning about the post-ingestive effects of particular foods has been conceptualized is termed “incentive value”. According to this view, the magnitude of the positive post-ingestive consequences of ingestion is a function of deprivation state. Therefore, animals assign incentive values to specific foods based upon the degree of food deprivation present when the foods are consumed [3,4,31,32]. During fasting, consumption of foods, particularly novel foods, will lead to assignment of a higher incentive value for that food than when the same food is consumed when an animal is sated. Over time, multiple experiences with foods under a variety of deprivation conditions will ultimately allow an animal to modulate their intake of a variety of foods based on the anticipated post-ingestive effects, or the incentive value they have assigned, given their deprivation level at a particular eating occasion. However, if an animal encounters a particular food only when food deprived, that food will maintain a consistently higher incentive value than foods that are sampled under both low and high deprivation. A higher incentive value, even in the absence of food deprivation, can subsequently elicit both increased food intake and increased appetitive approach behaviors [3].

Based on this notion, we have developed an experimental paradigm designed to assess the effects of hypothalamic peptides on the learning of these flavor → post-ingestive relationships. In this paradigm, animals are trained to associate a cue with a specific food (e.g., sucrose) under one deprivation condition. Animals are then exposed to this same food under either the same deprivation condition, a novel condition, or after being treated with a peptide of interest. They are then tested for their responding to the food-paired cue under the novel deprivation condition. Having been previously exposed to the food under this condition alters the animals responding during the test relative to those animals that were always given the food in the same deprivation condition, as the former group has had the opportunity to learn about the post-ingestive effects of the food under this state. The responding of the group treated with the peptide of interest (in the original deprivation condition) can then be compared to the animals in both groups to determine the effect of this peptide to alter what is learned about the post-ingestive consequences of the food.

The hypothalamic melanocortin peptide, alpha-melanocyte stimulating hormone (α -MSH), seemed an obvious candidate to test in this paradigms, as it reportedly increases the efficiency

of some forms of learning and memory [46,62,84,85]. With respect to learning about post-ingestive consequences, we observed that the α -MSH analogue MTII (which potently reduces food intake) was unable to support post-ingestive learning like that following the ingestion of food in a non-deprived state. These data are consistent with several general ideas: 1) That the processes underlying consumption of food and learning about the post-ingestive effects of food intake may be genuinely separable and 2) That specific neuropeptide systems might be involved in one, but not another of the processes.

3. Complications in differentiating the phases of ingestion

Obviously one important goal for these different behavioral techniques and conceptualizations is to elucidate the different physiological and biological controls of specific phases of ingestion. However, there are a number of issues that can complicate the interpretations of experimental results using several of the above paradigms. We would like to highlight two of these issues here: the effect of previous experience and the effect of test food selection.

3.1 Previous experience

As have others, we have hypothesized that specific hormones and peptides might play critical roles in specific ingestive processes. Multiple peptide systems have received significant attention along these lines. For example, neuropeptide-Y (which elicits robust increases in food intake) has previously been suggested to play an important role in the initiation of pre-ingestive or “appetitive” behaviors of food intake, but not in the consummatory phase of ingestion. As described above, a previous study conducted by Seeley et al. [74] concluded that the NPY acts through pre-ingestive, or appetitive, mechanisms, but not consummatory mechanisms, to increase food intake due to its ability to increase sucrose consumption from a lick-spout, but not when the solution was delivered intraorally.

We recently sought to replicate and extend these findings to other hypothalamic peptide systems [6]. The purpose of those experiments was to assess the effects of NPY, MCH, and orexin-A to elicit selectively appetitive or consummatory ingestive behaviors. Obviously the elucidation of distinct ingestive behavior phases for each candidate peptide would be of significant importance and value. Based on the previous research [74], we predicted that at least the effects of NPY would be limited to appetitive (pre-ingestive) driven sucrose intake (i.e., intake from a bottle on the homecage). However, to our surprise, we failed to confirm this hypothesis and instead observed that all neuropeptides under investigation were quite effective to increase intake of sucrose delivered directly into the mouth.

Unfortunately, this discrepancy seemed to present serious interpretative difficulty for either 1) the role of neuropeptides in such discrete behaviors, or 2) the existence of such “phases” of ingestion at all. On closer inspection, however, we noted an important difference between the studies: Prior to intraoral testing, Seeley [74] administered approximately 10 sessions of intraoral sucrose solution to habituate the rats to the experimental apparatus and procedure. In our recent study, no intraoral pretraining was administered. Thus, the rats’ first experience with sucrose was on the first day of testing with intracerebroventricular peptides. Importantly, we noted that comparison of the data from each study revealed that the previous [74] saline baseline was greater than twice that we observed on our first day of sucrose delivery. In this case, an unfortunate experimental error lead to a novel observation – the ability to interpret different effects of consummatory and appetitive responding may depend greatly on the previous experience of experimental subjects.

3.2 Test food composition

In any experimental analysis of food intake or feeding-related behaviors, a specific food or foods must be selected in order to conduct the test(s) of interest. In many cases, test foods are chosen on the basis of availability, cost or convenience. Often, sucrose is used as a test food because it is easy to prepare in solutions of various concentrations, it is easily measured when delivered via a graduated sipper tube, and it is readily accepted by rodents. While the experiment described above cautions researchers regarding the effects of animals prior experiences with test foods, we are gathering further data in our lab that suggest that the nutrient composition should also be taken into consideration when selecting foods for many of these behavioral assays.

Recently, our laboratory has been interested in the effects of hypothalamic feeding peptides on “reward”-related behaviors (meaning behaviors related to the palatability, hedonic, or other non-homeostatic motivational mechanisms). Specifically, previous evidence indicates that melanocortin peptides may act in mesolimbic regions to affect both food and non-food reward [15,16,48,55]. As discussed above, altering the motivational and rewarding aspects of food stimuli can strongly influence pre-ingestive behaviors. Thus, we set out to assess the effects of AgRP, an endogenous melanocortin receptor antagonist known to potently increase food intake, on operant progressive ratio responding and conditioned place preference [20,78]. Using sucrose as the reinforcer of choice yielded no effect of AgRP treatment on operant responding, a result which would lead us to conclude that melanocortins do not act to influence food intake by affecting motivational mechanisms. In the conditioned place preference test, animals given saline during training sessions displayed a significant preference for the environment paired with sucrose, while AgRP-treated animals showed no significant environment preference.

However, we also chose to conduct each of these experiments with an alternate reinforcer (peanut oil in the operant test and high-fat diet in the place preference study). In both cases, we observed substantially different results. Under these conditions, AgRP treatment increased operant responding for peanut oil, while a preference for the high-fat-paired environment was equivalent for both AgRP- and saline-treated animals. Clearly, these experiments indicate that the effects of melanocortins on appetitive behaviors can differ based on the nutritional composition of the food in question. Even more important, however, is the general observation regarding the importance of test-food selection when assessing the effects of neuropeptides on the different phases of ingestive behavior.

4. Conclusions and extensions

The history of research and behavioral analysis of ingestion spans many decades. Intricate theories and divisions of distinct behavioral ingestive phases have received much popularity and recent discoveries in neuroscience have bolstered support for these ideas. Nonetheless, our recent observations strongly indicate that behavioral work may be steeped in methodologies that foster one interpretation over another. For example, if we had recently included pre-exposure to the intra-oral administration paradigm, we might not have observed that NPY and other hypothalamic peptides increased IO administration, relative to vehicle treatment. Furthermore, had we used only sucrose as a reinforcer in recent experiments on operant responding and conditioned place preference, we would have come to vastly different conclusions regarding the effects of AgRP on motivation and reward than when a fat-based reinforcer was included. The important point is that while theories and conceptualizations of ingestive behaviors have been intricately developed and supported by myriad experimental data, they are theories and concepts nonetheless. They do not completely represent the true intricacies of the physiological and biological systems that give rise to the behaviors which are being recorded. These behaviors are influenced by the prior experiences of the animal and

environmental variables often unnoticed by experimenters. We suggest that the study of biological underpinnings of ingestion be coupled with consideration of the impact of these factors on the behavioral controls of food intake.

Acknowledgements

This work was supported by National Institutes of Health grant DK066223 to S.C. Benoit.

References

1. Ackroff K, Sclafani A. Energy density and macronutrient composition determine flavor preference conditioned by intragastric infusions of mixed diets. *Physiol Behav* 2006;89(2):250–60. [PubMed: 16854441]
2. Altizer AM, Davidson TL. The effects of NPY and 5-TG on responding to cues for fats and carbohydrates. *Physiol Behav* 1999;65:685–90. [PubMed: 10073468]
3. Balleine B. Instrumental performance following a shift in primary motivation depends on incentive learning. *J Exp Psychol Anim Behav Process* 1992;18(3):236–50. [PubMed: 1619392]
4. Balleine B, Dickinson A. Role of cholecystokinin in the motivational control of instrumental action in rats. *Behav Neurosci* 1994;108(3):590–605. [PubMed: 7917052]
5. Benoit SC, Air EL, Coolen LM, et al. The catabolic action of insulin in the brain is mediated by melanocortins. *J Neurosci* 2002;22(20):9048–52. [PubMed: 12388611]
6. Benoit SC, Clegg DJ, Woods SC, Seeley RJ. The role of previous exposure in the appetitive and consummatory effects of orexigenic neuropeptides. *Peptides* 2005;26(5):751–7. [PubMed: 15808905]
7. Benoit SC, Morell JR, Davidson TL. Lesions of the Amygdala Central Nucleus Interfere with Blockade of Satiation for Peanut Oil by Na-2-Mercaptoacetate. *Psychobiology* 2000;28:387–93.
8. Berridge K. Modulation of taste affect by hunger, caloric satiety, and sensory-specific satiety in the rat. *Appetite* 1991 Apr;16:103–20. [PubMed: 2064389]
9. Berridge K. Food reward: brain substrates of wanting and liking. *Neurosci Biobehav Rev* 1996;20:1–25. [PubMed: 8622814]
10. Berridge K, Grill H. Alternating ingestive and aversive consummatory responses suggest a two-dimensional analysis of palatability in rats. *Behav Neurosci* 1983 Aug;97:563–73. [PubMed: 6615632]
11. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev* 1998;28(3):309–69. [PubMed: 9858756]
12. Booth DA. Conditioned satiety in the rat. *J Comp Physiol Psychol* 1972;81(3):457–71. [PubMed: 4649186]
13. Breslin PAS, Davidson TL, Grill HJ. Conditioned reversal of reactions to normally-avoided tastes. *Physiology & Behavior* 1990;47:535–8. [PubMed: 2359765]
14. Brown C, Fletcher P, Coscina D. Neuropeptide Y-induced operant responding for sucrose is not mediated by dopamine. *Peptides* 1998;19:1667–73. [PubMed: 9880070]
15. Cabeza de Vaca S, Hao J, Afroz T, Krahne LL, Carr KD. Feeding, body weight, and sensitivity to non-ingestive reward stimuli during and after 12-day continuous central infusions of melanocortin receptor ligands. *Peptides* 2005;26(11):2314–21. [PubMed: 15894406]
16. Cabeza de Vaca S, Kim GY, Carr KD. The melanocortin receptor agonist MTII augments the rewarding effect of amphetamine in ad-libitum-fed and food-restricted rats. *Psychopharmacology (Berl)* 2002;161(1):77–85. [PubMed: 11967634]
17. Capaldi, ED. Hunger and the learning of flavor preferences. Bolles, RC., et al., editors. *The hedonics of taste*: Lawrence Erlbaum Associates, Inc; Hillsdale, NJ, USA: 1991. p. 127–42.
18. Capaldi ED, Hunter MJ, Lyn SA. Conditioning with taste as the CS in conditioned flavor preference learning. *Animal Learning & Behavior* 1997;25(4):427–36.
19. Capaldi ED, Myers DE, Campbell DH, Sheffer JD. Conditioned flavor preferences based on hunger level during original flavor exposure. *Animal Learning & Behavior* 1983;11(1):107–15.

20. Choi, DL.; Davis, JF.; Clegg, DJ.; Benoit, SC. Mice exhibit enhanced macronutrient-specific conditioned place preference under hypocaloric conditions. Society for the Study of Ingestive Behavior Annual Meeting; 2007; Steamboat Springs, CO. 2007.
21. Craig W. Appetites and aversions as constituents of instincts. *Biological Bulletin of Woods Hole* 1918;34:91–107.
22. Davidson T. The nature and function of interoceptive signals to feed: toward integration of physiological and learning perspectives. *Psychol Rev* 1993 Oct;100:640–57. [PubMed: 8255952]
23. Davidson T, Flynn F, Grill H. Comparison of the interoceptive sensory consequences of CCK, LiCl, and satiety in rats. *Behav Neurosci* 1988 Feb;102:134–40. [PubMed: 2833289]
24. Davidson T, Flynn F, Jarrard L. Potency of food deprivation intensity cues as discriminative stimuli. *J Exp Psychol Anim Behav Process* 1992 Apr;18:174–81. [PubMed: 1583446]
25. Davidson TL, Altizer AM, Benoit SC, Walls EK, Powley TL. Encoding and selective activation of “metabolic memories” in the rat. *Behav Neurosci* 1997;111:1014–30. [PubMed: 9383521]
26. Davidson TL, Benoit SC. The learned function of food-deprivation cues: A role for conditioned modulation. *Animal Learning & Behavior* 1996;24(1):46–56.
27. Davidson, TL.; Benoit, SC. Learning and eating. In: O'Donohue, WT., et al., editors. *Learning and behavior therapy*. Allyn & Bacon, Inc; Boston, MA, USA: 1998. p. 498-517.
28. Davidson TL, Carretta JC. Cholecystokinin, but not bombesin, has interoceptive sensory consequences like 1-h food deprivation. *Physiol Behav* 1993;53(4):737–45. [PubMed: 8511180]
29. Davidson TL, Kanoski SE, Tracy AL, Walls EK, Clegg D, Benoit SC. The interoceptive cue properties of ghrelin generalize to cues produced by food deprivation. *Peptides* 2005;26(9):1602–10. [PubMed: 16112399]
30. Delamater A, Sclafani A, Bodnar R. Pharmacology of sucrose-reinforced place-preference conditioning: effects of naltrexone. *Pharmacol Biochem Behav* 2000 Apr;65:697–704. [PubMed: 10764925]
31. Dickinson A, Balleine B. Motivational control of goal-directed action. *Animal Learning & Behavior* 1994;22(1):1–18.
32. Dickinson A, Balleine B. Motivational control of instrumental action. *Current Directions in Psychological Science* 1995;4(5):162–7.
33. Drucker D, Ackroff K, Sclafani A. Nutrient-conditioned flavor preference and acceptance in rats: effects of deprivation state and nonreinforcement. *Physiol Behav* 1994 Oct;56:701–7. [PubMed: 7800736]
34. Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, Bloom SR. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J Endocrinol* 1999;160(3):R7–12. [PubMed: 10077743]
35. Elizalde G, Sclafani A. Fat appetite in rats: flavor preferences conditioned by nutritive and non-nutritive oil emulsions. *Appetite* 1990 Dec;15:189–97. [PubMed: 2281949]
36. Fedorchak PM, Bolles RC. Hunger enhances the expression of calorie- but not taste-mediated conditioned flavor preferences. *J Exp Psychol Anim Behav Process* 1987;13(1):73–9. [PubMed: 3819653]
37. Ferguson SA, Paule MG. Progressive ratio performance varies with body weight in rats. *Behavioural Processes* 1997;40(2):177–82.
38. Figlewicz DP, Bennett JL, Naleid AM, Davis C, Grimm JW. Intraventricular insulin and leptin decrease sucrose self-administration in rats. *Physiol Behav* 2006;89(4):611–6. [PubMed: 17045623]
39. Figlewicz DP, Evans SB, Murphy J, Hoen M, Baskin DG. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res* 2003;964(1):107–15. [PubMed: 12573518]
40. Figlewicz DP, Higgins MS, Ng-Evans SB, Havel PJ. Leptin reverses sucrose-conditioned place preference in food-restricted rats. *Physiol Behav* 2001;73(1–2):229–34. [PubMed: 11399316]
41. Friedman MI, Edens NK, Ramirez I, Granneman J. Food intake in diabetic rats: isolation of primary metabolic effects of fat feeding. *Am J Physiol* 1983;249:R44–R51. [PubMed: 4014496]
42. Fulton S, Pissios P, Manchon RP, et al. Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 2006;51(6):811–22. [PubMed: 16982425]

43. Fulton S, Woodside B, Shizgal P. Modulation of brain reward circuitry by leptin. *Science* 2000;287:125–8. [PubMed: 10615045]
44. Furudono Y, Ando C, Yamamoto C, Kobashi M, Yamamoto T. Involvement of specific orexigenic neuropeptides in sweetener-induced overconsumption in rats. *Behav Brain Res* 2006;175(2):241–8. [PubMed: 17010451]
45. Garcia J, Koelling RA. Relation of cue to consequence in avoidance learning. *Psychonomic Science* 1966;4:123–4.
46. Handelsmann GE, O'Donohue TL, Forrester D, Cook W. Alpha-melanocyte stimulating hormone facilitates learning of visual but not auditory discriminations. *Peptides* 1983;4(2):145–8. [PubMed: 6622283]
47. Hodos W. Progressive ratio as a measure of reward strength. *Science* 1961;134:943–4. [PubMed: 13714876]
48. Hsu R, Taylor JR, Newton SS, et al. Blockade of melanocortin transmission inhibits cocaine reward. *Eur J Neurosci* 2005;21(8):2233–42. [PubMed: 15869520]
49. Jewett D, Cleary J, Levine A, Schaal D, Thompson T. Effects of neuropeptide Y on food-reinforced behavior in satiated rats. *Pharmacol Biochem Behav* 1992 Jun;42:207–12. [PubMed: 1631179]
50. Jewett D, Cleary J, Levine A, Schaal D, Thompson T. Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior. *Psychopharmacology* 1995 Aug;120:267–71.
51. Jewett DC, Schaal DW, Cleary J, Thompson T, Levine AS. The discriminative stimulus effects of neuropeptide Y. *Brain Res* 1991;561(1):165–8. [PubMed: 1797343]
52. Kanoski SE, Walls EK, Davidson TL. Interceptive “satiety” signals produced by leptin and CCK. *Peptides* 2007;28(5):988–1002. [PubMed: 17408804]
53. Kissileff HR, Van Itallie TB. Physiology of the control of food intake. *Annu Rev Nutr* 1982;2:371–418. [PubMed: 6764735]
54. Lindblom J, Kask A, Hagg E, Harmark L, Bergstrom L, Wikberg J. Chronic infusion of a melanocortin receptor agonist modulates dopamine receptor binding in the rat brain. *Pharmacol Res* 2002;45(2):119–24. [PubMed: 11846623]
55. Lindblom J, Schioth HB, Watanobe H, Suda T, Wikberg JE, Bergstrom L. Downregulation of melanocortin receptors in brain areas involved in food intake and reward mechanisms in obese (OLETF) rats. *Brain Res* 2000;852:180–5. [PubMed: 10661510]
56. Lindblom J, Wikberg JE, Bergstrom L. Alcohol-preferring AA rats show a derangement in their central melanocortin signalling system. *Pharmacol Biochem Behav* 2002;72(1–2):491–6. [PubMed: 11900824]
57. Lucas F, Sclafani A. Flavor preferences conditioned by intragastric fat infusions in rats. *Physiol Behav* 1989;46:403–12. [PubMed: 2623061]
58. Lucas F, Sclafani A. Food deprivation increases the rat's preference for a fatty flavor over a sweet taste. *Chem Senses* 1996 Apr;21:169–79. [PubMed: 8670695]
59. Lucas F, Sclafani A. Flavor preferences conditioned by high-fat versus high-carbohydrate diets vary as a function of session length. *Physiol Behav* 1999 May;66:389–95. [PubMed: 10357427]
60. Ludwig D, Mountjoy K, Tatro J, et al. Melanin-concentrating hormone: a functional melanocortin antagonist in the hypothalamus. *Am J Physiol* 1998 Apr;274:E627–33. [PubMed: 9575823]
61. Niswender KD, Schwartz MW. Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol* 2003;24:1–10. [PubMed: 12609497]
62. Nyakas C, Veldhuis HD, De Wied D. Beneficial effect of chronic treatment with Org 2766 and alpha-MSH on impaired reversal learning of rats with bilateral lesions of the parafascicular area. *Brain Res Bull* 1985;15(3):257–65. [PubMed: 2996719]
63. Percina S, Berridge KC. Opioid site in nucleus accumbens shell mediated eating and hedonic ‘liking’ for food: map based on microinjection fos plumes. *Brain Res* 2000;863(1–2):71–86. [PubMed: 10773195]
64. Perez C, Fanizza L, Sclafani A. Flavor preferences conditioned by intragastric nutrient infusions in rats fed chow or a cafeteria diet. *Appetite* 1999 Feb;32:155–70. [PubMed: 9989925]

65. Richardson NR, Roberts DC. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods* 1996;66(1):1–11. [PubMed: 8794935]
66. Saito Y, Nothacker H, Civelli O. Melanin-concentrating Hormone Receptor: An Orphan Receptor Fits the Key. *Trends in Endocrinology and Metabolism* 2000;11(8):299–303. [PubMed: 10996523]
67. Schwartz MW, Seeley RJ, Weigle DS, Burn P, Campfield LA, Baskin DG. Leptin increases hypothalamic proopiomelanocortin (POMC) mRNA expression in the rostral arcuate nucleus. *Diabetes* 1997;46:2119–23. [PubMed: 9392508]
68. Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661–71. [PubMed: 10766253]
69. Sclafani A. Conditioned food preferences and appetite. *Appetite* 1991 Aug;17:71–2. [PubMed: 1952919]
70. Sclafani A. Learned controls of ingestive behavior. *Appetite* 1997;29:153–8. [PubMed: 9344424]
71. Sclafani A, Nissenbaum J. Robust conditioned flavor preference produced by intragastric starch infusions in rats. *Am J Physiol* 1988 Oct;255:R672–5. [PubMed: 3140680]
72. Seeley R, Yagaloff K, Fisher S, et al. Melanocortin receptors in leptin effects. *Nature* 1997;390(27):349. [PubMed: 9389472]
73. Seeley RJ, Benoit SC, Davidson TL. The discriminative cues produced by NPY administration do not generalize to the interoceptive cues produced by food deprivation. *Physiology & Behavior* 1995;58:1237–41. [PubMed: 8623026]
74. Seeley RJ, Payne CJ, Woods SC. Neuropeptide Y fails to increase intraoral intake in rats. *Am J Physiol* 1995;268:R423–R7. [PubMed: 7864237]
75. Thorpe AJ, Cleary JP, Levine AS, Kotz CM. Centrally administered orexin A increases motivation for sweet pellets in rats. *Psychopharmacology (Berl)* 2005;182(1):75–83. [PubMed: 16075284]
76. Tordoff MG, Tepper BJ, Friedman MI. Food Flavor preferences produced by drinking glucose and oil in normal and diabetic rats: Evidence for conditioning based on fuel oxidation. *Physiol Behav* 1987;41:481–7. [PubMed: 3432403]
77. Torregrossa AM, Davis JD, Smith GP. Orosensory stimulation is sufficient and postingestive negative feedback is not necessary for neuropeptide Y to increase sucrose intake. *Physiol Behav* 2006;87(4):773–80. [PubMed: 16540131]
78. Tracy, AL.; Davis, JF.; Heiman, JU.; Clegg, DJ.; Davidson, TL.; Benoit, SC. Appetitive and motivational effects of agouti-related peptide (AgRP) on responding for fat and carbohydrate. Society for the Study of Ingestive Behavior Annual Meeting; 2006; Naples, FL: Appetite. 2006. p. 388
79. Tritos NA, Maratos-Flier E. Two important systems in energy homeostasis: melanocortins and melanin-concentrating hormone. *Neuropeptides* 1999;33(5):339–49. [PubMed: 10657511]
80. Ward SJ, Dykstra LA. The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). *Behav Pharmacol* 2005;16(5–6):381–8. [PubMed: 16148442]
81. Willie JT, Chemelli RM, Sinton CM, Yanagisawa M. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci* 2001;24:429–58. [PubMed: 11283317]
82. Woods SC, Schwartz MW, Baskin DG, Seeley RJ. Food intake and the regulation of body weight. *Annu Rev Psychol* 2000;51:255–77. [PubMed: 10751972]
83. Yamanaka A, Kunii K, Nambu T, et al. Orexin-induced food intake involves neuropeptide Y pathway. *Brain Res* 2000;859(2):404–9. [PubMed: 10719096]
84. Yehuda S. Effects of alpha-MSH, TRH and AVP on learning and memory, pain threshold, and motor activity: preliminary results. *Int J Neurosci* 1987;32(3–4):703–9. [PubMed: 3110086]
85. Yehuda S, Carasso RL, Mostofsky DI. The facilitative effects of alpha-MSH and melanin on learning, thermoregulation, and pain in neonatal MSG-treated rats. *Peptides* 1991;12(3):465–9. [PubMed: 1681521]
86. Yiin YM, Ackroff K, Sclafani A. Food deprivation enhances the expression but not acquisition of flavor acceptance conditioning in rats. *Appetite* 2005;45(2):152–60. [PubMed: 15946767]
87. Young RC, Gibbs J, Antin J, Holt J, Smith GP. Absence of satiety during sham feeding in the rat. *J Comp Physiol Psychol* 1974;87(5):795–800. [PubMed: 4430747]

88. Zhou Y, Spangler R, Schlussman SD, et al. Effects of acute “binge” cocaine on preprodynorphin, preproenkephalin, proopiomelanocortin, and corticotropin-releasing hormone receptor mRNA levels in the striatum and hypothalamic-pituitary-adrenal axis of mu-opioid receptor knockout mice. *Synapse* 2002;45(4):220–9. [PubMed: 12125043]
89. Zimanyi IA, Fathi Z, Poindexter GS. Central control of feeding behavior by neuropeptide Y. *Curr Pharm Des* 1998;4(4):349–66. [PubMed: 10197048]