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Food for Thought: Revisiting the Complexity of Food Intake

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The ability of hormones such as insulin, leptin, and cholecystokinin to alter food intake is influenced by intricate interactions between homeostatic and non-homeostatic factors. Consequently, when administered exogenously, the likelihood of these hormones influencing food intake is probabilistic, leading to difficulties replicating previously reported outcomes both within and between labs.

The act of eating is the behavioral result of diverse neural interactions, ultimately activating motor neurons in the brainstem to control salivating, biting, chewing, and swallowing required for consuming the food. These behaviors are dependent on both homeostatic (based on energy needs or deficits of specific nutrients) and non-homeostatic factors (environmental constraints, hedonics, palatability, opportunity, cognition/learning/experience, and the social situation). Terms including appetite, hunger, wanting and liking, satiety, and satiation are commonly used to summarize the net effect of these neural processes, but they often belie the complexity of the interacting neural circuitry and activity. Scientists examining these processes are generally limited to assessing the amount of food actually consumed by experimental subjects in a specified amount of time, in a controlled environment and under experimental conditions. When these conditions include administration of compounds (hormones, neurotransmitters, nutrients, etc.) hypothesized to influence food intake, outcomes have often been contradictory and difficult to interpret (Woods and Langhans, 2012). This short perspective highlights some of the problems and misinterpretations that have arisen in assessing the neurobiology of food intake.

Development of the Homeostatic Model of Food Intake

Several findings in the 1960s and 1970s led our lab to conclude that insulin, acting within the brain, should cause animals to eat less food and lose body weight over time. These findings included the demonstration that both basal and glucose-stimulated insulin secretion, and consequently insulin levels in the blood, are directly correlated with body weight/fat; that contrary to then-prevailing understanding, both insulin and insulin receptors are found in the brain; and that increased plasma insulin results in insulin crossing the blood-brain barrier, elevating insulin levels in the brain and cerebrospinal fluid (CSF). To assess our hypothesis, we initially infused low amounts of insulin into the CSF of baboons (via the lateral ventricles) and observed a dose-dependent reduction of food intake and body weight (Woods et al., 1979), and over the subsequent years we extended the basic observation to rats, mice, and marmots; many other investigators replicated the hypophagic action of insulin in the brain of many species and extended the finding to humans. When insulin is administered directly into the brain and reduces food intake, it does so without causing malaise or fatigue. Further, reducing insulin receptor activity in the brain by genetic or pharmacological means causes animals to eat more and gain weight. Thus, insulin came to be considered by some as an adiposity signal to the brain: when its levels increase it signifies an increase of body fat, and when they decrease it indicates a loss of body fat and changes of food intake are recruited to return body fat to normal (Begg and Woods, 2013). When leptin was discovered, it was found to share many of the same properties as insulin: it is secreted in direct proportion to body fat, and it penetrates the blood-brain barrier and acts on leptin receptors in the hypothalamus and other brain areas important in the control of metabolism. Further, experimentally induced increases of leptin locally in the brain elicit decreased food intake and body weight, and decreases of leptin activity in the brain result in overeating and weight gain. Such observations were consistent with a homeostatic view of body weight. When an individual diets (or is food-restricted), body weight and consequently plasma insulin and leptin decrease, the combined leptin and insulin signals in the brain also decrease, and the individual eats more food and regains lost weight. Analogously, eating more food than is required to maintain body weight results in weight gain, elevated plasma and brain insulin and leptin levels, and a tendency to eat less and lose weight. Our lab espoused this homeostatic model in reviews that have become highly influential and often cited (Schwartz et al., 2000; Woods et al., 1998), but in retrospect the model has proven to be overly simplistic and misleading, since it implies that hormonal influences over food intake are essentially hard-wired reflexes.

Replication Problems in the Study of Food Intake

As compelling as the homeostatic model is, many data fail to support it. Within our own lab, while many grad students and fellows administer insulin into the brain of rats or mice and observe reductions of food intake, others— with animals often taken from the same shipment and housed in the same room and using seemingly identical techniques and administered insulin—observe no change

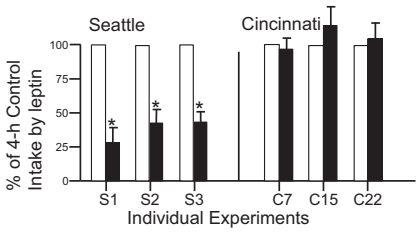

Figure 1. Intraventricular Leptin Reduces Food Intake in One Environment and Not in Another

Percent of 4 hr food intake following saline (open bars, 100%) or i3vt leptin (3.5 μg; solid bars) in three published experiments conducted in Seattle (S1–S3) and three unpublished experiments conducted in Cincinnati (C7, C15, C22) by the same experimenter and with all other parameters the same. Although only one dose and one time point are depicted, the outcomes were comparable across many doses and times; i.e., i3vt leptin did not reduce food intake in any of the first 28 experiments conducted in Cincinnati. * denotes $p < 0.05$ from saline. Details of the specific experiments depicted are in Woods and Langhans (2012).

of food intake relative to giving vehicle for the insulin. It is important to note that whether insulin "works" or doesn't work, there is no change of within-group variance. As a rule, all (or most) animals in a group respond or not; i.e., it is not as if a few respond such that including more subjects would result in a significant effect. Rather, animals administered insulin into the brain respond with comparable variance as controls.

This situation is obviously frustrating for trainees and has resulted in a lot of tears, with the occasional trainee leaving the lab with a sense of failure. But this phenomenon is not unique to our lab; numerous colleagues from around the world have informed us over the years that, try as they might, they have not been able to get centrally administered insulin to reduce food intake, or else they sometimes observe that effect and other times not, rendering it too unreliable for their purposes. Although experiments failing to replicate an effect rarely get published, there are occasional exceptions. For example, in one report a Herculean effort was made to document central insulin-induced hypophagia, but failed, stating, "Although we varied rat strain, stereotactic coordinates, formulations of insulin and vehicle, dose, volume, and time of injection, the anorectic effect of intracerebroventricular insulin could not be replicated" (p. R43) (Jessen et al., 2010).

In sum, dozens of reports have documented that insulin, when administered directly into the brain—and especially into the region of the mediobasal hypothalamus—reduces food intake, and, if continued chronically, body weight as well. This effect on food intake has been widely studied, in several species, under different experimental conditions, and using a wide range of doses. The hypophagic response to central insulin administration is more robust in males than in females, including in humans. It reduces food intake in animals fed relatively low-fat chow diets, but not in high-fat dietary obese animals or genetically obese animals, indicating that obesity results in central as well as peripheral insulin resistance. Further, considerable progress has been made in determining the underlying molecular mechanisms and neural circuitry of the response (Begg and Woods, 2013). In spite of this knowledge, when an experiment utilizing insulin administration into the brain and assessing food intake is planned, it cannot be predetermined whether or not the experiment will "work." Rather, some unknown factor(s) seems to dictate whether or not insulin, when administered into the brain, will reduce food intake or have no effect relative to a control administration. Over the years, our lab, and others with whom we have spoken, have systematically ruled out obvious potential confounds, including using an ineffective formulation or batch of insulin, the wrong dose, the wrong vehicle, adverse lab conditions (cleanliness, temperature, humidity, noise, etc.), animals that have been poorly handled or generally stressed, sickness or infection in the colony, disruption by animal care staff, or experimenter bias. In short, a pervasive and as yet unknown factor(s) simply negates the ability of centrally administered insulin to reduce food intake.

Failures to Replicate Are More Generalized

Although not as notorious as insulin, leptin can also be unreliable at reducing food intake when administered into the brain. Soon after leptin was discovered, it was found to reduce food intake. Randy Seeley, who was then in our group at the University of Washington, published several reports documenting that intraventricular leptin reduces food intake in rats (see Figure 1, left side). Our lab then moved from Seattle to the University of Cincinnati, including Seeley as well as the technician who had conducted the experiments with leptin in Seattle. After setting up the new lab, Seeley and the technician tried, but failed, to get rats to decrease their food intake when leptin was administered into the brain at the same dose and using the same parameters as in Seattle. In fact, no set of parameters of leptin administration was efficacious at reducing food intake for the first year and a half, as determined in more than 25 separate "failed" experiments (see Figure 1, right side, for representative data from 3 experiments). At some point, leptin mysteriously started working again and has reduced food intake relatively reliably (around 90% of the time) since. Other investigators have told us that they have around the same success rate with intraventricular leptin, and, importantly and like the case with insulin, the "failures" afflict the entire experimental group rather than a few animals within a larger group. As is also the case with insulin, failures to observe the hypophagia do not get published.

It is important to consider whether such failures to replicate an effect of a compound on food intake, one that was obtained in prior experiments, is publishable. When a finding is novel (e.g., when we found that insulin reduces food intake in baboons) and has not yet been widely replicated, there is a window of time when negative findings can be published. In modern times, this period of vulnerability to alternative explanations can last a year or two, but at some point dogma sets in and the scientific community accepts that a certain compound actually does have its stated effect. From then on, failures to observe the phenomenon become attributed to unknown factors and the experiment is rerun until the basic phenomenon occurs—and only the positive results are likely to appear in the literature. Negative (i.e., contrary) results are no longer newsworthy.

Insulin and leptin can be considered as adiposity signals that help maintain long-term body weight. One might therefore hypothesize that other classes of compounds influencing food intake are not subject to the same inconsistencies, but this is not the case. Similar problems have occurred with satiation signals,

molecules secreted by the gastrointestinal tract during meals that signal the nervous system to create a perception of fullness or satiation. The first satiation signal described, cholecystokinin (CCK), dose-dependently and acutely reduces meal size, with hundreds of reports documenting this effect. Nonetheless, prior to when dogma had been firmly established, there were several published failures to observe CCK-induced satiation including one report where CCK "worked" in some experiments but not in others. CCK-induced satiation is widely accepted as gospel today, although as discussed below there are caveats.

Since CCK, myriad gut peptides and other compounds have been suggested to be satiation factors. Rather than belabor the point, we cite two further examples. In 2002, Batterham, Bloom, and colleagues, in a well-controlled series of experiments using multiple approaches, published that peptide YY (PYY), a hormone secreted from the distal small intestine, reduces food intake in mice and in humans (Batterham et al., 2002). Because of the potential clinical importance of the observation, numerous groups began experimenting with PYY and food intake, not always with success, and in 2004 a publication from 42 authors representing 15 different labs claimed that PYY had no effect whatsoever on food intake (Tschöp et al., 2004). However, to date, in excess of 500 publications have investigated PYY and food intake, with most reporting that PYY reduces eating behavior.

Zhang et al. (2005) reported that obestatin, a peptide from the same gene that produces ghrelin, reduces food intake, and the basic findings were soon replicated by a group from Johnson & Johnson Pharmaceutical Research and Development (Lagaud et al., 2007). At the same time, another cadre of investigators from several labs could not replicate the basic finding, stating, "In the present study, we failed to observe any effect of obestatin on food intake, BW, body composition, energy expenditure, locomotor activity, respiratory quotient, or hypothalamic neuropeptides involved in energy-balance regulation. Therefore, the results presented here do not support a role of the obestatin/GPR-39 system in the regulation of energy balance" (p. 21) (Nogueiras et al., 2007). One important consequence of this was that Johnson & Johnson retracted their published article, stating, "The authors have found inaccuracies with the experimental data, specifically that recent feeding data obtained in the laboratory (in rats and mice) could not be reproduced using the same experimental protocol or even a more sophisticated system (BioDaq system). The long-term effects of obestatin on change in body weight (mice) could not be reproduced as well. The authors would like to apologize for this error" (p. 619) (Lagaud, 2009). Since then, there have been both reported failures and successes in replicating the original observation of obestatin-induced hypophagia.

Summaries of failures to replicate the effects of other compounds on food intake, including some peptides that are orexigenic, can be found in Woods and Langhans (2012). The important point is that administering a compound and assessing food intake is an iffy undertaking. Many factors can interfere, including some that are unknown. Thus, a failure to replicate your own previous results, or the findings of others, should not be taken as a sign that something was done wrong. In particular, failing to replicate one's own findings and consequently retracting a publication in this area of research as was done for obestatin, sets a high bar, and one that few could meet. Science should be based on solid data rather than on influential investigators swaying opinion.

Factors That May Account for Replication Issues

Anytime the nervous system is involved in the response to an input, the likelihood that the response will be manifest is not certain. For many responses, the range of probabilities of its occurrence can be quite high. At one end of a continuum is the passage of information across the neuromuscular junction. A motor nerve is activated and releases its neurotransmitter onto receptors in the junction, and the muscle cell contracts. This is as near to a one-to-one cause and effect as occurs. Moving back into the central nervous system, however, the motor nerve itself is innervated by multiple inputs from different circuits and neuronal types. Whether or not an action potential is elicited along the motor neuron axon to its junction with a skeletal muscle is consequently probabilistic, depending upon a complex calculus of the excitatory and inhibitory inputs it is receiving. When a circuit influencing a behavior includes only one or a very few synapses, such as the pain reflex, the probability is high that a painful stimulus will elicit the appropriate response. As the number of intervening synapses between an input such as insulin and a specific response such as eating less increases, the probability that the input will elicit a specific response is reduced. This may well be the case for hormones influencing food intake. Hormones such as CCK—influencing circuits in the hindbrain that are directly adjacent to the motor units controlling eating—have a relatively high, albeit not one hundred percent, likelihood of reducing meal size. In contrast, hormones such as insulin—which stimulates neurons in the mediobasal hypothalamus—elicit neural signals that must be filtered through many circuits before influencing food intake. Consistent with this notion, the proportion of time that CCK is found to reduce food intake is much higher than that for insulin. Leptin is intermediate between CCK and insulin, perhaps due to the numerous other circuits activated by central insulin involving glucose homeostasis, cognition, and others. If all of the data ever obtained in such experiments, both positive and negative, were published and available for meta-analysis, there would likely be a gradient of probabilities that a compound will influence food intake that parallels the number of intervening circuits.

Very few instances of eating are simple homeostatic reflexes initiated by a deficit of stored or available energy. Such reflexes do exist, for example when energy to the brain is acutely reduced (as occurs with sudden hypoglycemia induced by systemic insulin administration) or when brain cells are prevented from deriving energy from available glucose (as occurs when 2-deoxyglucose is acutely administered). These are states of emergency that can be evoked in laboratory experiments but that do not occur in normal eating situations. Chronic food deprivation results in loss of stored energy, but immediately available circulating energy nonetheless remains above the levels achieved by insulin or 2-deoxyglucose (Woods and Begg, in press).

Another potentially mitigating factor relates to learned responses. Ingestive

behaviors can be markedly influenced by factors that become associated with the ingested food; one classic example of this occurs with the phenomenon of conditioned taste aversions, whereby negative consequences such as visceral illness that are associated with ingesting a specific food or flavor results in future avoidance of that food or flavor. The ability of a hormone to reduce food intake can also be changed by experience. When CCK is repeatedly administered in an environment with stable and predictable cues present, its ability to reduce food intake in that environment is lessened over trials; yet, CCK continues to reduce food intake normally in other environments in the same animals (Goodison and Siegel, 1995). Thus, the presence or absence of some subtle environmental stimulus might prevent an animal from responding normally to CCK, or to any other compound that influences food intake, due to past experience. These examples demonstrate the complex interactions that occur between endogenous or exogenous cues and the effects of experimental interventions on a complex behavior. The overall point is that many behaviors are influenced by such a broad array of inputs that outcomes should be anticipated to vary among trials or experiments. In an important and revealing report, large cohorts of six different genetic strains of mice from the Jackson Laboratories were shipped to three different labs around North America and then analyzed in each lab on a battery of ostensibly identical behavioral tests. The correspondence of performance on the tests among the three sites was very low within and among strains, highlighting the inherent variability of behavioral outcomes even when every effort is made to ensure that experimental conditions are comparable (Crabbe et al., 1999).

Food intake is a motivated behavior, regulated by intricate interactions among homeostatic and non-homeostatic as well as exogenous and endogenous factors. It is highly influenced by experience and learning (Ramsay and Woods, 2014). Because of all of these factors, administered compounds purported to influence food intake do so in a probabilistic manner, which can lead to difficulties replicating previously reported outcomes.

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