Incubation of Sucrose Craving in Animal Models

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The "obesity epidemic" now has a global reach. Over 1 billion of the world's adult population is overweight with the number of obese above 300 million. Childhood overweight and obesity prevalence doubled from 1980 to 2006. Blame for the dramatic increase in overweight and obese individuals has fallen primarily on increased consumption of highly palatable, calorie-dense foods along with decreased physical activity. Almost by definition of overweight or obese, the increased caloric consumption is eating outside of caloric need.

Factors that drive this excess consumption include the "obesigenic environment," where not only are rich foods readily available but reminders of their existence are in place in media and in social environments, including schools and the workplace. Most people are well aware of how advertising and social situations can lead to eat when they are not hungry. These environmental cues are frustrating for healthy individuals watching their weight, but for obese individuals and/or individuals that suffer from compulsive overeating regardless of body weight, they can serve as an overwhelming push to eat—further contributing to their disease.

The propensity to seek and consume foods rich in energy is considered to be an adaptation to an environment where food is not consistently plentiful. To survive in such an environment, for example, across seasonal variation in vegetation, an individual would require the ability to identify food resources that have high nutritional value, remember the type and locations of the food, and in some instances ingest quantities of the food far in excess of current caloric need. Most studied in mammals, although rudimentary forms have been identified in other non-chordate phyla, including Drosophila and planaria, these adaptive behaviors map on to memory and motivational brain systems often referred to as the brain reward system. Elements of this system that include limbic brain regions tied to motivated behavioral output have drawn the interest of drug addiction researchers in the past several decades. Within this framework, much progress has been made in elucidating the neurobiology of drug addiction.

Drug self-administration by nonhuman animals, as a model of clinical addiction, has often been presented as a special case of motivated behavior. In fact, it is not uncommon to find food self-administration studies conducted in parallel with drugs to serve as a "control" to isolate drug-specific effects. In some cases there is the implication that the rewarding effects of food and drug are therefore served by different neurobehavioral systems. Certainly each has its particular substrates, for example, putative glucose-sensing neurons in the hypothalamus versus cocaine acting directly on dopamine transporter protein in the nucleus accumbens, but what is more likely is that the general neurobehavior is conserved. Interestingly, much of the original concept of the reward system came from non-drug research, in particular electrical brain self-stimulation and food self-administration. As drug addiction is characterized by extremes, it would be expected that an addiction to food would also lead to perturbations in the reward system.

Evidence for this has already been identified in rat studies. For example, a high-fat diet enhances the motivation to consume sucrose, and a dam fed a high-fat diet produces offspring that have hypothalamic neurons (part of the reward system) that are increased in sensitivity to glucose and production of orexigenic peptides, neurotransmitters that promote feeding. Furthermore, treatment of a food addiction would be a more daunting challenge compared to drug addiction in some respects as the reward system in question likely evolved selective for food. There would be less flexibility in this system in regard to food. Thereby food as a control
condition for drug addiction may have many limitations. Better yet, food may best serve as a target of addiction research in its own right.

It is clear that there are several features of uncontrolled eating that resemble criteria for addiction to drugs as defined by the American Psychiatric Association. For example, addiction to drugs is characterized by intense craving and ensuing compulsive drug-seeking. Individuals who have trouble avoiding excessive food intake also experience intense craving and will focus their behaviors on acquiring food. Much remains to be done to validate food addiction within the scientific and clinical communities, although progress is being made on both fronts.

To this end, from a basic science perspective, we and a handful of other laboratories have been examining sucrose as a substance with addictive qualities. In our laboratory we have focused on sucrose craving as the addiction behavior of interest. As indicated earlier, intense craving is part of the definition of addiction.

Craving is, however, a rather complicated phenomenon to define and therefore measure in humans. In addition, at least for alcohol, subjective indications of craving are often poor predictors of subsequent relapse. Tiffany has suggested a cognitive model to account for this discrepancy, where an unconscious reaction to drug-predictive stimuli engages drug seeking within this individual. This reaction then may or may not activate cognitive awareness and, if so, this would be experienced as craving. We and others argue that the unconscious reaction, an unconscious craving, is an important target for basic research with the goal of translational therapies for addiction. A recent brain imaging study lends support for unconscious reaction to either drug or nondrug appetitive stimuli. Not surprisingly, circuits within the reward system are activated upon exposure of an individual to these cues outside of conscious awareness.

It is reasonable to assume that this type of reaction could contribute to increased approach and consumption of food following exposure to food-predictive cues. This unconscious craving behavior would therefore be a key element of relapse behavior. As rats are without the self-awareness of humans, yet share the reward system, they provide a strong model system for study of craving, including food craving. Our particular approach has been to characterize sucrose-craving behavior in rats utilizing a rat model of relapse initially designed to examine cocaine relapse. Rats learn to self-administer either drug or food in daily self-administration sessions wherein responding on a lever delivers both the reward and a tone + light stimulus. Craving behavior is then examined in a subsequent test session wherein the reward is no longer available. In this session, rats will reliably respond for presentations of the tone + light stimulus. This responding serves as our operational definition of craving.

**INCUBATION OF CRAVING**

One element of craving that we have identified in the animal model to be of great potential relevance to human food addiction behaviors is how the length of time following reward self-administration influences reward craving. Specifically, over several weeks of abstinence from a reward, rats respond progressively more for the reward-paired cue upon returning to the reward self-administration environment. We first described this effect, an "incubation of craving" in rats that had self-administered cocaine. This first discovery was that rats in prolonged abstinence from cocaine self-administration will work harder pressing a lever for the presentation of a tone + light cue (no drug) that was previously paired with their cocaine compared to rats in very early abstinence (Fig. 32.1).

![Graph](image-url)

**FIGURE 32.1.** Incubation of cocaine craving in rats. Rats first had 10 days of 6 h/day access to cocaine self-administration (0.5 mg/kg per infusion) in operant chambers. After 1 to 60 days of forced abstinence in the home cage, rats were allowed to respond for a cocaine-paired stimulus in the operant chambers for 1 h. Asterisk indicates significant difference from day 1, p < 0.05, n = 9–11 per group.

FIGURE 32.2. Incubation of sucrose craving in rats. Rats first had 10 days of 6 h/day access to 10% sucrose self-administration in operant chambers. After 1, 7, or 30 days of forced abstinence in the home cages, rats were allowed to respond for a sucrose-paired stimulus in the operant chambers for 1 h. Asterisk indicates significant difference from day 1; p < 0.05, n = 8–9 per group.

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INCUBATION OF SUCROSE CRAVING

We subsequently observed incubation of craving for sucrose (Fig. 32.2). In these studies, rats had previously self-administered sucrose by drinking a drop of sucrose solution. As with the cocaine study, each delivery of sucrose depended on pressing a lever and each delivery was accompanied by a tone + light cue.

Incubation of sucrose craving is an extremely robust phenomenon. For example, we see this time-dependent increase in responding for a sucrose-paired cue even when the rats are tested multiple times (Fig. 32.3). Modern learning theory would predict that repeated exposure to the testing condition would lead to a decrease in responding over time, otherwise known as extinction.

In addition, prolonged access to sucrose ("satiation") up to the beginning of a test session was without effect at reducing incubation of sucrose craving (Fig. 32.4). This finding supports a hypothesis that craving in response to sucrose-paired cues becomes dissociated from sucrose itself. If this is true, it helps to explain how cues activate food craving and associated seeking (and intake) even when the individual feels "full."

We have also looked for evidence of incubation of craving with a nonnutrative sweetener as recent findings from both rat and human studies indicate that these sweeteners in "diet" foods may actually increase caloric intake from other sources.

We found an incubation of craving for sucralose (Splenda) in animals that had self-administered sucralose solution in the same procedure we have used to examine sucrose craving (Fig. 32.5).

As noted earlier, the brain substrates of drug and food craving have substantial overlap as they both characterize the brain reward system. The nexus of these cravings may be circuits in the brain that rely on the neurotransmitter dopamine. These circuits include connections both in the higher order thinking parts of the brain, the cortex, and in subcortical structures that mediate our behavioral output, including how we respond to signals in the environment indicating availability of drugs or food. Using our animal model of craving, we have performed several studies to identify the neural substrates of the incubation of craving for both cocaine and sucrose. For example, we found that
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FIGURE 32.4. Effect of 17 h (black bars) or 89 h (striped bar) free consumption of sucrose immediately prior to a sucrose craving test session after either 1, 7, or 30 days of forced abstinence from sucrose self-administration. Other than the "satiety" manipulation, training and testing parameters were as described in Figure 32.2. * indicates significant difference from day 1, basic effect, p < 0.05, n = 8–9 per group. Source: Reprinted from Physiology & Behavior, Vol. 84, JW. Grimm, et al. Incubation of sucrose craving: effects of reduced training and sucrose pre-loading, 7, Copyright (2005), with permission from Elsevier.

acute cocaine was less effective at potentiating craving in rats 1 month versus 1 day into forced abstinence.\textsuperscript{32} We interpret this finding as a tolerance of the response of the mesolimbic dopamine system to cocaine, perhaps due to already elevated levels of dopamine related to the incubation of craving. In other ongoing studies, we have found evidence indicating that the basolateral amygdala mediates cue-induced craving for both drug and food, and that the nucleus accumbens contributes to the time-dependent increase in craving for both drug and food.\textsuperscript{33} These brain regions are components of the subcortical circuits, noted earlier, that mediate how we respond to signals in the environment indicating availability of drugs or food. In addition, both of these structures are activated by the neurotransmitter dopamine. We have thus far found that while systemic injection of a dopamine D1 receptor antagonist preferentially decreases sucrose craving after 1 versus 30 days of forced abstinence, injection of the antagonist into subregions of the nucleus accumbens reduces sucrose craving to a similar extent at both forced abstinence time points.\textsuperscript{34} It is possible that incubation of craving is mediated by dopamine receptors in sites outside of the nucleus accumbens, and it also has been shown that neurotransmitter systems in other brain regions contribute to the effect. For example, our colleagues at the National Institutes of Health have found a critical role for metabotropic glutamate receptors in the central nucleus of the amygdala in the incubation of sucrose craving.\textsuperscript{35}

In other studies examining the incubation of sucrose craving at a more general behavioral and pharmacological level, we have found that incubation of sucrose craving can be attenuated by an opiate antagonist,\textsuperscript{36} and also by 1 month of environmental enrichment.\textsuperscript{37} In a recent study we have found that a conditioned taste aversion is effective at reducing sucrose craving only after several weeks of forced abstinence—indicating that some aspect(s) of the reward system changes along with, or due to, the incubation of craving.\textsuperscript{38}

**INCUBATION OF CRAVING AND FOOD ADDICTION**

Incubation of sucrose craving has not yet been documented in humans. It is fact, though, that many
obese dieters actually gain weight, and it is possible that incubation of craving accounts for some portion of diet recidivism. Incubation of craving for cocaine, heroin, and cigarette has been observed. Given the overlap between behavioral and neural substrates of drug and food craving, it is likely incubation of sucrose (and other food) craving occurs and has an important role in unhealthy eating behaviors. Related to this point, we have observed in several of our studies that actual sucrose consumption increases after several weeks of abstinence. If there is such a coemergence of incubation of sucrose craving and consumption in humans, it is not surprising that abstaining from sugar may become an unachievable goal in some food addicts. We hope that gaining a basic science understanding of behavioral and neurobiological aspects of the incubation of craving will be of use for developing effective therapies for food addiction and related diseases such as obesity.

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