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How Diet Can Alter Reward-Seeking Behavior

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by

Alisa Rose Kosheleff

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ABSTRACT OF THE DISSERTATION

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Doctor of Philosophy in Psychology

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The epidemic of chronic, preventable diseases such as obesity and diabetes remain a serious health concern, driven largely by 1) changes in the global food supply as the consumption of nutritive foods decreases and that of highly refined and processed foods increases, and 2) a subsequent inability to limit food consumption appropriately. While obesity remains at the forefront of preventable diseases, recent evidence suggests that it is strongly associated with insulin resistance and type 2 diabetes, so much so that the phrase “diabesity” has been coined to emphasize their comorbidity. Growing evidence suggests that the foods we eat can have serious consequences not just in the periphery (e.g., weight gain), but can alter our neurochemistry and behavior as well.

The experiments presented here probe this issue in two parts. First, we examine whether a junk food diet can alter the use of external and internal cues to guide reward seeking behavior. To this end, we used general and outcome-specific Pavlovian-to-instrumental transfer (PIT) tests to probe incentive motivation and decision making, respectively, and a test of outcome devaluation to examine the sensitivity of reward seeking to a decrease in outcome value, after either intermittent or ad libitum junk food exposure. We found that intermittent junk food exposure disrupts general and outcome-specific PIT, promoting reward seeking in response to cues only loosely paired with reward and inconsistent with outcomes predicted by the cue. Ad libitum junk food exposure suppresses reward seeking during a general PIT test, and disrupts outcome-specific PIT similarly to intermittent junk food exposure. Junk food exposure also disrupts the ability of internal, interoceptive cues about satiety state to adjust reward seeking in a test of outcome devaluation, irrespective of the pattern of junk food exposure.

We also examined whether an insulin-disrupting high fructose diet would alter incentive motivation in a general PIT test. We used fast-scan cyclic voltammetry to examine dopamine signaling during the PIT test, and in anesthetized animals to further assess dopamine reuptake kinetics. We found that insulin resistant rats were behaviorally and neurochemically sensitive to both reward-paired and "neutral" cues, demonstrating increased reward seeking and phasic dopamine release in response to both types of cues. We also found that dopamine reuptake was prolonged in insulin resistant rats, and that treatment with the insulin receptor sensitizing drug pioglitazone normalized reuptake and incentive motivation.

The dissertation of Alisa Rose Kosheleff is approved.

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Ostlund, S.B., **Kosheleff, A.R.**, & Maidment, N.T. (2014). Differential effects of systemic cholinergic receptor blockade on Pavlovian incentive motivation and goal-directed action selection. *Neuropsychopharmacology*, *39*, 1490–1497.

Ostlund, S.B., **Kosheleff, A.R.**, Maidment, N.T., & Murphy, N.P. (2013). Decreased consumption of sweet fluids in mu opioid receptor knockout mice: a microstructural analysis of licking behavior. *Psychopharmacology, 229*(1), 105-113.

Ostlund, S.B., **Kosheleff, A.R.**, & Maidment, N.T. (2012). Relative response cost determines the sensitivity of instrumental reward seeking to dopamine receptor blockade. *Neuropsychopharmacology, 37*, 2653-2660.

Kosheleff, A.R., Rodriguez, D., O'Dell, S.J., Marshall, J.F., & Izquierdo, A. (2012). Comparison of single-dose and extended methamphetamine administration on reversal learning in rats. *Psychopharmacology, 224*(3), 459-467.

Kosheleff, A.R., Grimes, M., O'Dell, S.J., Marshall, J.F., & Izquierdo, A. (2012). Work aversion and associated changes in dopamine and serotonin transporter after methamphetamine exposure in rats. *Psychopharmacology, 219*(2), 411-420.

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Kosheleff, A.R.,* Tsan, L., Gomez-Pinilla, F., Ostlund, S.B., Murphy, N.P., Maidment, N.T. Chronic fructose consumption enhances cue-triggered cocaine- and food-seeking. The College on Problems of Drug Dependence. 11-16 June, 2016. Palm Springs, CA. (Oral presentation.)

Kosheleff, A.R.,* Tsan, L., Zhuang, Y., Gomez-Pinilla, F., Ostlund, S.B., Murphy, N.P., Maidment, N.T. A high-fructose diet alters brain insulin signaling, incentive motivation and dopamine release. 46th Society for Neuroscience Annual Conference, San Diego, CA. 12-16 Nov 2016.

Kosheleff, A.R.,* Quizon, K., Zhou, J., Hsueh, J., Le, A., Ostlund, S.B., Maidment, N.T., Murphy, N.P. Junk food consumption induces abnormal food-seeking responses to environmental stimuli in rats. 45th Society for Neuroscience Annual Conference, Chicago, IL. 17-21 Oct 2015.

Kosheleff, A.R.,* Zhou, J., Hsueh, J., Le, A., Quizon, K., Ostlund, S.B., Maidment, N.T., Murphy, N.P. Chronic junk food consumption induces aberrant cue-induced food-seeking in rats. 46th Annual International Narcotics Research Conference, Pheonix, AZ. 15-19 June 2015.

Ostlund, S.B.,* LeBlanc, K.H., **Kosheleff, A.R.**, Wassum, K.M., & Maidment, N.T. (2013). Repeated cocaine exposure enhances incentive motivation through exaggerated phasic mesolimbic dopamine signaling. 43rd Society for Neuroscience Annual Conference, San Diego, CA. 9-13 Nov 2013.

Mendez, I.A., * **Kosheleff, A.R.**, Ostlund, S.B., Murphy, N.P., & Maidment, N. T. Role of mu opioid receptors and beta-endorphin in sucrose palatability. 42nd Society for Neuroscience Annual Conference, New Orleans, LA. 13-17 Oct 2012.

Kosheleff, A., Rodriguez, D. L., Grimes, M., Sterns, A., Carlos, K., & Izquierdo, A.* Comparison of escalating and single doses of methamphetamine on visual discrimination retention and reversal learning in rats. 41st Society for Neuroscience Annual Conference, Washington, D.C. 12-16 Nov 2011.

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Chapter 1

General Introduction

While researchers have long studied the neurochemistry guiding our food choices, only recently has the reverse been explored: how might our food choices alter our neurochemistry, and, consequently, behavior? Indeed, contemporary Westernized diets have shifted from home made, whole food meals to pre-packaged, refined, and fast foods. These poor quality diets are typically high fat, high sugar, and are extremely palatable [1,2]. Like all natural and drug rewards, these foods have direct action on the mesolimbic dopamine system, promoting dopamine release in the nucleus accumbens (NAc). Such dopamine signaling rapidly encodes the motivational significance of events, and, with experience over time, dopamine signaling gradually shifts from reward delivery to the earliest reward predictor [3,4]. These predictors, or cues, signal reward availability, and can invigorate reward-seeking behavior, such as driving food-seeking and "relapse," such as that seen in restricted (i.e., dieting) eaters. With repeated experience, neuroadaptations can alter how the mesolimbic dopamine system responds to rewards: it can become "sensitized" to environmental stimuli [5], or downregulated [6]. Whether palatable, westernized junk foods can facilitate food "addiction" remains unclear, and the neurochemical and behavioral consequences of these diets remain an active area of investigation.

With the proliferation of cheap, poor quality, and refined Westernized diets, metabolic disorders have increased dramatically across the globe, ultimately resulting in increased morbidity and shorter lifespans as sufferers develop heart disease, ischemic strokes, and diabetes [7,8]. Insulin dysfunction, such as that implicated in type 2 diabetes, has also recently been incriminated in brain disorders, such as Alzheimer's [9] and Parkinson's [10,11] diseases, and more recently, a role for insulin has been revealed in regulating mesolimbic dopamine [11–14]. An anorexigenic peptide, insulin has long been known to act in the hypothalamus as a satiety signal to *decrease*

appetite and feeding [15,16], and recent evidence has shown that insulin can also act in other areas of the brain to influence feeding behavior in more subtle and complex ways. Specifically, insulin's action on ventral tegmental area (VTA) dopamine neurons has been implicated in the regulation of non-homeostatic (i.e., not driven by caloric or metabolic necessity) and hedonic (i.e., pleasure-driven) feeding (reviewed in [17]). However, insulin's interactions with the dopamine system, a strong mediator of reward-seeking behaviors, and its behavioral consequences remain unclear. In brief, it is possible that diet-induced dysfunctional insulin signaling at VTA dopamine neurons potentiates dopamine signaling in the ventral striatum, which may exacerbate maladaptive reward-seeking behavior, such as that seen in drug or food addiction.

The Mesolimbic Dopamine System and Reward

Dopamine neurons project from the VTA to the NAc

Dopamine neurons in the VTA project to the NAc, where they mediate the reinforcing, incentive and motivational components of reward (reviewed in [18,19]). This mesolimbic dopamine system is thought to be the primary target of natural (e.g., sex, food) and pharmacological (e.g., cocaine, amphetamine) reinforcers. Upon food consumption or drug exposure, dopamine neurons in the VTA, projecting via the medial forebrain bundle to their terminals in the ventral striatum, increase dopamine signaling in the NAc. Behaviorally relevant stimuli (such as unpredicted rewards or reward-paired cues) can activate VTA dopamine neurons, thereby driving phasic dopamine release [20] over and above the sustained low "background" tonic dopamine signaling regulated by cortical prefrontal afferents (reviewed in [21]). Because the concentration of

dopamine released in the NAc is associated with the perceived value of the reward [22,23], novel or highly palatable foods are likely to elicit more dopamine than predictable or bland ones, while pharmacological drugs (e.g., cocaine, dopamine agonists) are likely to elicit artificially large concentrations of mesolimbic dopamine.

Phasic dopamine release in the striatum provides very fast (on the order of milliseconds) and dramatic increases in dopamine concentration in the synaptic cleft [24] which quickly saturates postsynaptic D1 receptors [25]. The high-affinity dopamine transporter (DAT) quickly removes dopamine from the extracellular space back into the presynaptic neuron, terminating dopamine signaling. Drugs of abuse disrupt the homeostasis of the dopamine system by causing an increase in extracellular dopamine concentrations, and in the case of psychostimulants, by interfering with dopamine reuptake by blocking the DAT. Interestingly, increasing burst firing of dopamine neurons does not appear to considerably alter total (i.e., synaptic and extra-synaptic) dopamine concentrations in the NAc (as measured by microdialysis), presumably due to DAT's high affinity for dopamine and quick reuptake action; if uptake is pharmacologically prohibited (i.e., via a DAT inhibitor), dopamine concentrations increase significantly [26].

Dopamine signaling mediates food reinforcement

Mesolimbic dopamine has been strongly implicated in the motivation to work for food rewards. Early experiments found that ablating or decreasing function of dopamine neurons abolishes and decreases food seeking and consumption, respectively [27–34]. Conversely, food rewards increase extracellular NAc dopamine concentrations proportionally to the perceived incentive value (which can include quality and quantity) of the reward [23,35]. When lever pressing for

food reward, extracellular NAc dopamine concentrations (as measured by microdialysis) are higher when rats are tested hungry (i.e., when motivational state is upregulated) versus sated [36]. During a sated test, NAc shell dopamine levels were positively correlated with effort, where higher dopamine levels were associated with more vigorous lever pressing [36], again suggesting that reward valuation (as measured by lever pressing behavior) is strongly associated with dopamine concentrations. Research using in vivo fast-scan cyclic voltammetry (FSCV), in which fluctuating extracellular NAc dopamine can be tracked in real time during tests of reward-seeking and incentive motivation, strengthened the finding that dopamine concentration is mediated by the perceived value of the reward, and subsequently guides reward-seeking behaviors [37–39]. Taken together, these data suggest that NAc dopamine is involved in encoding the motivational value of rewards, which can be altered by changes in motivational state (i.e., from sated to hungry).

Mesolimbic Insulin Signaling

CNS insulin impacts the dopamine system

Recent work has confirmed a link between postingestive insulin signaling and the dopamine system. Insulin is made exclusively by pancreatic beta cells and excreted into circulation in response to elevated blood glucose (e.g., after eating a meal). It is transported into the brain via a saturable, receptor-mediated, active transport system [40–42], and, as a result, CNS insulin concentrations are highly correlated with those in the peripheral, circulating bloodstream [43]. In the CNS, insulin has a variety of roles beyond facilitating glucose uptake, including mediating feeding behavior, neurodevelopment, neurogenesis, neurotransmitter release, receptor trafficking

and neurotransmitter reuptake (reviewed in [44]). Insulin receptors are found extensively throughout the brain [45], including, importantly, on dopamine neurons in the VTA [12], where their activation subsequently activates PI3K, which is required for insulin to influence feeding [46], and Akt, which regulates dopamine signaling and homeostasis [47,48]. Further, a genetic variant of Akt has been associated with schizophrenia and methamphetamine abuse, disorders in which dopamine dysfunction is strongly implicated [49,50], and genetic manipulation of Akt phosphorylation in mice can also dysregulate dopamine activity [51], further highlighting a role for insulin in the dopamine system.

Functional consequences of mesolimbic insulin signaling: implications for the DAT

As discussed above, insulin receptors are heavily expressed throughout the brain, including on dopamine neurons in the VTA. However, the full functional significance of insulin receptor expression on dopamine neurons remains unclear. Studies report seemingly contradictory evidence that insulin can both potentiate firing of dopaminergic neurons [52,53], and depress excitatory VTA afferents [13,54], though this may be due to insulin's differential effects on tonic versus burst firing, as well as regional differences (e.g., dorsal versus ventral striatum), and the involvement of mediating circuitry (i.e., cholinergic interneuron involvement [53]) [55].

A growing body of evidence has shown that activation of insulin receptors results in increased DAT expression and activity, thus increasing dopamine reuptake and clearance. Through activation of the PI3K and Akt signaling pathways, insulin exerts a positive influence on DAT function by facilitating its translocation to the synaptic membrane surface [14,48,54,56,57]. Tyrosine kinase inhibitors, which also block the receptors activated by insulin and insulin-like

growth factors, reduce dopamine clearance by redistributing DAT away from the cell membrane, thus decreasing DAT surface expression [58]. Inhibiting downstream components of the insulin signaling pathway, such as PI3K or Akt, also reduces DAT surface expression and dopamine clearance [48,59], as does feeding a high-fat diet (which impairs Akt) [57,60]. Chronic hyperinsulinemia, whether due to chronic i.c.v infusion [61] or in genetically modified rats predisposed to obesity and hyperinsulinemia (i.e., FA/FA Zucker rats) [61], increases DAT mRNA in the ventral midbrain. Further, insulin administered directly into the VTA can also reduce somatodendritic dopamine signaling, an effect that is abolished when insulin is co-administered with a selective DAT inhibitor or in DAT KO mice [54]. This decrease in somatodendritic dopamine in the VTA is likely due to an increase in DAT trafficking to the membrane and expression, which allows increased dopamine reuptake into the cell, thus ultimately lowering dopamine concentrations [54]. Taken together, these data show that normal insulin function is important for reliable DAT-regulated dopamine clearance.

Because insulin is released from the pancreas in response to feeding (i.e., caloric load) and is transported from the periphery to the brain, CNS insulin closely mirrors concentrations in the periphery [40–42]. Therefore, fasting is likely to produce both PNS and CNS hypoinsulinemia. In food-restriction models of hypoinsulinemia, DAT-mediated dopamine clearance and reuptake is severely reduced both in vitro [14] and in vivo [62], but is easily restored by adding insulin to the in vitro suspension [14] or returning rats to food access [62]. Streptozotocin administration is another common model of hypoinsulinemia and diabetes, as it destroys pancreatic beta cells, leading to a loss of insulin production. These models, too, show decreased dopamine clearance and reuptake in both in vivo chronoamperometry and in vitro striatal synaptosomal preparations

[63], which can be reversed by chronic amphetamine administration [63]. Insulin can also block an amphetamine-induced change in DAT expression [48]; because one of amphetamine's primary effects is to redistribute DAT away from the plasma membrane [64,65], the use of chronic amphetamine in this experiment provides evidence that insulin's actions on dopamine are likely to occur via the DAT (i.e., when DAT activity is blocked by amphetamine, insulin counters this effect, and increases DAT translocation to the membrane thus increasing dopamine reuptake). Williams et al. [66] confirmed these results, showing that streptozotocin (which eliminates insulin signaling) decreases striatal DAT cell-surface expression, significantly reducing amphetamine's ability to reverse-transport dopamine from the cytosol into the extracellular space as a result of insulin-induced DAT downregulation, an effect that can be reversed with local insulin infusions into the striatum [66,67]. These data show that artificial manipulations of CNS insulin signaling, whether pharmacological or as a result of fasting, can have serious consequences for the mesolimbic dopamine system. How these manipulations subsequently influence compulsive reward seeking warrant further study.

Dopamine and Behavior

Dopamine responds to reward-paired cues

Pavlovian conditioned stimuli influence behavior

A key component of reward learning is the acquisition of Pavlovian conditioned associations between the primary rewards themselves (e.g., food, drugs) and relevant stimuli in the environment (i.e., a context or discrete cue). With repeated exposure, stimuli associated with reward (that occur predictively or simultaneously) become conditioned stimuli (CSs), capable of

signaling reward availability. The invigorating and motivating effects of CSs are frequently used in conjunction with instrumental processes in experimental paradigms to model maladaptive reward seeking behaviors. Importantly, contextual cues (e.g., the operant box) can also become CSs, capable of predicting reward, and invigorating or reinforcing behavior. In the context of addiction and compulsive reward-seeking, these cues can also trigger reward cravings [68,69], driving efforts to procure reward and resulting in relapse [70,71]. This process, whereby reward-associated cues become motivational and capable of eliciting reward-seeking, is termed incentive motivation.

While the precise neurocircuitry governing Pavlovian and instrumental processes in the context of compulsive reward seeking remain unclear, the contemporary incentive sensitization hypothesis states that, with repeated pairings, Pavlovian CSs (e.g., contexts, people, actions associated with reward) come to activate the same neural circuitry and neurochemistry as the primary reinforcer (e.g., food, drugs) [72,73]. This shared ability to facilitate mesolimbic dopamine transmission is what enables reward-paired cues to become rewarding, and capable of driving pathological reward-seeking such as that seen in addiction and compulsive overeating [74–76]. Reward-associated cues are then able to increase activity of VTA dopamine projections to the NAc, eventually increasing motivation and driving reward-seeking [77–79]. A large body of evidence has implicated the mesolimbic dopamine system as responsible for the transition of environmental stimuli into reward-associated conditioned stimuli through Pavlovian conditioning (reviewed in [80]).

Pavlovian conditioned stimuli influence dopamine signaling

Seminal electrophysiology work by Schultz and colleagues in non-human primates demonstrated that midbrain dopamine neurons fire in response to primary rewards *and* conditioned reinforcers, and that dopamine activity is increased with unexpected reward delivery [3,81–83]. After repeated reward-cue pairings, dopamine signaling shifts temporally from the primary reward distally to the CS onset [84]. The magnitude of the CS-induced dopamine response may reflect the motivational properties of the stimulus, as larger dopamine neuron activation was negatively correlated with the animal's reaction time to procure the reward [85].

Instrumental responding for rewards can also elicit dopamine release in the NAc, for both drug rewards [86,87] and food [88,89]. Specifically, dopamine neurons in the VTA increase their firing rate as rats approach and press a lever previously associated with heroin delivery, only to decrease firing once heroin delivery began [90]. This "anticipatory" role for mesolimbic dopamine is likely to work for the earliest predictive cue, not just those immediately preceding reward delivery. While recording dopamine via FSCV, Wassum and colleagues [91] found, using an instrumental action sequence for food reward (lever 1 → lever 2 → food reward), that NAc dopamine is increased upon delivery of an unexpected reward, such as that during the initial acquisition of the task. As the action-outcome contingency is learned, this dopamine release shifts to the earliest actions (i.e., first the proximal, then distal lever press), and ceases to occur upon reward delivery. Finally, the amplitude of the dopamine signal predicts the latency to complete the action sequence, suggesting again (as above) that dopamine signaling likely also tracks the incentive value of the reward [91]. Taken together, these data suggest that mesolimbic dopamine tracks not just the incentive value of reward, but reward-paired stimuli that might facilitate optimized reward-seeking.

Dopamine influences behavior in response to Pavlovian conditioned stimuli

Indeed, dopamine signaling in response to reward-paired cues and in anticipation of active reward-seeking strongly suggests dopaminergic mediation of cue-invigorated reward-seeking. VTA inactivation reduces instrumental responding for cues associated with cocaine [92] and food reward [34,93], while leaving consumption unaffected [34], and dramatically reduces firing in the NAc in response to Pavlovian CSs (while not affecting neural activity associated with operant responding and checking the food cup) [94]. Many CS-induced behavioral effects can be reduced or abolished by dopamine blockade or lesions in the VTA: administration of systemic dopamine antagonists reduces CS-induced locomotor activity [95,96], and blocks conditioned place preference for food, amphetamine and cocaine reward [97–99]. Further, local NAc dopamine blockade prevents the ability of a CS to maintain responding [94,100–102], and reduces Pavlovian conditioned goal approach to a food-paired cue [103]. Predictably, increasing mesolimbic dopamine neurotransmission tends to enhance the effects of CSs on behavior: systemic administration of D2 agonists [104,105] or amphetamine [105] potentiates instrumental responding for a conditioned reinforcer, as do local infusions of amphetamine into the NAc [106].

Similar neuroadaptations underlie drug and food addiction

The reward hypofunction hypothesis proposes that a deficiency in dopamine signaling underlies an individual's susceptibility to drug and food addiction [6,107,108]. Imaging studies in humans show strong evidence of decreased DAT density and D2 receptor availability after chronic drug and alcohol use [109–111] (reviewed in [112]), and decreased D2 signaling in rodents [113] and monkeys [114] is associated with increased psychostimulant use. Similarly, overexpression of

D2 receptors reduced alcohol intake in rats [115]. Obese individuals (who some argue might be "food addicts" due to their compulsive eating) show parallel decreases in D2 receptor availability in VTA projection target areas such as the striatum, thought to reflect decreased availability, a decrease that negatively correlates with BMI [116,117]. Women who gained weight (versus those that didn't) also show decreased activity in VTA target areas in response to palatable foods [118]. Likewise, rodents fed a palatable diet [6,119] or are genetically predisposed to obesity [120] also show decreased D2 receptor levels, thought to reflect decreased receptor availability. Using viral-mediated RNA interference, Johnson and Kenny [6] decreased D2 receptor signaling in the striatum and found that, after D2 receptor knockdown, rats decreased responding for rewarding brain stimulation almost immediately after starting a diet of various calorically dense, palatable foods. Johnson and Kenny suggest this is indicative of 'reward hypofunctionality' in rats with decreased striatal D2 receptor activity and a junk food diet, whereas in control rats such a diet would produce similar results, but only after several weeks of junk food exposure and the onset of obesity. While D2 receptor downregulation is the most notable effect after overeating, other deficits in dopamine transmission are also reported after junk food access and obesity. Rats genetically predisposed to obesity have lower basal and evoked dopamine concentrations in the NAc than those resistant to weight gain [121], and rats overfed on a high-fat [122] or cafeteria [123] diet show lower basal and evoked dopamine in the NAc compared to lab chow-only rats. Taken together, these data suggest drug and food addiction share many characteristics, though the full implications remain understudied. Given their neurochemical and behavioral similarities, it is possible that food and drug addiction may share similar risks and complications from a poor diet resulting in insulin dysregulation, and the ensuing disruptions to the mesolimbic dopamine signaling, resulting in a hypersensitivity to reward-paired cues.

Reward Wanting: Drug- and Food-Driven Changes in Incentive Sensitization

Incentive sensitization promotes reward craving

Perhaps one of the most difficult aspects of drug abuse or disordered, compulsive eating is the persistent cravings, which, in the case of drug abuse, can persist even after years of abstinence. Robinson and Berridge have proposed a theory of incentive sensitization [124,125], whereby drug 'wanting' and craving may be due to neuroadaptations hastened by repeated drug exposure. Their theory proposes that the most important change occurring as a result of repeated drug use is a pathological sensitization to drugs and their associated stimuli, whereby drug-paired CSs become hypersalient, excessively attractive, pathologically wanted, and capable of eliciting approach behavior and reward-seeking [124,125]. This sensitization is thought to bias attentional processes towards reward-associated stimuli, and drive the compulsive drug 'wanting' and craving associated with compulsive reward-seeking and addiction.

Incentive sensitization engages a process of incentive salience, whereby neural representations of CSs are transformed from mere information into "an attractive and 'wanted' incentive that can 'grab attention'" [126, page 313]. Importantly, this process of incentive salience also engages motivational mechanisms, transforming CSs into objects of attraction that animals are willing to work for. Incentive motivation occurs when cues, having repeatedly been paired with reward, become imbued with incentive salience and capable of driving reward-seeking. This is thought to occur predominantly via the neural circuitry that mediates Pavlovian conditioned responding, which can manifest as either unconscious wanting or explicit, conscious craving, capable of lasting for years, even after long periods of abstinence. The incentive sensitization theory makes

it clear that addiction and compulsive reward seeking is more than just aberrant learning promoting strong stimulus-response (S-R) habits, which, by definition, do not engage motivational mechanisms - a necessary component of compulsive behavior [127]. Ultimately, CSs (i.e., reward-paired cues) imbued with incentive salience share three fundamental qualities (reviewed in [128]): 1) they become "wanted" and can elicit approach behavior (measured by sign-tracking or Pavlovian conditioned approach), 2) they can drive reward seeking for their unconditioned reinforcers (i.e., food, drug) as a result of cue-induced 'wanting', 3) they can become conditioned reinforcers, reinforcing instrumental behaviors.

While initially described with drug abuse in mind, the incentive sensitization model of reward addiction may be well suited to food addiction models as well. Growing evidence from animal models suggests that, like drugs of abuse, poor diets (i.e., high-fat, high-sugar and refined foods) may have long-term consequences for behavior and cognition, making it difficult to determine whether a hypersensitivity to food-paired cues precedes maladaptive eating in humans, or emerges as a result of it. Indeed, poor quality diets have been shown to produce deficits in hippocampal-dependent learning and memory [129–131], promote a shift from goal-directed to habitual responding [132–134], and alter reward liking and craving [6,135–141]. While specific mechanisms are unclear, growing evidence supports a role for mesolimbic dopamine dysfunction [6,142,143], though the hippocampus may also be preferentially vulnerable to the deleterious effects of junk foods [144–146]. Importantly, food-paired cues also become highly salient, capable of producing strong physiological reactions (e.g., increased salivation) and cravings (i.e., cue-reactivity) [147]. Reward-paired cues are well known to potentiate non-homeostatic (i.e., hunger state-independent) feeding, in both rats [148–150] and humans [151,152], as well as

maladaptive overeating [153] in both obese and normal-weight restrained eaters (i.e., such as when dieting) [154,155, reviewed in ,156].

Pavlovian-to-instrumental transfer is mediated by mesolimbic dopamine signaling

Attempting to assess changes in the incentive salience of reward-paired cues and model cue-induced relapse in the laboratory is best done with the Pavlovian-to-instrumental transfer (PIT) paradigm, which controls for alternative explanations for cue-induced behaviors, such as habitual stimulus-response reactions or conditioned 'liking' [127,157]. In this task, the subject is trained on an instrumental action (e.g., a lever press) to procure a reward (e.g., a food pellet). In a separate phase, the subject is conditioned to expect a reward during a cue (e.g., a tone). At test, the cue is presented, and the subject is given the opportunity to engage in the instrumental action. Increased instrumental reward seeking during the cue is interpreted as increased incentive motivation. The degree to which a reward paired cue can drive reward seeking is a well known component of drug relapse – people in drug recovery are advised to stay away from people, places or items associated with their drug use, as such items can induce cravings and provoke relapse. Food addiction also faces similar, but perhaps even more difficult, challenges: while drug addicts in recovery can alter their environment to stay away from drugs and drug-paired cues altogether, the food addict can not altogether eliminate all foods from their environment, and must instead avoid specific food triggers that spur excessive consumption or consumption of problematic items.

Incentive motivation and the degree to which rewards can drive behavior are subject to the control of the mesolimbic dopamine system. Systemic administration of dopamine receptor

antagonists [158,159] abolish the PIT effect, as do lesions of the NAc [160] or inactivation of the VTA [93,161]. Specifically, inactivation of the NAc core attenuates cue-induced lever pressing for alcohol [162] or food [163,164], but had no effect on Pavlovian goal-approach to the food cup [163]. The role of the NAc shell with regards to PIT remains less clear, as conflicting studies have found both attenuation and no effect on PIT following dopamine antagonism [165] or lesioning [164], respectively, and NAc shell inactivation [163,166] and potentiation [157] have both increased PIT responding. These conflicting results may be due to differences in methodology or subtle anatomical differences (i.e., medial versus lateral shell; for discussion see [167]).

Food seeking as a proxy for drug seeking

Drugs of abuse that potentiate and sensitize mesolimbic dopamine signaling (such as psychostimulants) tend to potentiate PIT. Frequently, the effects of drugs on PIT are modeled in food-deprived animals using food reward, where it has been found that experimenter-delivered, non-contingent amphetamine [168] or cocaine [169] administration increases PIT for food rewards. Interestingly, in rats self-administering intravenous cocaine (rather than experimenter-administered drug), PIT for food rewards was dramatically increased, compared to unsignaled, noncontingent intravenous cocaine or saline administration [170], suggesting the volition to take the drug may confer different neuroadaptations than noncontingent administration. The use of food rewards, however, is highly criticized for low construct validity if the question at issue is how *drugs* potentiate hypersensitivity to *drug*-paired cues. Therefore, models employing drug self-administration can be preferable during tests of incentive salience and motivation. While few studies showing this effect for drug rewards exist, LeBlanc et al. [171] did find, using a

seeking-taking action sequence, that rats will increase their reward seeking and taking (i.e., they will increase lever pressing on both components of the action sequence) upon presentation with a cocaine-paired cue. Other studies have found that intravenous cocaine self-administration will elicit sign-tracking, suggesting drug-paired cues can acquire incentive salience as a consequence of Pavlovian conditioning [172], and cocaine-sensitized rats will work for conditioned reinforcement of a previously cocaine-paired cue [173]. While these results support many similarities between food and drug reward studies, the molecular and chemical adaptations that occur uniquely after drug use (for reviews, see [174,175]) (and, importantly, drug *self-administration* [176]) might be best-modeled using drug, not food, reward. However, PIT for drug rewards can be a finicky task, and is overwhelmingly more easily modeled using food rewards. Given the similarities drug and food rewards have on neurochemistry and reward-seeking behavior, PIT for food rewards remains a well-established assay of incentive motivation.

Specific Aims

Our contemporary food environment is overwhelmed with a variety of refined, high-sucrose and high-fat foods, engineered to be highly palatable [1,2]. Such foods, when consumed in excess and combined with low levels of physical activity, are well known to alter insulin signaling, driving insulin insensitivity and ultimately resulting in type 2 diabetes [177–179] which may be consequential for dopamine signaling and reward seeking behaviors. Non-homeostatic food consumption is a ubiquitous problem [180–182], as food is readily available and overeating lacks the extreme stigma of drug overconsumption and abuse [183] (though obesity is not immune from its own stigma in many areas [184,185]).

Sufferers of eating disorders such as binge eating and compulsive overeating frequently consume beyond homeostatic need, well into satiety. Efforts at restraint can prove difficult, and such a tendency is implicated in the obesity epidemic [186]. Eating despite satiety may have several etiologies, including an increased susceptibility to the motivational effects of reward-paired cues, an inability to appropriately interpret and use interoceptive cues about hunger state, an inability to use the representation of an outcome to guide reward seeking appropriately. Whether junk foods can "sensitize" reward systems sufficiently to increase incentive motivation and disrupt adaptive reward seeking is unclear. *In Aim 1 (Chapters 2 and 3), I will investigate whether a junk food diet can alter the use of external and internal cues to guide reward seeking behavior.*

Like in the periphery, CNS insulin receptors can become desensitized to insulin binding, becoming insulin insensitive and, eventually, resistant [187]. If DAT function is decreased as a result of neuronal insulin resistance, and phasic dopamine signaling is extended as a result of decreased reuptake, the simultaneous presentation of reward-paired cues may have a more profound impact on associative learning processes. As a result, reward-paired cues might more quickly become salient, motivating, and capable of eliciting reward-seeking. Insulin resistance may result in a hypersensitivity to reward-paired cues as a result of decreased DAT expression, increased dopamine signaling, and repeated exposure to environmental stimuli paired with the drug reward. In this way, our insulin-resistance-promoting Westernized food ecosystem may, in turn, invigorate the motivational capacity of food-paired cues to drive further aberrant and maladaptive reward seeking. *In Aim 2 (Chapter 4), I will investigate whether diet-induced insulin resistance will increase dopamine sensitivity and incentive motivation for reward-paired cues.*

References

1. Qi, L.; Cornelis, M. C.; Zhang, C.; van Dam, R. M.; Hu, F. B. Genetic predisposition, Western dietary pattern, and the risk of type 2 diabetes in men. *Am. J. Clin. Nutr.* **2009**, *89*, 1453–1458.

2. Chen, L.; Magliano, D. J.; Zimmet, P. Z. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. *Nat. Rev. Endocrinol.* **2011**, *8*, 228–236.

3. Schultz, W. Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. *J. Neurophysiol.* **1986**, *56*, 1439–61.

4. Hollerman, J. R.; Schultz, W. Dopamine neurons report an error in the temporal prediction of reward during learning. *Nat. Neurosci.* **1998**, *1*, 304–309.

5. Avena, N. M.; Rada, P.; Hoebel, B. G. Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci. Biobehav. Rev.* **2008**, *32*, 20–39.

6. Johnson, P. M.; Kenny, P. J. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* **2010**, *13*, 635–41.

7. Biessels, G.-J.; ter Braak, E. W.; Erkelens, D. W.; Hijman, R. Cognitive function in patients with type 2 diabetes mellitus. *Neurosci. Res. Commun.* **2001**, *28*, 11–22.

8. Bjørgaas, M.; Gimse, R.; Vik, T.; Sand, T. Cognitive function in Type 1 diabetic children with and without episodes of severe hypoglycaemia. *Acta Paediatr.* **1997**, *86*, 148–153.

9. Barbagallo, M.; Dominguez, L. J. Type 2 diabetes mellitus and Alzheimer's disease. *World J. Diabetes* **2014**, *5*, 889.

10. Aviles-Olmos, I.; Limousin, P.; Lees, A.; Foltynie, T. Parkinson's disease, insulin resistance and novel agents of neuroprotection. *Brain* **2013**, *136*, 374–384.

11. Morris, J. K.; Bomhoff, G. L.; Gorres, B. K.; Davis, V. A.; Kim, J.; Lee, P.-P.; Brooks, W. M.; Gerhardt, G. A.; Geiger, P. C.; Stanford, J. A. Insulin resistance impairs nigrostriatal dopamine function. *Exp. Neurol.* **2011**, *231*, 171–180.

12. Figlewicz, D. P. D. .; Evans, S. . B.; Murphy, J.; Hoen, M.; Baskin, D. . G. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res.* **2003**, *964*, 107–115.

13. Labouèbe, G.; Liu, S.; Dias, C.; Zou, H.; Wong, J. C. Y.; Karunakaran, S.; Clee, S. M.; Phillips, A. G.; Boutrel, B.; Borgland, S. L. Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids. *Nat. Neurosci.* **2013**, *16*, 300–8.

14. Patterson, T. A.; Brot, M. D.; Zavosh, A.; Schenk, J. O.; Szot, P.; Figlewicz, D. P. Food deprivation decreases mRNA and activity of the rat dopamine transporter. *Neuroendocrinology* **1998**, *68*, 11–20.

15. Debons, A. F.; Krimsky, I.; From, A.; Cloutier, R. J. Rapid effects of insulin on the

hypothalamic satiety center. *Am. J. Physiol.* **1969**, *217*, 1114–8.

16. Debons, A. F.; Krimsky, I.; From, A. A direct action of insulin on the hypothalamic satiety center. *Am. J. Physiol.* **1970**, *219*, 938–43.

17. Meye, F. J.; Adan, R. A. H. Feelings about food: the ventral tegmental area in food reward and emotional eating. *Trends Pharmacol. Sci.* **2014**, *35*, 31–40.

18. Ikemoto, S. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res. Rev.* **2007**, *56*, 27–78.

19. Berridge, K. C. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl).* **2007**, *191*, 391–431.

20. Grace, A. A.; Bunney, B. S. The control of firing pattern in nigral dopamine neurons: burst firing. *J. Neurosci.* **1984**, *4*, 2877–90.

21. Grace, A. A. The tonic/phasic model of dopamine system regulation: its relevance for understanding how stimulant abuse can alter basal ganglia function. *Drug Alcohol Depend.* **1995**, *37*, 111–129.

22. Smith, G. P.; Schneider, L. H. Relationships between mesolimbic dopamine function and eating behavior. *Ann. N. Y. Acad. Sci.* **1988**, *537*, 254–61.

23. Martel, P.; Fantino, M. Influence of the amount of food ingested on mesolimbic dopaminergic system activity: A microdialysis study. *Pharmacol. Biochem. Behav.* **1996**, *55*, 297–302.

24. Garris, P. A.; Wightman, R. M. Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an in vivo voltammetric study. *J. Neurosci.* **1994**, *14*, 442–50.

25. Sesack, S. R.; Aoki, C.; Pickel, V. M. Ultrastructural localization of D2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. *J. Neurosci.* **1994**, *14*, 88–106.

26. Floresco, S. B.; West, A. R.; Ash, B.; Moore, H.; Grace, A. A. Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat. Neurosci.* **2003**, *6*, 968–73.

27. Wise, R. A. Catecholamine theories of reward: A critical review. *Brain Res.* **1978**, *152*, 215–247.

28. Wise, R. A.; Schwartz, H. V. Pimozide attenuates acquisition of lever-pressing for food in rats. *Pharmacol. Biochem. Behav.* **1981**, *15*, 655–656.

29. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand. Suppl.* **1971**, *367*, 95–122.

30. Wise, R. A.; Raptis, L. Effects of naloxone and pimozide on initiation and maintenance measures of free feeding. *Brain Res.* **1986**, *368*, 62–8.

31. Geary, N.; Smith, G. P. Pimozide decreases the positive reinforcing effect of sham fed sucrose in the rat. *Pharmacol. Biochem. Behav.* **1985**, *22*, 787–790.

32. Wise, R. A.; Spindler, J.; Legault, L. Major attenuation of food reward with performance-sparing doses of pimozide in the rat. *Can. J. Psychol.* **1978**, *32*, 77–85.

33. Xenakis, S. The effects of pimozide on the consumption of a palatable saccharin-glucose solution in the rat. *Pharmacol. Biochem. Behav.* **1981**, *15*, 435–442.

34. Ikemoto, S.; Panksepp, J. Dissociations between appetitive and consummatory responses by pharmacological manipulations of reward-relevant brain regions. *Behav. Neurosci.* **1996**, *110*, 331–45.

35. Martel, P.; Fantino, M. Mesolimbic dopaminergic system activity as a function of food reward: A microdialysis study. *Pharmacol. Biochem. Behav.* **1996**, *53*, 221–6.

36. Ostlund, S. B.; Maidment, N. T. Dopamine receptor blockade attenuates the general incentive motivational effects of noncontingently delivered rewards and reward-paired cues without affecting their ability to bias action selection. *Neuropsychopharmacology* **2012**, *37*, 508–19.

37. Sugam, J. A.; Day, J. J.; Wightman, R. M.; Carelli, R. M. Phasic nucleus accumbens dopamine encodes risk-based decision-making behavior. *Biol. Psychiatry* **2012**, *71*, 199–205.

38. Phillips, P. E. M.; Stuber, G. D.; Heien, M. L. A. V.; Wightman, R. M.; Carelli, R. M. Subsecond dopamine release promotes cocaine seeking. *Nature* **2003**, *422*, 614–618.

39. Gan, J. O.; Walton, M. E.; Phillips, P. E. M. Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. *Nat. Neurosci.* **2009**, *13*, 25–27.

40. Woods, S. C.; Seeley, R. J.; Baskin, D. G.; Schwartz, M. W. Insulin and the blood-brain barrier. *Curr. Pharm. Des.* **2003**, *9*, 795–800.

41. Schwartz, M. W.; Bergman, R. N.; Kahn, S. E.; Taborsky, G. J.; Fisher, L. D.; Sipols, A. J.; Woods, S. C.; Steil, G. M.; Porte, D. Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. Quantitative aspects and implications for transport. *J. Clin. Invest.* **1991**, *88*, 1272–81.

42. Baura, G. D.; Foster, D. M.; Porte, D.; Kahn, S. E.; Bergman, R. N.; Cobelli, C.; Schwartz, M. W. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. *J. Clin. Invest.* **1993**, *92*, 1824–30.

43. Schwartz, M. W.; Sipols, A.; Kahn, S. E.; Lattemann, D. F.; Taborsky, G. J.; Bergman, R. N.; Woods, S. C.; Porte, D. Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid. *Am. J. Physiol.* **1990**, *259*, E378-83.

44. Daws, L. C.; Avison, M. J.; Robertson, S. D.; Niswender, K. D.; Galli, A.; Saunders, C. Insulin signaling and addiction. *Neuropharmacology* **2011**, *61*, 1123–8.

45. Woods, S. C.; D'Alessio, D. A. Central control of body weight and appetite. *J. Clin. Endocrinol. Metab.* **2008**, *93*, S37-50.

46. Niswender, K. D.; Gallis, B.; Blevins, J. E.; Corson, M. A.; Schwartz, M. W.; Baskin, D. G. Immunocytochemical Detection of Phosphatidylinositol 3-kinase Activation by Insulin and Leptin. *J. Histochem. Cytochem.* **2003**, *51*, 275–283.

47. Beaulieu, J.-M.; Gainetdinov, R. R.; Caron, M. G. The Akt-GSK-3 signaling cascade in the actions of dopamine. *Trends Pharmacol. Sci.* **2007**, *28*, 166–72.

48. Garcia, B. G.; Wei, Y.; Moron, J. A.; Lin, R. Z.; Javitch, J. A.; Galli, A. Akt is essential for insulin modulation of amphetamine-induced human dopamine transporter cell-surface redistribution. *Mol. Pharmacol.* **2005**, *68*, 102–9.

49. Emamian, E. S.; Hall, D.; Birnbaum, M. J.; Karayiorgou, M.; Gogos, J. A. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat. Genet.* **2004**, *36*, 131–7.

50. Ikeda, M.; Iwata, N.; Suzuki, T.; Kitajima, T.; Yamanouchi, Y.; Kinoshiya, Y.; Sekine, Y.; Iyo, M.; Harano, M.; Komiyama, T.; Yamada, M.; Sora, I.; Ujike, H.; Inada, T.; Ozaki, N. Positive association of AKT1 haplotype to Japanese methamphetamine use disorder. *Int. J. Neuropsychopharmacol.* **2006**, *9*, 77–81.

51. Siuta, M. A.; Robertson, S. D.; Kocalis, H.; Saunders, C.; Gresch, P. J.; Khatri, V.; Shiota, C.; Kennedy, J. P.; Lindsley, C. W.; Daws, L. C.; Polley, D. B.; Veenstra-Vanderweele, J.; Stanwood, G. D.; Magnuson, M. A.; Niswender, K. D.; Galli, A. Dysregulation of the norepinephrine transporter sustains cortical hypodopaminergia and schizophrenia-like behaviors in neuronal rictor null mice. *PLoS Biol.* **2010**, *8*, e1000393.

52. Könner, A. C.; Hess, S.; Tovar, S.; Mesaros, A.; Sánchez-Lasheras, C.; Evers, N.; Verhagen, L. A. W.; Brönneke, H. S.; Kleinridders, A.; Hampel, B.; Kloppenburg, P.; Brüning, J. C. Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. *Cell Metab.* **2011**, *13*, 720–8.

53. Stouffer, M. A.; Woods, C. A.; Patel, J. C.; Lee, C. R.; Witkovsky, P.; Bao, L.; Machold, R. P.; Jones, K. T.; de Vaca, S. C.; Reith, M. E. A.; Carr, K. D.; Rice, M. E. Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. *Nat. Commun.* **2015**, *6*, 8543.

54. Mebel, D. M.; Wong, J. C. Y.; Dong, Y. J.; Borgland, S. L. Insulin in the ventral tegmental area reduces hedonic feeding and suppresses dopamine concentration via increased reuptake. *Eur. J. Neurosci.* **2012**, *36*, 2336–46.

55. Liu, S.; Borgland, S. L. Regulation of the mesolimbic dopamine circuit by feeding peptides. *Neuroscience* **2015**, *289*, 19–42.

56. Speed, N. K.; Matthies, H. J. G.; Kennedy, J. P.; Vaughan, R. A.; Javitch, J. A.; Russo, S. J.; Lindsley, C. W.; Niswender, K.; Galli, A. Akt-dependent and isoform-specific regulation of dopamine transporter cell surface expression. *ACS Chem. Neurosci.* **2010**, *1*, 476–81.

57. Speed, N.; Saunders, C.; Davis, A. R.; Owens, W. A.; Matthies, H. J. G.; Saadat, S.; Kennedy, J. P.; Vaughan, R. A.; Neve, R. L.; Lindsley, C. W.; Russo, S. J.; Daws, L. C.; Niswender, K. D.; Galli, A. Impaired striatal Akt signaling disrupts dopamine homeostasis and

increases feeding. *PLoS One* **2011**, *6*, e25169.

58. Doolen, S.; Zahniser, N. R. Protein tyrosine kinase inhibitors alter human dopamine transporter activity in Xenopus oocytes. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 931–8.

59. Carvelli, L.; Morón, J. A.; Kahlig, K. M.; Ferrer, J. V.; Sen, N.; Lechleiter, J. D.; Leeb-Lundberg, L. M. F.; Merrill, G.; Lafer, E. M.; Ballou, L. M.; Shippenberg, T. S.; Javitch, J. A.; Lin, R. Z.; Galli, A. PI 3-kinase regulation of dopamine uptake. *J. Neurochem.* **2002**, *81*, 859–869.

60. Cone, J. J.; Chartoff, E. H.; Potter, D. N.; Ebner, S. R.; Roitman, M. F. Prolonged high fat diet reduces dopamine reuptake without altering DAT gene expression. *PLoS One* **2013**, *8*, e58251.

61. Figlewicz, D. P.; Szot, P.; Chavez, M.; Woods, S. C.; Veith, R. C. Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res.* **1994**, *644*, 331–334.

62. Sevak, R. J.; Koek, W.; Owens, W. A.; Galli, A.; Daws, L. C.; France, C. P. Feeding conditions differentially affect the neurochemical and behavioral effects of dopaminergic drugs in male rats. *Eur. J. Pharmacol.* **2008**, *592*, 109–15.

63. Owens, W. A.; Sevak, R. J.; Galici, R.; Chang, X.; Javors, M. A.; Galli, A.; France, C. P.; Daws, L. C. Deficits in dopamine clearance and locomotion in hypoinsulinemic rats unmask novel modulation of dopamine transporters by amphetamine. *J. Neurochem.* **2005**, *94*, 1402–1410.

64. Kahlig, K. M.; Galli, A. Regulation of dopamine transporter function and plasma membrane expression by dopamine, amphetamine, and cocaine. *Eur. J. Pharmacol.* **2003**, *479*, 153–158.

65. Saunders, C.; Ferrer, J. V; Shi, L.; Chen, J.; Merrill, G.; Lamb, M. E.; Leeb-Lundberg, L. M.; Carvelli, L.; Javitch, J. A.; Galli, A. Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 6850–5.

66. Williams, J. M.; Owens, W. A.; Turner, G. H.; Saunders, C.; Dipace, C.; Blakely, R. D.; France, C. P.; Gore, J. C.; Daws, L. C.; Avison, M. J.; Galli, A. Hypoinsulinemia regulates amphetamine-induced reverse transport of dopamine. *PLoS Biol.* **2007**, *5*, e274.

67. Figlewicz, D. P.; Brot, M. D.; McCall, A. L.; Szot, P. Diabetes causes differential changes in CNS noradrenergic and dopaminergic neurons in the rat: a molecular study. *Brain Res.* **1996**, *736*, 54–60.

68. Childress, A. R.; Mozley, P. D.; McElgin, W.; Fitzgerald, J.; Reivich, M.; O'Brien, C. P. Limbic Activation During Cue-Induced Cocaine Craving. *Am. J. Psychiatry* **1999**, *156*, 11–18.

69. Ehrman, R. N.; Robbins, S. J.; Childress, A. R.; O'Brien, C. P. Conditioned responses to cocaine-related stimuli in cocaine abuse patients. *Psychopharmacology (Berl).* **1992**, *107*, 523–529.

70. Stewart, J.; de Wit, H.; Eikelboom, R. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychol. Rev.* **1984**, *91*, 251–258.

71. Wallace, B. Psychological and environmental determinants of relapse in crack cocaine smokers. *J. Subst. Abuse Treat.* **1989**, *6*, 95–106.

72. Bradberry, C. W.; Barrett-Larimore, R. L.; Jatlow, P.; Rubino, S. R. Impact of Self-Administered Cocaine and Cocaine Cues on Extracellular Dopamine in Mesolimbic and Sensorimotor Striatum in Rhesus Monkeys. *J. Neurosci.* **2000**, *20*, 3874–3883.

73. Ito, R.; Dalley, J. W.; Howes, S. R.; Robbins, T. W.; Everitt, B. J. Dissociation in Conditioned Dopamine Release in the Nucleus Accumbens Core and Shell in Response to Cocaine Cues and during Cocaine-Seeking Behavior in Rats. *J. Neurosci.* **2000**, *20*, 7489–7495.

74. Adinoff, B. Neurobiologic Processes in Drug Reward and Addiction. *Harv. Rev. Psychiatry* **2009**.

75. Wang, G.-J.; Volkow, N. D.; Thanos, P. K.; Fowler, J. S. Similarity Between Obesity and Drug Addiction as Assessed by Neurofunctional Imaging. *J. Addict. Dis.* **2004**, *23*, 39–53.

76. Dalley, J. W.; Everitt, B. J. Dopamine receptors in the learning, memory and drug reward circuitry. *Semin. Cell Dev. Biol.* **2009**, *20*, 403–10.

77. Stewart, J. Review. Psychological and neural mechanisms of relapse. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2008**, *363*, 3147–58.

78. Zellner, M. R.; Ranaldi, R. How conditioned stimuli acquire the ability to activate VTA dopamine cells: A proposed neurobiological component of reward-related learning. *Neurosci. Biobehav. Rev.* **2010**, *34*, 769–780.

79. Wise, R. A. Dopamine, learning and motivation. *Nat. Rev. Neurosci.* **2004**, *5*, 483–94.

80. Robinson, T. E.; Berridge, K. C. The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction* **2000**, *95 Suppl 2*, S91-117.

81. Ljungberg, T.; Apicella, P.; Schultz, W. Responses of monkey dopamine neurons during learning of behavioral reactions. *J. Neurophysiol.* **1992**, *67*, 145–63.

82. Schultz, W.; Apicella, P.; Ljungberg, T. Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J. Neurosci.* **1993**, *13*, 900–13.

83. Nishino, H.; Ono, T.; Muramoto, K.; Fukuda, M.; Sasaki, K. Neuronal activity in the ventral tegmental area (VTA) during motivated bar press feeding in the monkey. *Brain Res.* **1987**, *413*, 302–13.

84. Day, J. J.; Roitman, M. F.; Wightman, R. M.; Carelli, R. M. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci.* **2007**, *10*, 1020–8.

85. Satoh, T.; Nakai, S.; Sato, T.; Kimura, M. Correlated coding of motivation and outcome of

decision by dopamine neurons. *J. Neurosci.* **2003**, *23*, 9913–23.

86. Di Ciano, P.; Blaha, C. D.; Phillips, A. G. Conditioned changes in dopamine oxidation currents in the nucleus accumbens of rats by stimuli paired with self-administration or yoked-administration of d-amphetamine. *Eur. J. Neurosci.* **1998**, *10*, 1121–1127.

87. Gratton, A.; Wise, R. A. Drug- and behavior-associated changes in dopamine-related electrochemical signals during intravenous cocaine self-administration in rats. *J. Neurosci.* **1994**, *14*, 4130–46.

88. Roitman, M. F.; Stuber, G. D.; Phillips, P. E. M.; Wightman, R. M.; Carelli, R. M. Dopamine operates as a subsecond modulator of food seeking. *J. Neurosci.* **2004**, *24*, 1265–71.

89. Blackburn, J. R.; Pfaus, J. G.; Phillips, A. G. Dopamine functions in appetitive and defensive behaviours. *Prog. Neurobiol.* **1992**, *39*, 247–79.

90. Kiyatkin, E. A.; Rebec, G. V Activity of presumed dopamine neurons in the ventral tegmental area during heroin self-administration. *Neuroreport* **1997**, *8*, 2581–5.

91. Wassum, K. M.; Ostlund, S. B.; Maidment, N. T. Phasic mesolimbic dopamine signaling precedes and predicts performance of a self-initiated action sequence task. *Biol. Psychiatry* **2012**, *71*, 846–54.

92. Di Ciano, P.; Everitt, B. J. Contribution of the ventral tegmental area to cocaine-seeking maintained by a drug-paired conditioned stimulus in rats. *Eur. J. Neurosci.* **2004**, *19*, 1661–7.

93. Murschall, A.; Hauber, W. Inactivation of the ventral tegmental area abolished the general excitatory influence of Pavlovian cues on instrumental performance. *Learn. Mem.* **2006**, *13*, 123–6.

94. Yun, I. A.; Wakabayashi, K. T.; Fields, H. L.; Nicola, S. M. The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. *J. Neurosci.* **2004**, *24*, 2923–33.

95. Blackburn, J. R.; Phillips, A. G.; Fibiger, H. C. Dopamine and preparatory behavior: I. Effects of pimozide. *Behav. Neurosci.* **1987**, *101*, 352–360.

96. Blackburn, J. R.; Phillips, A. G.; Jakubovic, A.; Fibiger, H. C. Dopamine and preparatory behavior: II. A neurochemical analysis. *Behav. Neurosci.* **1989**, *103*, 15–23.

97. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Dopaminergic substrates of amphetamine-induced place preference conditioning. *Brain Res.* **1982**, *253*, 185–93.

98. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Attenuation by haloperidol of place preference conditioning using food reinforcement. *Psychopharmacology (Berl).* **1982**, *77*, 379–82.

99. Spyraki, C.; Nomikos, G. G.; Varonos, D. D. Intravenous cocaine-induced place preference: attenuation by haloperidol. *Behav. Brain Res.* **1987**, *26*, 57–62.

100. Cador, M.; Taylor, J. R.; Robbins, T. W. Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. *Psychopharmacology (Berl).*

1991, *104*, 377–385.

101. Nicola, S. M.; Taha, S. A.; Kim, S. W.; Fields, H. L. Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. *Neuroscience* **2005**, *135*, 1025–33.

102. Wakabayashi, K. T.; Fields, H. L.; Nicola, S. M. Dissociation of the role of nucleus accumbens dopamine in responding to reward-predictive cues and waiting for reward. *Behav. Brain Res.* **2004**, *154*, 19–30.

103. Di Ciano, P.; Cardinal, R. N.; Cowell, R. A.; Little, S. J.; Everitt, B. J. Differential Involvement of NMDA, AMPA/Kainate, and Dopamine Receptors in the Nucleus Accumbens Core in the Acquisition and Performance of Pavlovian Approach Behavior. *J. Neurosci.* **2001**, *21*, 9471–9477.

104. Beninger, R. J.; Ranaldi, R. The effects of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. *Behav. Pharmacol.* **1992**, *3*, 155–163.

105. Ranaldi, R.; Pantalony, D.; Beninger, R. J. The D1 agonist SKF 38393 attenuates amphetamine-produced enhancement of responding for conditioned reward in rats. *Pharmacol. Biochem. Behav.* **1995**, *52*, 131–137.

106. Taylor, J. R.; Robbins, T. W. Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharmacology (Berl).* **1984**, *84*, 405–412.

107. Wang, G.-J.; Volkow, N. D.; Logan, J.; Pappas, N. R.; Wong, C. T.; Zhu, W.; Netusll, N.; Fowler, J. S. Brain dopamine and obesity. *Lancet* **2001**, *357*, 354–357.

108. Reinholz, J.; Skopp, O.; Breitenstein, C.; Bohr, I.; Winterhoff, H.; Knecht, S. Compensatory weight gain due to dopaminergic hypofunction: new evidence and own incidental observations. *Nutr. Metab. (Lond).* **2008**, *5*, 35.

109. Chang, L.; Alicata, D.; Ernst, T.; Volkow, N. Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. *Addiction* **2007**, *102 Suppl*, 16–32.

110. Lee, B.; London, E. D.; Poldrack, R. a; Farahi, J.; Nacca, A.; Monterosso, J. R.; Mumford, J. A.; Bokarius, A. V; Dahlbom, M.; Mukherjee, J.; Bilder, R. M.; Brody, A. L.; Mandelkern, M. a Striatal dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. *J. Neurosci.* **2009**, *29*, 14734–40.

111. Volkow, N. D.; Ding, Y. S.; Fowler, J. S.; Wang, G. J.; Logan, J.; Gatley, S. J.; Hitzemann, R.; Smith, G.; Fields, S. D.; Gur, R. Dopamine transporters decrease with age. *J. Nucl. Med.* **1996**, *37*, 554–9.

112. Kenny, P. J.; Voren, G.; Johnson, P. M. Dopamine D2 receptors and striatopallidal transmission in addiction and obesity. *Curr. Opin. Neurobiol.* **2013**, *23*, 535–8.

113. Dalley, J. W.; Fryer, T. D.; Brichard, L.; Robinson, E. S. J.; Theobald, D. E. H.; Lääne, K.;

Peña, Y.; Murphy, E. R.; Shah, Y.; Probst, K.; Abakumova, I.; Aigbirhio, F. I.; Richards, H. K.; Hong, Y.; Baron, J.-C.; Everitt, B. J.; Robbins, T. W. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science (80-.).* **2007**, *315*, 1267–70.

114. Morgan, D.; Grant, K. A.; Gage, H. D.; Mach, R. H.; Kaplan, J. R.; Prioleau, O.; Nader, S. H.; Buchheimer, N.; Ehrenkaufer, R. L.; Nader, M. A. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat. Neurosci.* **2002**, *5*, 169–74.

115. Thanos, P. K.; Volkow, N. D.; Freimuth, P.; Umegaki, H.; Ikari, H.; Roth, G.; Ingram, D. K.; Hitzemann, R. Overexpression of dopamine D2 receptors reduces alcohol self-administration. *J. Neurochem.* **2001**, *78*, 1094–103.

116. Wang, Q.; Jolly, J. P.; Surmeier, J. D.; Mullah, B. M.; Lidow, M. S.; Bergson, C. M.; Robishaw, J. D. Differential dependence of the D1 and D5 dopamine receptors on the G protein gamma 7 subunit for activation of adenylylcyclase. *J. Biol. Chem.* **2001**, *276*, 39386–93.

117. Barnard, N. D.; Noble, E. P.; Ritchie, T.; Cohen, J.; Jenkins, D. J. A.; Turner-McGrievy, G.; Gloede, L.; Green, A. A.; Ferdowsian, H. D2 dopamine receptor Taq1A polymorphism, body weight, and dietary intake in type 2 diabetes. *Nutrition* **2009**, *25*, 58–65.

118. Stice, E.; Yokum, S.; Blum, K.; Bohon, C. Weight gain is associated with reduced striatal response to palatable food. *J. Neurosci.* **2010**, *30*, 13105–9.

119. Colantuoni, C.; Schwenker, J.; McCarthy, J.; Rada, P.; Ladenheim, B.; Cadet, J. L.; Schwartz, G. J.; Moran, T. H.; Hoebel, B. G. Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport* **2001**, *12*, 3549–52.

120. Volkow, N. D.; Wang, G.-J.; Telang, F.; Fowler, J. S.; Thanos, P. K.; Logan, J.; Alexoff, D.; Ding, Y.-S.; Wong, C.; Ma, Y.; Pradhan, K. Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. *Neuroimage* **2008**, *42*, 1537–43.

121. Geiger, B. M.; Behr, G. G.; Frank, L. E.; Caldera-Siu, A. D.; Beinfeld, M. C.; Kokkotou, E. G.; Pothos, E. N. Evidence for defective mesolimbic dopamine exocytosis in obesity-prone rats. *FASEB J.* **2008**, *22*, 2740–6.

122. Davis, J. F.; Tracy, A. L.; Schurdak, J. D.; Tschöp, M. H.; Lipton, J. W.; Clegg, D. J.; Benoit, S. C. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. *Behav. Neurosci.* **2008**, *122*, 1257–63.

123. Geiger, B. M.; Haburcak, M.; Avena, N. M.; Moyer, M. C.; Hoebel, B. G.; Pothos, E. N. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience* **2009**, *159*, 1193–9.

124. Robinson, T.; Berridge, K. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.* **1993**, *18*, 247–291.

125. Robinson, T. E.; Berridge, K. C. The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction* **2000**, *95*, 91–117.

126. Berridge, K. C.; Robinson, T. E. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* **1998**, *28*, 309–369.

127. Robinson, T. E.; Berridge, K. C. Addiction. *Annu. Rev. Psychol.* **2003**, *54*, 25–53.

128. Robinson, T. E.; Berridge, K. C. Review. The incentive sensitization theory of addiction: some current issues. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2008**, *363*, 3137–46.

129. Jurdak, N.; Kanarek, R. B. Sucrose-induced obesity impairs novel object recognition learning in young rats. *Physiol. Behav.* **2009**, *96*, 1–5.

130. Kanoski, S. E.; Davidson, T. L. Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. *Physiol. Behav.* **2011**, *103*, 59–68.

131. Beilharz, J. E.; Maniam, J.; Morris, M. J. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain. Behav. Immun.* **2014**, *37*, 134–41.

132. Furlong, T. M.; Jayaweera, H. K.; Balleine, B. W.; Corbit, L. H. Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *J. Neurosci.* **2014**, *34*, 5012–22.

133. Ahn, S.; Phillips, A. G. Repeated cycles of restricted food intake and binge feeding disrupt sensory-specific satiety in the rat. *Behav. Brain Res.* **2012**, *231*, 279–285.

134. Tantot, F.; Parkes, S. L.; Marchand, A. R.; Boitard, C.; Naneix, F.; Layé, S.; Trifilieff, P.; Coutureau, E.; Ferreira, G. The effect of high-fat diet consumption on appetitive instrumental behavior in rats. *Appetite* **2017**, *108*, 203–211.

135. Vendruscolo, L. F.; Gueye, A. B.; Vendruscolo, J. C. M.; Clemens, K. J.; Mormède, P.; Darnaudéry, M.; Cador, M. Reduced alcohol drinking in adult rats exposed to sucrose during adolescence. *Neuropharmacology* **2010**, *59*, 388–394.

136. Shin, A. C.; Townsend, R. L.; Patterson, L. M.; Berthoud, H.-R. "Liking" and "wanting" of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R1267-80.

137. Lesser, E. N.; Arroyo-Ramirez, A.; Mi, S. J.; Robinson, M. J. F. The impact of a junk-food diet during development on "wanting" and "liking." *Behav. Brain Res.* **2017**, *317*, 163–178.

138. Buda-Levin, A.; Wojnicki, F. H. E. E.; Corwin, R. L. Baclofen reduces fat intake under binge-type conditions. *Physiol. Behav.* **2005**, *86*, 176–84.

139. Avena, N. M.; Hoebel, B. G. A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience* **2003**, *122*, 17–20.

140. Duca, F. A.; Swartz, T. D.; Covasa, M. Effect of diet on preference and intake of sucrose in obese prone and resistant rats. *PLoS One* **2014**, *9*, e111232.

141. Vendruscolo, L. F.; Gueye, A. B.; Darnaudéry, M.; Ahmed, S. H.; Cador, M. Sugar overconsumption during adolescence selectively alters motivation and reward function in adult

rats. *PLoS One* **2010**, *5*, e9296.

142. Vollbrecht, P. J.; Mabrouk, O. S.; Nelson, A. D.; Kennedy, R. T.; Ferrario, C. R. Pre-existing differences and diet-induced alterations in striatal dopamine systems of obesity-prone rats. *Obesity (Silver Spring).* **2016**, *24*, 670–677.

143. Robinson, M. J.; Burghardt, P. R.; Patterson, C. M.; Nobile, C. W.; Akil, H.; Watson, S. J.; Berridge, K. C.; Ferrario, C. R. Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology* **2015**.

144. Lindqvist, A.; Mohapel, P.; Bouter, B.; Frielingsdorf, H.; Pizzo, D.; Brundin, P.; Erlanson-Albertsson, C. High-fat diet impairs hippocampal neurogenesis in male rats. *Eur. J. Neurol.* **2006**, *13*, 1385–1388.

145. Martire, S. I.; Maniam, J.; South, T.; Holmes, N.; Westbrook, R. F.; Morris, M. J. Extended exposure to a palatable cafeteria diet alters gene expression in brain regions implicated in reward, and withdrawal from this diet alters gene expression in brain regions associated with stress. *Behav. Brain Res.* **2014**, *265*, 132–41.

146. Molteni, R.; Barnard, R. J.; Ying, Z.; Roberts, C. K.; Gómez-Pinilla, F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* **2002**, *112*, 803–14.

147. Jansen, A. A learning model of binge eating: Cue reactivity and cue exposure. *Behav. Res. Ther.* **1998**, *36*, 257–272.

148. Weingarten, H. P. Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science* **1983**, *220*, 431–3.

149. Boggiano, M. M.; Dorsey, J. R.; Thomas, J. M.; Murdaugh, D. L. The Pavlovian power of palatable food: lessons for weight-loss adherence from a new rodent model of cue-induced overeating. *Int. J. Obes. (Lond).* **2009**, *33*, 693–701.

150. Petrovich, G. D.; Ross, C. A.; Gallagher, M.; Holland, P. C. Learned contextual cue potentiates eating in rats. *Physiol. Behav.* **2007**, *90*, 362–7.

151. Jansen, A.; Havermans, R. C.; Nederkoorn, C. Cued Overeating. In *Handbook of Behavior, Food and Nutrition*; Preedy, V. R.; Watson, R. R.; Martin, C. R., Eds.; Springer New York: New York, NY, 2011; pp. 1431–1443.

152. Cornell, C. E.; Rodin, J.; Weingarten, H. Stimulus-induced eating when satiated. *Physiol. Behav.* **1989**, *45*, 695–704.

153. Jansen, A.; Theunissen, N.; Slechten, K.; Nederkoorn, C.; Boon, B.; Mulkens, S.; Roefs, A. Overweight children overeat after exposure to food cues. *Eat. Behav.* **2003**, *4*, 197–209.

154. Fedoroff, I. C.; Polivy, J.; Herman, C. P. The Effect of Pre-exposure to Food Cues on the Eating Behavior of Restrained and Unrestrained Eaters. *Appetite* **1997**, *28*, 33–47.

155. Jansen, A.; van den Hout, M. On being led into temptation: "Counterregulation" of dieters

after smelling a “preload.” *Addict. Behav.* **1991**, *16*, 247–253.

156. Wardle, J. Conditioning processes and cue exposure in the modification of excessive eating. *Addict. Behav.* **1990**, *15*, 387–393.

157. Wyvell, C. L.; Berridge, K. C. Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward “wanting” without enhanced “liking” or response reinforcement. *J. Neurosci.* **2000**, *20*, 8122–30.

158. Wassum, K. M.; Ostlund, S. B.; Balleine, B. W.; Maidment, N. T. Differential dependence of Pavlovian incentive motivation and instrumental incentive learning processes on dopamine signaling. *Learn. Mem.* **2011**, *18*, 475–483.

159. Dickinson, A.; Smith, J.; Mirenowicz, J. Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behav. Neurosci.* **2000**, *114*, 468–83.

160. de Borchgrave, R.; Rawlins, J. N. P.; Dickinson, A.; Balleine, B. W. Effects of cytotoxic nucleus accumbens lesions on instrumental conditioning in rats. *Exp. brain Res.* **2002**, *144*, 50–68.

161. Corbit, L. H.; Janak, P. H.; Balleine, B. W. General and outcome-specific forms of Pavlovian-instrumental transfer: the effect of shifts in motivational state and inactivation of the ventral tegmental area. *Eur. J. Neurosci.* **2007**, *26*, 3141–3149.

162. Chaudhri, N.; Sahuque, L. L.; Schairer, W. W.; Janak, P. H. Separable roles of the nucleus accumbens core and shell in context- and cue-induced alcohol-seeking. *Neuropsychopharmacology* **2010**, *35*, 783–91.

163. Floresco, S. B.; McLaughlin, R. J.; Haluk, D. M. Opposing roles for the nucleus accumbens core and shell in cue-induced reinstatement of food-seeking behavior. *Neuroscience* **2008**, *154*, 877–84.

164. Hall, J.; Parkinson, J. A.; Connor, T. M.; Dickinson, A.; Everitt, B. J. Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. *Eur. J. Neurosci.* **2001**, *13*, 1984–1992.

165. Lex, A.; Hauber, W. Dopamine D1 and D2 receptors in the nucleus accumbens core and shell mediate Pavlovian-instrumental transfer. *Learn. Mem.* **2008**, *15*, 483–91.

166. Di Ciano, P.; Benham-Hermetz, J.; Fogg, A. P.; Osborne, G. E. C. Role of the prelimbic cortex in the acquisition, re-acquisition or persistence of responding for a drug-paired conditioned reinforcer. *Neuroscience* **2007**, *150*, 291–8.

167. Ikemoto, S.; Qin, M.; Liu, Z.-H. The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the accumbens core, shell, and olfactory tubercle valid? *J. Neurosci.* **2005**, *25*, 5061–5.

168. Wyvell, C. L.; Berridge, K. C. Incentive sensitization by previous amphetamine exposure: increased cue-triggered “wanting” for sucrose reward. *J. Neurosci.* **2001**, *21*, 7831–40.

169. LeBlanc, K. H.; Maidment, N. T.; Ostlund, S. B. Repeated cocaine exposure facilitates the expression of incentive motivation and induces habitual control in rats. *PLoS One* **2013**, *8*, e61355.

170. Leblanc, K. H.; Maidment, N. T.; Ostlund, S. B. Impact of repeated intravenous cocaine administration on incentive motivation depends on mode of drug delivery. *Addict. Biol.* **2013**.

171. LeBlanc, K. H.; Ostlund, S. B.; Maidment, N. T. Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behav. Neurosci.* **2012**, *126*, 681–9.

172. Uslaner, J. M.; Acerbo, M. J.; Jones, S. A.; Robinson, T. E. The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. *Behav. Brain Res.* **2006**, *169*, 320–324.

173. Di Ciano, P. Facilitated Acquisition but Not Persistence of Responding for a Cocaine-Paired Conditioned Reinforcer Following Sensitization with Cocaine. *Neuropsychopharmacology* **2007**, *33*, 1426–1431.

174. Kelley, A. E. Memory and addiction: shared neural circuitry and molecular mechanisms. *Neuron* **2004**, *44*, 161–79.

175. Nestler, E. J.; Aghajanian, G. K. Molecular and Cellular Basis of Addiction. *Science (80-.).* **1997**, *278*, 58–63.

176. Chen, B. T.; Bowers, M. S.; Martin, M.; Hopf, F. W.; Guillory, A. M.; Carelli, R. M.; Chou, J. K.; Bonci, A. Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. *Neuron* **2008**, *59*, 288–97.

177. Basciano, H.; Federico, L.; Adeli, K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr. Metab. (Lond).* **2005**, *2*, 5.

178. Thorp, A. A.; Owen, N.; Neuhaus, M.; Dunstan, D. W. Sedentary Behaviors and Subsequent Health Outcomes in Adults. *Am. J. Prev. Med.* **2011**, *41*, 207–215.

179. Hu, F. B. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* **2011**, *34*, 1249–57.

180. Adam, T. C.; Epel, E. S. Stress, eating and the reward system. *Physiol. Behav.* **2007**, *91*, 449–458.

181. Borer, K. T. Nonhomeostatic control of human appetite and physical activity in regulation of energy balance. *Exerc. Sport Sci. Rev.* **2010**, *38*, 114–21.

182. Mela, D. J. Eating for pleasure or just wanting to eat? Reconsidering sensory hedonic responses as a driver of obesity. *Appetite* **2006**, *47*, 10–17.

183. Davis, C. From passive overeating to "food addiction": a spectrum of compulsion and severity. *ISRN Obes.* **2013**, 1–20.

184. Puhl, R. M.; Heuer, C. A. The stigma of obesity: A review and update. *Obesity* **2009**, *17*, 941–964.

185. Puhl, R. M.; Heuer, C. A. Obesity stigma: important considerations for public health. *Am. J. Public Health* **2010**, *100*, 1019–28.

186. Yanovski, S. Z. Binge eating disorder and obesity in 2003: Could treating an eating disorder have a positive effect on the obesity epidemic? *Int. J. Eat. Disord.* **2003**, *34*, S117–S120.

187. Agrawal, R.; Gomez-Pinilla, F. "Metabolic syndrome" in the brain: deficiency in omega-3 fatty acid exacerbates dysfunctions in insulin receptor signalling and cognition. *J. Physiol.* **2012**, *590*, 2485–99.

Chapter 2

Pattern Of Access Determines Influence Of Junk Food Diet On Cue Sensitivity And Palatability

Introduction

Non-homeostatic eating behavior is strongly motivated by the rewarding effects of palatability and variety in flavor and texture [1]. Overindulgence in such foods has been heavily implicated in the obesity epidemic sweeping developed nations [2], in which obesity rates have more than doubled in the last 30 years [3,4]. Importantly, for many overweight and obese individuals, efforts to control their body weight prove challenging, with craving and compulsive consumption being major culprits [5]. In some cases, compulsive overeating can become so extreme that it has been compared with drug addiction, as they share many characteristics such as escalating intake over time despite negative consequences, such as foot shock in rats [6,7] or negative health or social consequences in humans [8–12].

A key component of maladaptive reward seeking is the acquisition of Pavlovian associations between the primary rewards themselves (e.g., food, drugs) and predictive stimuli in the environment (i.e., a context or discrete cue). With repeated pairings, such cues can come to trigger reward cravings [13,14] and drive efforts to procure reward [15–17]. This process, whereby reward-associated cues acquire motivational properties that allow them to become capable of eliciting reward seeking, is termed Pavlovian incentive motivation, frequently referred to in the literature as 'wanting'. The motivational influence of drug-paired cues is well documented in the drug addiction literature, where drug-paired cues have been shown to potentiate drug seeking for alcohol [18], nicotine [19], cocaine [20], and morphine [21], and sensitivity to such cues is considered to underlie a vulnerability to craving, compulsive drug seeking and, consequently, addiction [22,23]. Importantly, food-paired cues also become highly

salient, capable of producing strong physiological reactions (e.g., increased salivation) and cravings [24]. Food-paired cues are well known to potentiate non-homeostatic (i.e., hunger state-independent) feeding, in both rats [25–27] and humans [28,29], as well as maladaptive overeating [30] in both obese and normal-weight restrained eaters (i.e., such as when dieting) [31–33].

Growing evidence from animal models suggests that, like drugs of abuse, poor diets (i.e., high-fat, high-sugar and refined foods) may have long-term consequences for behavior and cognition, making it difficult to determine whether a hypersensitivity to food-paired cues precedes maladaptive eating in humans, or emerges as a result of it. Indeed, poor quality diets have been shown to produce deficits in hippocampal-dependent learning and memory [34–36], promote a shift from goal-directed to habitual responding [37–39], and alter reward liking and craving [6,40–46]. While specific mechanisms are unclear, growing evidence supports a role for mesolimbic dopamine dysfunction [6,47,48], though the hippocampus may also be preferentially vulnerable to the deleterious effects of junk foods [49–51]. As alluded to above, behavioral responses to reward can be dissociated into 'wanting' - attributing motivational salience to reward-related stimuli, and 'liking' – the hedonic pleasure experienced by consuming reward [52], and each has been shown to be impacted by such diets [6,40–46]. A recent study [48] supports a role for both pre-existing individual differences and junk-food-driven changes in reward seeking and liking: rats later identified as susceptible to junk-food-induced obesity show stronger pre-existing conditioned approach behavior than obesity-resistant rats, while junk food exposure, regardless of weight gain, dampened the hedonic impact of palatable foods. Other studies have shown that the *pattern* of consumption may also matter: sugar-binging rats display

addiction-like behaviors not seen in rats with ad libitum sugar access or control rats [43,44]. These data indicate that factors such as *how* the diet is consumed, in addition to individual predisposition to weight gain, must be considered when investigating diet-induced changes in behavior.

Here, we investigated whether a junk food diet could alter reward seeking and liking. Because of the differences between continuous overconsumption and binge eating, we used both ad libitum (24 h) and restricted, intermittent (2 h) daily access to junk food. To probe these behavioral effects, we used the Pavlovian-to-instrumental transfer (PIT) paradigm (a test of cue-evoked incentive motivation, or *wanting*, for food) and microstructural analysis of licking behavior for a palatable solution (a measure of reward *liking*) [53,54]. PIT was employed because of the power of this approach to parse the incentive motivational impact of cues from their conditioned reinforcing effects [55–57]. Since cue-invigorated food-seeking and consumption *when hungry* may be considered adaptive, and we were specifically interested in *maladaptive* food-seeking behavior, i.e., eating in the absence of hunger, our focus was on tests conducted when rats were sated on home chow, although tests were also conducted under the more conventional hungry condition. Our focus on the sated condition was also based on reports that cues invigorate food consumption in humans in the sated state [29,58], and that this may contribute to overeating and obesity [59–61]. We also examined how diet-induced weight gain relates to cue-evoked reward 'wanting' and 'liking' by comparing across high- and low-weight-gainers. We hypothesized that the incentive motivational properties of reward-paired cues in the sated state would increase with junk food exposure, and that, based on the literature cited above, intermittent-fed rats would be particularly vulnerable to this effect.

Methods

Phase	Duration	Procedure
Magazine Training	1 d	Noncontingent reward
Instrumental Training	10 d	Lever-press → Reward
Pavlovian Conditioning	10 d	CS^+ → Reward
Diet Exposure	7, 21, or 42 d	Control, Intermittent or 24 h Ad Libitum exposure
Return to Food Restriction	3 d	2 h chow per day
Instrumental Retraining	3 d	Lever-press → Reward
Pavlovian Re-Conditioning	1 d	A.M.: CS^o → No reward P.M.: CS+ → Reward
Instrumental Extinction	1 d	Press → No reward
PIT & Lick Test 1	1 d	Lever extended with CS^+ and CS^o (both unrewarded)
Instrumental Retraining	3 d	Lever-press → Reward
Pavlovian Re-Conditioning	1 d	A.M.: CS^o → No reward P.M.: CS+ → Reward
Instrumental Extinction	1 d	Press → No reward
PIT & Lick Test 2	1 d	Lever extended with CS^+ and CS^o (both unrewarded)

Table 1. Experimental Timeline

Subjects and apparatus

Adult (10 weeks old) male Sprague-Dawley rats (n = 79) were pair-housed for the duration of the experiment. Rats were food restricted to 85% of their free-feeding body weight during initial behavioral training. All behavioral training and testing took place in sound- and light-attenuating operant chambers (Med Associates, VT) equipped with a retractable lever, a white noise generator, a clicker audio generator, a food cup capable of delivering liquids, and a contact lickometer system capable of recording licking behavior. All experimental procedures were approved by the UCLA Institutional Animal Care and Use Committee and were in accord with the National Research Council Guide for the Care and Use of Laboratory Animals. See **Table 1** for a summary of the training and testing timeline described in detail below.

Initial behavioral training

To maximize detection of a facilitatory effect of junk food exposure on incentive motivation, we trained naïve rats using a sub-threshold PIT paradigm known to support minimal cue-evoked responding under normal home chow diet conditions [62–64]. A 50% sweetened condensed milk

(SCM) solution was used as a reward stimulus during all training phases. After 1 day of magazine training, rats underwent 10 days of instrumental training during which they learned to press a lever to receive a 0.1ml infusion of SCM (delivered over 2 sec). Daily instrumental training lasted for 30 minutes or until 30 reinforcements were earned. Lever pressing was continuously reinforced for the first session, and was then shifted to a variable interval (VI) schedule, which was increased every day beginning with VI-5s on Day 1, then progressing each day to VI-10s on Day 2, VI-15s on Day 3, VI-25s on Day 4, VI-35s on Day 5, and VI-45 s on Days 6-10. Rats were then given 10 days of Pavlovian conditioning with the lever withdrawn, during which time the SCM delivery was paired with the offset of a 30-sec auditory cue (click or white noise; CS^{+}). Daily Pavlovian conditioning lasted for 10 cue presentations (trials) per session, each separated by a variable 2.5 min interval. Analysis of initial behavioral training data is presented in Supplementary Materials (Supplemental Fig. 1).

Junk food diet

Following initial training, rats were assigned to one of three diet groups: Control, Intermittent, or Ad Libitum, and one of three diet durations: 1, 3, or 6 weeks. During this time, all rats received unlimited access to chow and water, while the two treatment groups (Intermittent and Ad Libitum) also received access to two junk foods (one sweet, one savory) each day for either 2 h only (Intermittent) or for 24 h (Ad Libitum). The junk foods differed from day to day and consumption of each food type was measured daily along with body weight. Junk foods included cookies, chocolates, cheese, and hot dogs, among others (see Supplementary Materials for a full list). Animals were assigned to the various food exposure groups in a manner that ensured comparable levels of lever pressing across groups based on the initial behavioral training data.

Consumption patterns during this phase of the study are presented in Supplementary Materials (Supplemental Fig. 3).

Behavioral retraining

On the last day of the diet exposure phase, all food was removed and rats were given access to chow only for two hours a day for the remainder of the experiment. After three days of such food restriction, rats were briefly retrained, beginning with 3 days of instrumental retraining on a VI 45s schedule, then 1 day of Pavlovian conditioning. On the Pavlovian re-conditioning day, rats were trained in two sessions. In the first session, rats were presented with a new auditory sound (click or white noise) *not* previously paired with SCM, which would serve as the control stimulus (CS^o). The CS^o was presented in the same manner as the CS^+ only no SCM was delivered. Approximately 2 h after this session, rats were given a Pavlovian conditioning session identical to that in the initial behavioral training phase (i.e., with the CS^+ and SCM deliveries). The following day, rats underwent 1 day of instrumental extinction, which involved 30 min of access to the lever *without* any SCM infusions, in order to suppress response rates. Analysis of behavioral retraining data is presented in Supplementary Materials (Supplemental Fig. 4).

Pavlovian-to-instrumental transfer testing

In the PIT test, rats were given continuous access to the lever but no rewards were delivered. The CS^+ and CS^o were presented non-contingently (i.e., cue onset and offsets occurred regardless of lever pressing) 4 times each for 30 sec at a time, in ABBA order, separated by 3.5-min intervals. As explained in the Introduction, we were specifically interested in how reward-paired cues might invigorate instrumental reward-seeking *when sated*, but since PIT tests are more

commonly conducted in a hungry state, we ran two PIT tests (one under conventional food-deprived, hungry conditions, and one under sated conditions, order counterbalanced between diets and durations), separated by a day of rest (i.e., no behavioral training) and 5 days of behavioral retraining and extinction as outlined above. Immediately prior to each PIT test, rats were individually housed for 1 h in a new, clean homecage, where all rats had access to water, and rats undergoing their sated test also had ad libitum access to chow.

Licking microstructure

To quantify reward 'liking', immediately after each PIT test, rats were given an opportunity to lick, non-contingently, from a spout in the operant chamber delivering 50% SCM for 5 min in order to assess their licking microstructure. A contact lickometer (Med Associates, VT) was used to measure individual licking responses. The program timer controlling session length did not begin until each rat initiated licking, allowing a full 5 min of access from when the spout was first licked. All licks were recorded and parsed into bouts, defined as any continuous series of licks separated by less than 1 sec [65].

Weight gain and abdominal adipose tissue measurement

To determine the impact of junk food consumption on body composition, body weights were taken daily and abdominal white fat was collected and weighed following euthanasia by isoflurane overdose the day after the final PIT test.

Data analysis

Data were analyzed by ANOVA using SPSS (IBM, Armonk, NY). Effects were defined as statistically significant when $p < 0.05$, and significant interactions were further assessed via multiple pairwise comparisons using a logical extension of Fisher's protected least significant difference procedure for controlling family-wise Type I error rates [66]. Outliers were detected using Extreme Studentized Deviate (criterion $p < 0.01$). All data are expressed as means ± standard error of the mean (SEM). As noted above, and in the Introduction, we were specifically interested in the capacity of cues to alter reward wanting and liking in the *sated* state. Therefore, sated PIT and licking data were analyzed, *a priori*, independently of those from the hungry state tests, and are presented in detail in the Results section. Brief descriptions of the results of the hungry tests are also provided in the Results, but readers are referred to the Supplementary Materials for a full description of the analyses of these hungry tests (see Supplementary Fig. 6 and 7).

Weight gain as a factor in PIT and licking analysis: As recent reports link individual susceptibility to obesity with cue-sensitivity [48] and striatal neuroadaptations [47], we divided rats into low and high 'weight-gainers' (irrespective of diet or duration) using 2-group K-means clustering [48] based on the percentage of weight gained during the experiment (weight gained / start weight). This weight-gain factor was included in PIT and lick analyses.

PIT analysis: Because high baseline responding can obscure the expression of PIT by engaging ceiling effects [67,68], we employed a targeted analysis of pre-CS response rates to ensure homogenous baseline reward-seeking. Two outliers were removed on the basis of their pre-cue

(i.e., baseline) lever presses: one from the Controls, 3-week group, and one from the Ad Libitum, 1-week group. These animals were also excluded from analyses of food cup entries. A univariate ANOVA confirmed that pre-cue, baseline response rates did not differ significantly between diets or durations, nor was there any interaction between these two factors (all F's < 1.00, all $p > 0.05$ ns; mean rate of lever pressing per 30 sec: 0.60 ± 0.07 SEM). Therefore, data are presented as elevation scores from baseline responding wherein the number of lever presses during the 30 sec immediately preceding the cue period (i.e., pre-cue baseline responding) was subtracted from the total number of lever presses during the 30 sec cue period. Cue, diet, duration and weight-gain effects were analyzed using repeated-measures analyses of variance (rmANOVA), with paired- and independent-sample post-hoc t-tests where appropriate. Time in the food cup was analyzed in the same manner.

Lick analysis: Immediately after the PIT test, rats were given a 5-min SCM exposure test, during which all licks were recorded. When drinking palatable solutions, rodents take occasional pauses of varying lengths, resulting in distinct bouts of licking behavior [69]. The average bout length, in particular, is considered to reflect the experienced palatability/hedonic impact of the solution, especially during periods of short access, such that involvement of post-ingestive processes is precluded [65,70]. Thus, in addition to total number of licks, we also assessed the average bout length, where a bout is a series of licks in which each lick is separated by 1 second or less. One statistical outlier with significantly lower total licks (due to equipment malfunction) was removed from this analysis (Intermittent, 6-week group). As for PIT data, cue, diet, duration and weight-gain effects were analyzed using repeated-measures analyses of variance (rmANOVA), with paired- and independent-sample post-hoc t-tests where appropriate.

Results

Weight gain and adipose tissue content

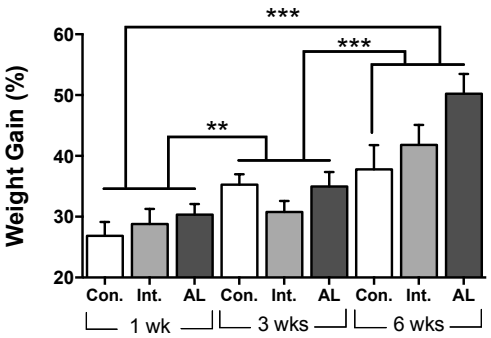

Figure 1. Changes in Body Weight. Increases in body weight, expressed as a percentage of individual starting weight. *Con. = Control group; Int. = Intermittent group; AL = Ad Libitum group.*

Full details of these measures are provided in Supplementary Materials; only features potentially pertinent to interpretation of the results of the main focus of the study, namely effect of diet on cue-induced food seeking and palatability, are described here for clarity. Multivariate ANOVAs showed no difference among the various diet and duration groups in starting weight (mean 299.68 g, ± 1.72 SEM), but significant differences were apparent in weight gained by the day of euthanasia as a percentage of starting weight (**Fig. 1**). As expected, rats exposed to all diets for longer periods prior to euthanasia gained more weight (main effect of duration $F(2,70) = 22.36$, $p < 0.001$). Specifically, 6 week rats gained more weight than 1 week ($t(49) = 5.89$, $p < 0.001$) or 3 week ($t(53) = 4.20$, $p < 0.001$) rats, and 3 week rats gained more weight than 1 week rats ($t(50) = 2.81$, $p < 0.01$). There was also a significant main effect of diet ($F(2,70) = 3.52$, $p < 0.05$), where Ad Libitum rats gained more weight than Controls ($t(49) = 2.05$, $p < 0.05$). There was no diet x duration interaction. Abdominal adipose tissue content, expressed as a percentage of total body weight, was significantly higher in Ad Libitum rats compared with the other two diet groups at 6 weeks (Supplementary Materials Fig. 2B).

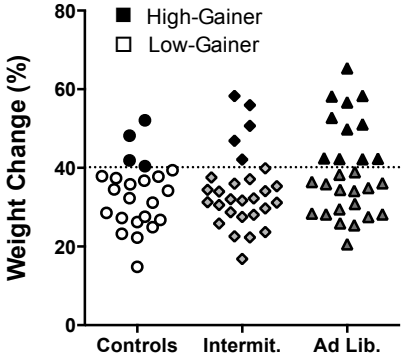

Figure 2. Individual Differences in Weight Gain. Rats were split into low vs. high weight-gainers using 2 group k-means clustering.

Incorporation of weight gain as a factor in PIT and licking analyses: Using 2-group K-means clustering [48] based on the percentage of weight gained during the experiment (weight gained / start weight), we divided rats into low versus high "weight-gainers" (**Fig. 2**). A total of 20 High-Weight-Gainers were identified (Control n = 4, Intermittent n = 5, Ad Libitum n = 11) and 59 Low-Weight-Gainers (Control n = 19, Intermittent = 23, Ad Libitum n = 17). Weight gain status (High vs. Low) was included as a factor in both PIT and licking microstructure analyses.

Pavlovian-to-instrumental (PIT) testing

To determine the impact of junk food exposure on *sated* cue-evoked reward seeking, we sated rats on home chow for 1 h (consumption data presented in Supplementary Materials Fig. 5), then presented the CS^+ and CS^o noncontingently, allowing rats the opportunity to lever press in the absence of any reward deliveries.

An ANOVA of lever-press activity with factors: cue (CS^+ vs. CS^o, repeated measure), diet (Controls vs. Intermittent vs. Ad Libitum), duration (1 vs. 3 vs. 6 weeks), and weight gain (High vs. Low) revealed a significant main effect of cue ($F(1,62) = 6.03$, $p < 0.05$), and a significant cue x diet interaction ($F(2,62) = 3.49$, $p < 0.05$), but no main effect of, or interactions with, duration or weight gain. Further analyses were therefore conducted on data collapsed across duration and weight gain (**Fig. 3A**). One-sample t-tests (versus 0) revealed that Control rats significantly increased their responding during the CS^+ ($t(21) = 5.54$, $p < 0.001$), but not the CS^o (as expected), and paired t-tests confirmed that responding during the CS^+ was significantly higher from that during the CS^o ($t(21) = 4.43$, $p < 0.001$). Intermittent rats significantly increased

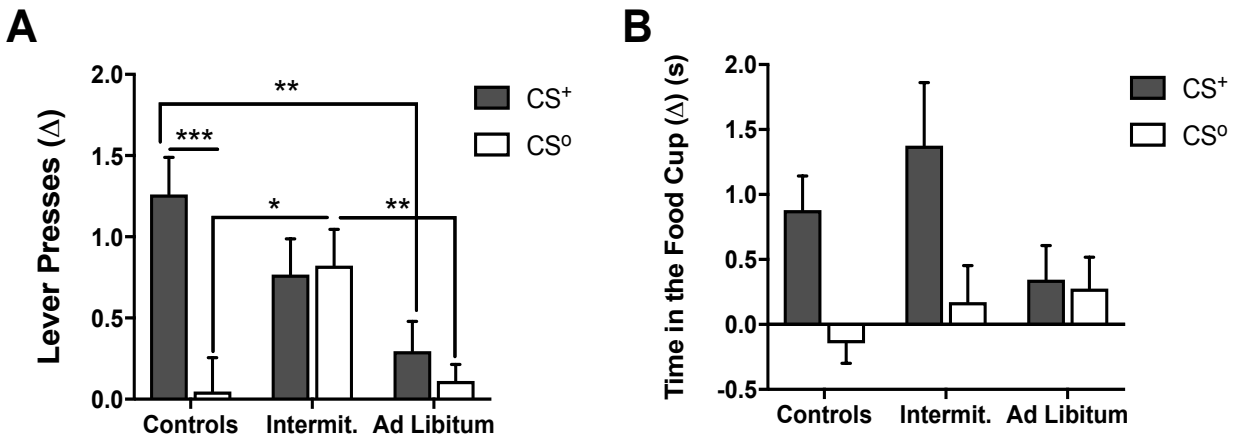

Figure 3. Pavlovian-to-Instrumental Transfer Test. (A) Increase in lever pressing from pre-cue (baseline) responding per 30 sec, averaged across 4 30-s CS presentations. (B) Increase in time (in sec) spent in the food cup from baseline per 30 sec, averaged across 4 30-s CS presentations. *CS^+ = reward-paired cue; CS^o = neutral cue.*

their responding for both the CS^+ ($t(27) = 3.50$, $p < 0.01$) and the CS^o ($t(27) = 3.68$, $p < 0.01$) to a similar degree, and a paired t-test showed no significant difference between the two cues, suggesting a nonspecific food-seeking effect of both cues. In contrast, Ad Libitum rats failed to significantly increase lever pressing in response to either cue and there was no significant difference between the cues. Independent samples t-tests revealed that Control rats increased their lever-pressing in response to the CS^+ more than Ad Libitum rats ($t(47) = 3.35$, $p < 0.01$), but not more than Intermittent rats, while Intermittent rats increased responding to the CS^o more than Controls ($t(48) = 2.48$, $p < 0.05$) and Ad Libitum rats ($t(53) = 2.86$, $p < 0.01$). In summary, intermittent junk food exposure potentiates food seeking even in response to a neutral cue, suggesting a generalization of CS^+-enhanced reward-seeking to less predictive, but otherwise similar, stimuli, while Ad Libitum junk food exposure abolishes cue-invigorated reward-seeking.

An ANOVA conducted on time in the food cup revealed no main effects or interactions. However, in order to permit comparison with lever-press data, food cup entry data were similarly collapsed across duration and weight gain (**Fig. 3B**). While this analysis revealed no significant effects of any factor, trends are apparent. Similar to lever pressing, cues elicited minimal food

cup approach in Ad Libitum rats. In contrast to lever pressing, however, Intermittent (and Control) rats appeared to selectively increase their time at the food cup in response to the CS^{+} versus the CS^{o}. This suggests that Intermittent rats are not impaired in their ability to discriminate the cues.

An identical analysis of lever pressing during the hungry test failed to reveal a statistically significant effect of cue, diet or diet duration, although trends similar to the statistically significant results of the sated test are apparent i.e. greater cue differentiation in controls than in the Intermittent and Ad Libitum groups and lower general responses to cues in the Ad Libitum group (Supplementary Materials Fig. 6A). There was a significant main effect of cue on cue-invigorated food cup entries, with more time spent in the food cup during the CS^{+} versus the CS^{o} across all groups, but again, no significant effects of diet or diet duration (Supplementary Materials Fig. 6B). Full statistical analyses are presented in Supplementary Materials.

Lick analysis

Immediately after the PIT test, rats were given a 5-min SCM exposure test, during which all licks were recorded. We conducted a multivariate (total licks and bout length) ANOVA with the factors diet (Controls vs. Intermittent vs. Ad Libitum), duration (1 vs. 3 vs. 6 weeks), and weight gain (High vs. Low).

Total Licks: This analysis revealed a significant effect of diet ($F(2,63) = 10.61$, $p < 0.001$), a significant effect of weight gain ($F(1,63) = 4.49$, $p < 0.05$), and a significant diet x weight gain interaction ($F(2,63) = 5.05$, $p < 0.01$), but no effect of, or interaction with, duration. Data,

collapsed across duration, are shown in **Fig. 4A**. Follow-up comparisons revealed that High-Weight Gainer licked more than Low-Weight Gainers within Controls ($t(20) = 2.79$, $p < 0.05$) and Intermittent ($t(26) = 3.42$, $p < 0.01$) groups, an effect that was noticeably absent in the Ad Libitum rats. Intermittent rats licked more than Controls whether they were Low-Weight Gainers ($t(39) = 2.35$, $p < 0.05$), or High-Weight Gainers ($t(13) = 2.18$, $p < 0.05$). High-weight gaining Intermittent rats also licked more than high-weight gaining Ad Libitum rats ($t(14) = 3.81$, $p < 0.01$). Post-hoc comparisons on the simple main effects revealed that Intermittent rats licked more than Controls ($t(48) = 2.98$, $p < 0.01$) and Ad Libitum rats ($t(53) = 3.21$, $p < 0.01$), while High Weight Gainers licked more than Low Weight Gainers ($t(76) = 1.75$, $p < 0.05$).

Bout Length: Bout length showed a similar pattern to total licks across groups. There was a significant effect of diet ($F(2,63) = 20.79$, $p < 0.01$), a significant effect of weight gain ($F(1,63) = 15.31$, $p < 0.01$) and a significant diet x weight gain interaction ($F(2,63) = 18.02$, $p < 0.01$). Again, there was no main effect of, or interaction with, duration, and data collapsed across this variable are presented in **Figure 4B**. Follow up comparisons revealed that bout length was longer in High Weight Gainers than Low Weight Gainers in both Control ($t(21) = 2.41$, $p < 0.05$) and

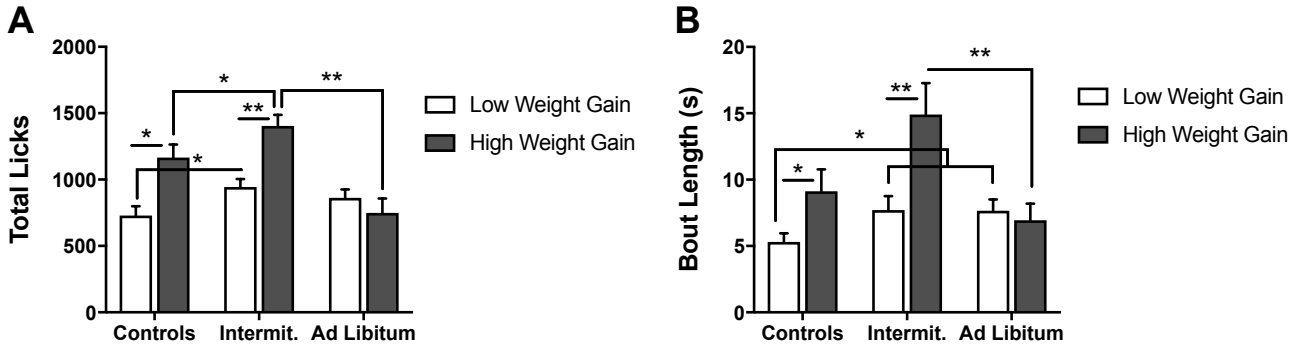

Figure 4. Licking Microstructure Analysis. (A) Total number of licks and (B) bout length (in seconds) during a 5-min sated lick test for sweetened condensed milk immediately after the PIT test.

Intermittent rats ($t(25) = 3.69$, $p < 0.01$), an effect that was noticeably absent in the Ad Libitum group. Among Low Weight Gainers, Ad Libitum ($t(33) = 2.21$, $p < 0.05$) and Intermittent ($t(33) = 2.16$, $p = 0.04$) rats exhibited longer bouts than Controls. Among High Weight Gainers, Intermittent rats exhibited longer bouts than Ad Libitum rats ($t(14) = 3.57$, $p < 0.01$). Post-hoc comparisons on the simple main effects revealed that Intermittent rats had longer bouts than Controls ($t(48) = 2.52$, $p < 0.05$), while High Weight Gainers had longer bouts than Low Weight Gainers ($t(76) = 2.49$, $p < 0.05$).

An identical analysis of licking microstructure during the hungry test failed to reveal any group differences in total licks (Supplementary Fig. 7A). There was, however, a significant main effect of diet on bout length, but follow-up tests narrowly ($P = 0.07$) failed to support evidence of longer bouts in Intermediate rats relative to controls (Supplementary Fig. 7B). Full statistical analyses are presented in Supplementary Materials.

Discussion

We probed how a junk food diet influences cue-evoked reward seeking and reward palatability, using the PIT test and licking microstructure analysis, respectively. Our focus was on the results of tests conducted under sated conditions because of their relevance to maladaptive food-seeking behavior i.e. over-eating. We found that junk food consumption resulted in the emergence of different patterns of behavior under sated conditions depending on the schedule of junk food exposure (intermittent versus ad libitum access). We also demonstrated that the hedonic impact of the SCM reward differs depending on both the schedule of diet exposure and weight gain. Contrary to our initial hypothesis, animals provided ad libitum access to junk food were

insensitive to the instrumental invigorating effect of SCM-paired cues observed in their chow-fed counterparts (**Fig. 3A**). Moreover, their Pavlovian conditioned approach to the food cup also appeared to be suppressed under sated conditions (**Fig. 3B**). This was apparent despite the fact that the hedonic impact of the reward was similar to chow-fed animals on the basis of lick-bout length measure (**Fig. 4B**). Animals with restricted daily access to junk food, on the other hand, pressed the lever more vigorously over baseline in response to the reward-paired cue, as predicted, but contrary to our initial hypothesis, this was also the case in the presence of the neutral cue, suggesting a generalization of the excitatory effects of the CS^+ to other, similar stimuli (**Fig. 3A**) despite a trend towards a reward-paired-cue-specific food cup approach response (**Fig. 3B**). Interestingly, palatability responses were highest of all among high-weight-gaining intermittent access rats (**Fig. 4**). While some similar trends were apparent under hungry conditions these generally failed to attain statistical significance, perhaps reflecting increased variability in responses associated with the heightened behavioral state.

Ad libitum junk food exposure decreases responsiveness to reward-paired cues under sated conditions

Rats provided ad libitum access to varied, highly palatable foods, in addition to regular chow, were generally not susceptible to the instrumental invigorating effects of reward-paired cues seen in their chow-fed counterparts, when sated (**Fig. 3A)**. Notably, this was not explained by employment of the alternative strategy of checking the food cup **(Fig. 3B),** and is consistent with previous studies reporting deficits in reward processing in rodents chronically exposed to poor quality and junk food diets, as indicated by increased brain self-stimulation thresholds [6], decreased conditioned place preference for amphetamine [71], decreased ethanol consumption

[40], and decreased motivation for reward on a progressive ratio task [72]. Interestingly, decreased motivation for food on progressive ratio [46,72] or incentive runway [73] tasks is seen in several conditions associated with poor quality diets and obesity, such as after junk food exposure, with [46,72] or without [73] weight gain, and even in obesity-prone rats in the absence of obesity or junk food exposure [73]. The lack of a statistically significant main effect of weight gain on cue-induced food seeking, or an interaction of weight gain with diet on this measure in our study argues against a conclusion that the dietary effects we observed on cue-induced lever-pressing were secondary to metabolic effects of weight gain alone, but rather supports a more direct effect of diet on the motivational influence of cues. However, since the ad libitum junk food-fed rats gained more body fat (statistically significant following 6 weeks of exposure) than control or intermittent junk food-fed rats, secondary metabolic effects remain a possible cause of the apparent motivational deficit in the Ad Libitum group.

We found no evidence that ad libitum junk food-fed rats found SCM significantly less palatable than their chow-fed counterparts (**Fig. 4)**. Indeed, low weight-gainers in this group 'liked' SCM *more* than their chow-fed counterparts. It is noteworthy, however, that high weight-gaining rats in this group trended towards lower palatability responses than their high weight-gaining chow-fed counterparts (**Fig. 4)**. Further, unlike chow-fed and intermittent junk food-fed animals, high weight-gaining rats among those exposed to an ad libitum junk food diet did not show evidence of elevated palatability responses relative to low-weight gainers fed the same diet **(Fig. 4)**. Thus, while this diet tended to produce the highest weight gains **(Fig. 1),** this is likely not due to increased palatability of sweet/fatty food or to increased cue-precipitated incentive motivation. Collectively, these observations may be considered somewhat in agreement with studies

elsewhere suggesting that increases in palatability may not explain the development of obesity: that is, obese humans experience reduced sweetness [74], and obese [73] and obesity-prone [45] rats "like" low concentrations of sucrose and fat less than lean or obesity-resistant rats, respectively, an effect that can be normalized with weight loss (but not with acute food deprivation) [41].

Growing evidence points to disruption of the dopamine system as a likely neuroadaptation mediating the reduction in incentive motivation observed in our ad libitum junk food-fed rats: chronic consumption of poor quality, junk food diets produces lower basal and evoked DA in the rat NAc [71,75], and downregulated D2 receptors (D2R) [6,76], similar to the decreased D2 receptors reported in pathologically obese humans [77–79]. Diet-induced downregulation of the mesolimbic dopamine system may function as a satiety-signal by reducing the motivational impact of food-paired cues when sufficient food has already been consumed: striatal dopamine signaling is required to maintain feeding behavior [80] and to attribute salience to environmental cues associated with reward [81]. Conversely, it has also been reported that diet-induced D2R downregulation is also associated with compulsive-like feeding and increased reward seeking [6], possibly in an attempt to restore homeostasis to an underactive reward system, similar to the allostatic model of drug addiction [82].

Interestingly, previous work has shown that cafeteria diet-induced obese rats displayed no increase in extracellular DA in response to standard lab chow (in contrast to controls), and only showed such increases in response to a cafeteria-diet "challenge" [75]. Such a finding may be pertinent to why our Ad Libitum group displayed little instrumental responding for a 50% SCM

solution – a food reward that supported cue-evoked food-seeking in the other diet groups, but that may hold little value for rats accustomed to a richer, more varied diet. The decreased incentive motivation seen in ad libitum-exposed rats may also reflect the emergence of a depression-like phenotype, as obesity is associated with increased risk of mood disorders, including depression [83], which is partly characterized by decreased interest or pleasure and changes in appetite [84]. The mesolimbic dopamine system has been implicated in the etiology of mood disorders [85], in addition to mediating appetitive behaviors such as food liking, craving, and seeking [80,86]. A high-fat diet can induce a depression-like phenotype in mice, indicated by increased behavioral despair [87]. Although the latter study employed a longer period of exposure (12 weeks) than used here, poor quality diets (i.e., high sucrose, high fat, junk foods, etc.) have been shown to effect changes in behavior within the timeframe of our study [37,88–91], including anxiety [92], which is highly comorbid with depression [93]. The brief withdrawal from the junk-food diet used in our experimental design may also have contributed to the expression of such a phenotype.

Intermittent junk food access produces indiscriminate cue responsivity and increased reward palatability under sated conditions

Emerging evidence suggests a strong role for the pattern of diet consumption (i.e., binge eating versus constant "grazing") in the susceptibility to maladaptive eating, where restricted and binge eating are associated with addiction-like behaviors [43,44]. Like the relationship between drug-paired cues and drug relapse, food-paired cues can potentiate non-homeostatic eating in intermittent-fed rats [26]. Here, we modeled restricted eating by using intermittent (2h/day) junk food exposure. We found that, unlike their ad libitum junk food access counterparts, these rats

displayed significantly increased lever pressing in response to reward-paired cues, when sated. However, this invigoration was no greater than that observed in chow-fed animals. Rather, the distinguishing characteristic of the Intermittent group was their equal lever invigoration response to a neutral cue. While they did not discriminate between the two cues in terms of their lever pressing, there was a noticeable trend towards such a discrimination with respect to food cup entries during cue presentation, suggesting an intact ability to discern the two cues. It also suggests that they remained susceptible to the conditioned effects of the reward-paired cue even if this does not induce them to expend significant effort in an attempt to procure the reward. Their indiscriminate lever pressing suggests an increased susceptibility to the excitatory effects of environmental cues when sated, including generalizing to those that are similar to, but distinct from, those previously paired with reward.

As alluded to above, sensitization of mesolimbic dopamine transmission is strongly implicated in the invigoration of reward seeking precipitated by reward-paired cues [64,81,94]. Intermittent sucrose access has been shown to repeatedly release DA in the NAc shell [95,96], alter the expression [97] and availability [76] of DA receptors, and facilitate locomotor sensitization to a DA agonist [44,98,99] suggesting that such dietary interventions may impact the dopaminergic systems involved in learning about and responding to reward-paired cues [100–103]. Interestingly, DA neurons will fire in response to familiar stimuli non-predictive of reward, but to a lesser degree than firing in response to cues predicting reward, suggesting DA neurons may support stimulus generalization [104,105]. This is notable because our intermittent-fed rats appeared to overgeneralize the excitatory response-invigorating effects of the CS^{+} to the seemingly neutral CS^{o} stimulus. Although the CS^{o} was never directly paired with food reward, it

may have acquired (or been attributed) latent motivational properties due to it perceptual similarity to the CS^{+}, or through its second-order relationship with reward, in that it was presented in a context strongly associated with food reward. Regardless, it is not uncommon for "neutral" or ambiguous cues to acquire incentive motivational properties. For instance, previous studies have shown that cues that are presented in a random fashion with respect to food reward can still acquire the ability to stimulate food-seeking behavior [106]. Similarly, cues that signal the cancelation of food access acquire the ability to potentiate feeding [107], even though such a relationship might be expected to support inhibitory rather than excitatory learning. Although the CS^{o} stimulus used in the current experiment did not elicit an overt motivational influence over reward seeking in the control (chow) condition, intermittent junk food exposure appeared to instigate or uncover this underlying motivational influence, either through over-attribution of incentive salience to the CS^{o}, or through a nonspecific reduction in the motivational threshold for the elicitation of reward-seeking behavior. Interestingly, it has been shown that intermittent exposure to cocaine [63,81] or amphetamine [64] can also potentiate cue-triggered food-seeking behavior. Further research will be needed to determine how such effects relate to the motivational effects of junk food exposure, including whether they depend on a common set of neuroadaptations. Indeed, this hypergeneralization and hypersensitivity to reward-paired contexts and cues is a hallmark of both drug addiction and binge eating disorder [10,108], and growing evidence suggests remarkable parallels between drug addiction and food binging [10]. For instance, rats provided intermittent access to a sweet solution (thus enabling food binging) show similarities to rodents in drug-abuse paradigms, exhibiting escalating intake [76], increased motivation to obtain sucrose [109], naloxone- and food-deprivation-induced signs of withdrawal

[110], and accelerated development of habitual behavior [37]. Notably, sugar-bingeing rats show cross-sensitization with amphetamine, while rats with ad libitum sugar access do not [44].

The intermittent-fed rats' indiscriminant lever pressing may also be due to decreased response inhibition or increased impulsivity, both of which are strongly associated with binge eating [111–113] and addiction [114–116] disorders. Recent reports indicate that rodents exhibit increased impulsivity after high-fat, high-sugar, and palatable diets [117], an effect that can be passed on to offspring as a result of an "unfavorable intrauterine nutritional environment" [118]. While trait impulsivity has been thought to play a *causative* role in these disorders [114,119], drug use is thought to also exacerbate impulsivity and disrupt response inhibition [120], creating a vicious cycle of impulsive drug seeking [116]. Given the behavioral and neurochemical similarities between drug addiction and binge eating disorder [121], it is possible that an impulsive phenotype may be both a product of intermittent palatable feeding, and a driving factor in humans with binge eating disorder.

The second notable characteristic of the intermittent junk food access rats is the elevated palatability measure (lick bout length) (**Fig. 4**). In particular, rats that gained the most weight on the intermittent diet access appeared to 'like' the SCM more than respective control-fed or ad libitum-fed rats. (While the difference between High Weight Gainer Controls and High Weight Gainer Intermittent rats failed to reach significance with this measure (bouts: $p = 0.096$), the total licks comparison was significant.) Our results are consistent with previously reported evidence of increased reward 'liking' after 5 weeks of palatable-food binging [122]. Limited-access diets are known to potentiate not only dopamine [95,96] but also opioid activity [76], neurochemical

systems known to positively regulate motivation and reward-learning [101], and palatability/hedonia [86], respectively. Given overwhelming evidence that palatable food consumption can be induced and abolished by opioid agonists and antagonists, respectively [65,123,124], it is possible that intermittent junk food access upregulates opioid systems, potentiating reward 'liking'. In fact, this effect is consistent with reports that binge eating in humans is associated with a "gain-of-function" mutation in the mu-opioid receptor gene, which is also associated with increased self-reported food liking [125].

Summary

Access to a junk food diet produced profound alterations in cue-induced food seeking and food "liking" under sated conditions that varied with the pattern of access provided to the junk food. Rats provided ad libitum access were generally unresponsive to reward-paired cues when sated despite apparently 'liking' the SCM to a similar degree or, in the case of low weight-gainers, significantly *more* than chow-fed animals. The deficit in these animals was therefore primarily motivational rather than hedonic. Unsurprisingly, these animals tended to gain more weight than the other groups but neither motivational nor palatability differences could account for within-group variability in weight gain. On the other hand, restricted junk food access induced development of a *cue generalization* phenotype in sated animals. In these animals, as in ad libitum chow-fed controls, within-group differences in weight gain could potentially be accounted for by the degree to which the SCM reward was "liked" upon consumption. The data underline the importance of the *pattern* of consumption as a factor impacting diet-behavior interactions and are particularly interesting in the context of research highlighting different subtypes of overeating and obesity. For some individuals, overeating is a steady, perhaps

habitual action characterized by frequent snacking, large portion sizes, and poor quality foods [126]. For others, it can be compulsive and *driven*, characterized by food binges and marked distress about overeating, as in the case of binge eating disorder [127]. Our data suggest that such intermittent junk food "binges" may cause cues that are only loosely associated with eating to take on motivational significance when sated, and may also increase the hedonic impact of palatable food, which may be of particular relevance to binge eating.

References

1. Rolls, B. J.; Rolls, E. T.; Rowe, E. A.; Sweeney, K. Sensory specific satiety in man. *Physiol. Behav.* **1981**, *27*, 137–142.

2. Hill, J. O.; Peters, J. C.; Flegal, K. M.; Carrol, M. D.; Kuczmaraki, R. J.; Johnson, C. L.; Foreyt, J. P.; Goodrick, G. K.; Troiano, R. P.; Flegal, K. M.; Lean, M. E. J.; James, W. P. T.; Sclafani, A.; Salmon, D. M.; Flatt, J. P.; Rolls, B. J.; Hammer, V. A.; Westerterp-Plantenga, M. S.; Stubbs, R. J.; Harbron, C. G.; Murgatroyd, P. R.; Prentice, A. M.; Bell, E. A.; Ernst, N. D.; Birch, L. L.; Johnson, S. L.; Andersen, G.; Peters, J. C.; Schulte, M. C.; Klem, M. L.; Wing, R. R.; McGuire, M. T.; Seagle, H. M.; Hill, J. O. Environmental contributions to the obesity epidemic. *Science* **1998**, *280*, 1371–4.

3. Fryar, C. D.; Carroll, M. D.; Ogden, C. L. *Prevalence of overweight, obesity, and extreme obesity among adults: United States, Trends 1960–1962 through 2007–2008*; 2014.

4. Ogden, C. L.; Carroll, M. D.; Kit, B. K.; Flegal, K. M. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA* **2014**, *311*, 806–14.

5. Heymsfield, S. B.; Harp, J. B.; Reitman, M. L.; Beetsch, J. W.; Schoeller, D. A.; Erondu, N.; Pietrobelli, A. Why do obese patients not lose more weight when treated with low-calorie diets? A mechanistic perspective. *Am. J. Clin. Nutr.* **2007**, *85*, 346–54.

6. Johnson, P. M.; Kenny, P. J. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* **2010**, *13*, 635–41.

7. Avena, N. M.; Rada, P.; Hoebel, B. G. Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci. Biobehav. Rev.* **2008**, *32*, 20–39.

8. Gearhardt, A. N.; Yokum, S.; Orr, P. T.; Stice, E.; Corbin, W. R.; Brownell, K. D. Neural correlates of food addiction. *Arch. Gen. Psychiatry* **2011**, *68*, 808–16.

9. Gearhardt, A. N.; Corbin, W. R.; Brownell, K. D. Food addiction: an examination of the diagnostic criteria for dependence. *J. Addict. Med.* **2009**, *3*, 1–7.

10. Davis, C.; Carter, J. C. Compulsive overeating as an addiction disorder. A review of theory and evidence. *Appetite* **2009**, *53*, 1–8.

11. Colles, S. L.; Dixon, J. B.; O'Brien, P. E. Loss of control is central to psychological disturbance associated with binge eating disorder. *Obesity (Silver Spring).* **2008**, *16*, 608–14.

12. Heatherton, T. F.; Baumeister, R. F. Binge eating as escape from self-awareness. *Psychol. Bull.* **1991**, *110*, 86–108.

13. Childress, A. R.; Mozley, P. D.; McElgin, W.; Fitzgerald, J.; Reivich, M.; O'Brien, C. P. Limbic Activation During Cue-Induced Cocaine Craving. *Am. J. Psychiatry* **1999**, *156*, 11–18.

14. Ehrman, R. N.; Robbins, S. J.; Childress, A. R.; O'Brien, C. P. Conditioned responses to cocaine-related stimuli in cocaine abuse patients. *Psychopharmacology (Berl).* **1992**, *107*, 523–

529.

15. Stewart, J.; de Wit, H.; Eikelboom, R. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychol. Rev.* **1984**, *91*, 251–258.

16. Wallace, B. Psychological and environmental determinants of relapse in crack cocaine smokers. *J. Subst. Abuse Treat.* **1989**, *6*, 95–106.

17. Volkow, N. D.; Wang, G.-J.; Baler, R. D. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn. Sci.* **2011**, *15*, 37–46.

18. Chaudhri, N.; Sahuque, L. L.; Schairer, W. W.; Janak, P. H. Separable roles of the nucleus accumbens core and shell in context- and cue-induced alcohol-seeking. *Neuropsychopharmacology* **2010**, *35*, 783–91.

19. Liu, X.; Caggiula, A. R.; Yee, S. K.; Nobuta, H.; Sved, A. F.; Pechnick, R. N.; Poland, R. E. Mecamylamine attenuates cue-induced reinstatement of nicotine-seeking behavior in rats. *Neuropsychopharmacology* **2007**, *32*, 710–8.

20. LeBlanc, K. H.; Ostlund, S. B.; Maidment, N. T. Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behav. Neurosci.* **2012**, *126*, 681–9.

21. Doherty, J.; Ogbomnwan, Y.; Williams, B.; Frantz, K. Age-dependent morphine intake and cue-induced reinstatement, but not escalation in intake, by adolescent and adult male rats. *Pharmacol. Biochem. Behav.* **2009**, *92*, 164–172.

22. Robinson, T.; Berridge, K. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.* **1993**, *18*, 247–291.

23. Robinson, T. E.; Berridge, K. C. The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction* **2000**, *95 Suppl 2*, S91-117.

24. Jansen, A. A learning model of binge eating: Cue reactivity and cue exposure. *Behav. Res. Ther.* **1998**, *36*, 257–272.

25. Weingarten, H. P. Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science* **1983**, *220*, 431–3.

26. Boggiano, M. M.; Dorsey, J. R.; Thomas, J. M.; Murdaugh, D. L. The Pavlovian power of palatable food: lessons for weight-loss adherence from a new rodent model of cue-induced overeating. *Int. J. Obes. (Lond).* **2009**, *33*, 693–701.

27. Petrovich, G. D.; Ross, C. A.; Gallagher, M.; Holland, P. C. Learned contextual cue potentiates eating in rats. *Physiol. Behav.* **2007**, *90*, 362–7.

28. Jansen, A.; Havermans, R. C.; Nederkoorn, C. Cued Overeating. In *Handbook of Behavior, Food and Nutrition*; Preedy, V. R.; Watson, R. R.; Martin, C. R., Eds.; Springer New York: New York, NY, 2011; pp. 1431–1443.

29. Cornell, C. E.; Rodin, J.; Weingarten, H. Stimulus-induced eating when satiated. *Physiol. Behav.* **1989**, *45*, 695–704.

30. Jansen, A.; Theunissen, N.; Slechten, K.; Nederkoorn, C.; Boon, B.; Mulkens, S.; Roefs, A. Overweight children overeat after exposure to food cues. *Eat. Behav.* **2003**, *4*, 197–209.

31. Fedoroff, I. C.; Polivy, J.; Herman, C. P. The Effect of Pre-exposure to Food Cues on the Eating Behavior of Restrained and Unrestrained Eaters. *Appetite* **1997**, *28*, 33–47.

32. Jansen, A.; van den Hout, M. On being led into temptation: "Counterregulation" of dieters after smelling a "preload." *Addict. Behav.* **1991**, *16*, 247–253.

33. Wardle, J. Conditioning processes and cue exposure in the modification of excessive eating. *Addict. Behav.* **1990**, *15*, 387–393.

34. Jurdak, N.; Kanarek, R. B. Sucrose-induced obesity impairs novel object recognition learning in young rats. *Physiol. Behav.* **2009**, *96*, 1–5.

35. Kanoski, S. E.; Davidson, T. L. Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. *Physiol. Behav.* **2011**, *103*, 59–68.

36. Beilharz, J. E.; Maniam, J.; Morris, M. J. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain. Behav. Immun.* **2014**, *37*, 134–41.

37. Furlong, T. M.; Jayaweera, H. K.; Balleine, B. W.; Corbit, L. H. Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *J. Neurosci.* **2014**, *34*, 5012–22.

38. Ahn, S.; Phillips, A. G. Repeated cycles of restricted food intake and binge feeding disrupt sensory-specific satiety in the rat. *Behav. Brain Res.* **2012**, *231*, 279–285.

39. Tantot, F.; Parkes, S. L.; Marchand, A. R.; Boitard, C.; Naneix, F.; Layé, S.; Trifilieff, P.; Coutureau, E.; Ferreira, G. The effect of high-fat diet consumption on appetitive instrumental behavior in rats. *Appetite* **2017**, *108*, 203–211.

40. Vendruscolo, L. F.; Gueye, A. B.; Vendruscolo, J. C. M.; Clemens, K. J.; Mormède, P.; Darnaudéry, M.; Cador, M. Reduced alcohol drinking in adult rats exposed to sucrose during adolescence. *Neuropharmacology* **2010**, *59*, 388–394.

41. Shin, A. C.; Townsend, R. L.; Patterson, L. M.; Berthoud, H.-R. "Liking" and "wanting" of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R1267-80.

42. Lesser, E. N.; Arroyo-Ramirez, A.; Mi, S. J.; Robinson, M. J. F. The impact of a junk-food diet during development on "wanting" and "liking." *Behav. Brain Res.* **2017**, *317*, 163–178.

43. Buda-Levin, A.; Wojnicki, F. H. E. E.; Corwin, R. L. Baclofen reduces fat intake under binge-type conditions. *Physiol. Behav.* **2005**, *86*, 176–84.

44. Avena, N. M.; Hoebel, B. G. A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience* **2003**, *122*, 17–20.

45. Duca, F. A.; Swartz, T. D.; Covasa, M. Effect of diet on preference and intake of sucrose in

obese prone and resistant rats. *PLoS One* **2014**, *9*, e111232.

46. Vendruscolo, L. F.; Gueye, A. B.; Darnaudéry, M.; Ahmed, S. H.; Cador, M. Sugar overconsumption during adolescence selectively alters motivation and reward function in adult rats. *PLoS One* **2010**, *5*, e9296.

47. Vollbrecht, P. J.; Mabrouk, O. S.; Nelson, A. D.; Kennedy, R. T.; Ferrario, C. R. Pre-existing differences and diet-induced alterations in striatal dopamine systems of obesity-prone rats. *Obesity (Silver Spring)*. **2016**, *24*, 670–677.

48. Robinson, M. J.; Burghardt, P. R.; Patterson, C. M.; Nobile, C. W.; Akil, H.; Watson, S. J.; Berridge, K. C.; Ferrario, C. R. Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology* **2015**.

49. Lindqvist, A.; Mohapel, P.; Bouter, B.; Frielingsdorf, H.; Pizzo, D.; Brundin, P.; Erlanson-Albertsson, C. High-fat diet impairs hippocampal neurogenesis in male rats. *Eur. J. Neurol.* **2006**, *13*, 1385–1388.

50. Martire, S. I.; Maniam, J.; South, T.; Holmes, N.; Westbrook, R. F.; Morris, M. J. Extended exposure to a palatable cafeteria diet alters gene expression in brain regions implicated in reward, and withdrawal from this diet alters gene expression in brain regions associated with stress. *Behav. Brain Res.* **2014**, *265*, 132–41.

51. Molteni, R.; Barnard, R. J.; Ying, Z.; Roberts, C. K.; Gómez-Pinilla, F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* **2002**, *112*, 803–14.

52. Berridge, K. C.; Robinson, T. E.; Aldridge, J. W. Dissecting components of reward: “liking”, “wanting”, and learning. *Curr. Opin. Pharmacol.* **2009**, *9*, 65–73.

53. Davis, J. D.; Smith, G. P. Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. *Behav. Neurosci.* **1992**, *106*, 217–228.

54. Hsiao, S.; Fan, R. J. Additivity of taste-specific effects of sucrose and quinine: microstructural analysis of ingestive behavior in rats. *Behav. Neurosci.* **1993**, *107*, 317–26.

55. Ostlund, S. B.; Balleine, B. W. On habits and addiction: An associative analysis of compulsive drug seeking. *Drug Discov. Today Dis. Model.* **2008**, *5*, 235–245.

56. Dickinson, A.; Balleine, B. Motivational control of goal-directed action. *Anim. Learn. Behav.* **1994**, *22*, 1–18.

57. Berridge, K. C.; Robinson, T. E. Parsing reward. *Trends Neurosci.* **2003**, *26*, 507–513.

58. Birch, L. L.; McPhee, L.; Sullivan, S.; Johnson, S. Conditioned meal initiation in young children. *Appetite* **1989**, *13*, 105–13.

59. Lowe, M. R.; Butryn, M. L. Hedonic hunger: A new dimension of appetite? *Physiol. Behav.* **2007**, *91*, 432–439.

60. Yeomans, M. R.; Blundell, J. E.; Leshem, M. Palatability: response to nutritional need or

need-free stimulation of appetite? *Br. J. Nutr.* **2004**, *92 Suppl 1*, S3-14.

61. Hetherington, M. M. Cues to overeat: psychological factors influencing overconsumption. *Proc. Nutr. Soc.* **2007**, *66*, 113–123.

62. Ostlund, S. B.; Kosheleff, A. R.; Maidment, N. T. Differential Effects of Systemic Cholinergic Receptor Blockade on Pavlovian Incentive Motivation and Goal-Directed Action Selection. *Neuropsychopharmacology* **2013**, *39*, 1490–1497.

63. LeBlanc, K. H.; Maidment, N. T.; Ostlund, S. B. Repeated cocaine exposure facilitates the expression of incentive motivation and induces habitual control in rats. *PLoS One* **2013**, *8*, e61355.

64. Wyvell, C. L.; Berridge, K. C. Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. *J. Neurosci.* **2001**, *21*, 7831–40.

65. Ostlund, S. B.; Kosheleff, A. R.; Maidment, N. T.; Murphy, N. P. Decreased consumption of sweet fluids in mu opioid receptor knockout mice: a microstructural analysis of licking behavior. *Psychopharmacology (Berl).* **2013**, *229*, 105–113.

66. Levin, J. R.; Serlin, R. C.; Seaman, M. A. A controlled, powerful multiple-comparison strategy for several situations. *Psychol. Bull.* **1994**, *115*, 153–159.

67. Lovibond, P. F. Appetitive pavlovian-instrumental interactions: Effects of inter-stimulus interval and baseline reinforcement conditions. *Q. J. Exp. Psychol. Sect. B* **1981**, *33*, 257–269.

68. Campese, V.; McCue, M.; Lázaro-Muñoz, G.; Ledoux, J. E.; Cain, C. K. Development of an aversive Pavlovian-to-instrumental transfer task in rat. *Front. Behav. Neurosci.* **2013**, *7*, 176.

69. Davis, J. D. The Microstructure of Ingestive Behavior. *Ann. N. Y. Acad. Sci.* **1989**, *575*, 106–121.

70. Higgs, S.; Cooper, S. J. Effects of benzodiazepine receptor ligands on the ingestion of sucrose, intralipid, and maltodextrin: an investigation using a microstructural analysis of licking behavior in a brief contact test. *Behav. Neurosci.* **1998**, *112*, 447–57.

71. Davis, J. F.; Tracy, A. L.; Schurdak, J. D.; Tschöp, M. H.; Lipton, J. W.; Clegg, D. J.; Benoit, S. C. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. *Behav. Neurosci.* **2008**, *122*, 1257–63.

72. Blaisdell, A. P.; Lau, Y. L. M.; Telminova, E.; Lim, H. C.; Fan, B.; Fast, C. D.; Garlick, D.; Pendergrass, D. C. Food quality and motivation: a refined low-fat diet induces obesity and impairs performance on a progressive ratio schedule of instrumental lever pressing in rats. *Physiol. Behav.* **2014**, *128*, 220–5.

73. Shin, A. C.; Zheng, H.; Pistell, P. J.; Berthoud, H.-R. Roux-en-Y gastric bypass surgery changes food reward in rats. *Int. J. Obes. (Lond).* **2011**, *35*, 642–51.

74. Bartoshuk, L. M.; Duffy, V. B.; Hayes, J. E.; Moskowitz, H. R.; Snyder, D. J. Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives. *Philos. Trans.*

R. Soc. Lond. B. Biol. Sci. **2006**, *361*, 1137–48.

75. Geiger, B. M.; Haburcak, M.; Avena, N. M.; Moyer, M. C.; Hoebel, B. G.; Pothos, E. N. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience* **2009**, *159*, 1193–9.

76. Colantuoni, C.; Schwenker, J.; McCarthy, J.; Rada, P.; Ladenheim, B.; Cadet, J. L.; Schwartz, G. J.; Moran, T. H.; Hoebel, B. G. Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport* **2001**, *12*, 3549–52.

77. de Weijer, B. A.; van de Giessen, E.; van Amelsvoort, T. A.; Boot, E.; Braak, B.; Janssen, I. M.; van de Laar, A.; Fliers, E.; Serlie, M. J.; Booij, J. Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Res.* **2011**, *1*, 37.

78. Volkow, N. D.; Wang, G.-J.; Telang, F.; Fowler, J. S.; Thanos, P. K.; Logan, J.; Alexoff, D.; Ding, Y.-S.; Wong, C.; Ma, Y.; Pradhan, K. Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. *Neuroimage* **2008**, *42*, 1537–43.

79. Wang, G.-J.; Volkow, N. D.; Logan, J.; Pappas, N. R.; Wong, C. T.; Zhu, W.; Netusll, N.; Fowler, J. S. Brain dopamine and obesity. *Lancet* **2001**, *357*, 354–357.

80. Salamone, J. D.; Mahan, K.; Rogers, S. Ventrolateral striatal dopamine depletions impair feeding and food handling in rats. *Pharmacol. Biochem. Behav.* **1993**, *44*, 605–610.

81. Ostlund, S. B.; Leblanc, K. H.; Kosheleff, A. R.; Wassum, K. M.; Maidment, N. T. Phasic mesolimbic dopamine signaling encodes the facilitation of incentive motivation produced by repeated cocaine exposure. *Neuropsychopharmacology* **2014**, *39*, 1–9.

82. Koob, G. F.; Le Moal, M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* **2001**, *24*, 97–129.

83. Simon, G. E.; Von Korff, M.; Saunders, K.; Miglioretti, D. L.; Crane, P. K.; van Belle, G.; Kessler, R. C. Association between obesity and psychiatric disorders in the US adult population. *Arch. Gen. Psychiatry* **2006**, *63*, 824–30.

84. American Psychiatric Association *Diagnostic and statistical manual of mental disorders*; 5th ed.; American Psychiatric Publishing: Arlington, VA, 2013.

85. Nestler, E. E. J.; Carlezon, W. J. W. A. The mesolimbic dopamine reward circuit in depression. *Biol. Psychiatry* **2006**, *59*, 1151–9.

86. Berridge, K. C. Food reward: Brain substrates of wanting and liking. *Neurosci. Biobehav. Rev.* **1996**, *20*, 1–25.

87. Sharma, S.; Fulton, S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int. J. Obes.* **2012**, *37*, 382–389.

88. Kendig, M. D.; Boakes, R. A.; Rooney, K. B.; Corbit, L. H. Chronic restricted access to 10% sucrose solution in adolescent and young adult rats impairs spatial memory and alters sensitivity

to outcome devaluation. *Physiol. Behav.* **2013**, *120*, 164–72.

89. la Fleur, S. E.; Vanderschuren, L. J. M. J.; Luijendijk, M. C.; Kloeze, B. M.; Tiesjema, B.; Adan, R. A. H. A reciprocal interaction between food-motivated behavior and diet-induced obesity. *Int. J. Obes. (Lond).* **2007**, *31*, 1286–94.

90. Reichelt, A. C.; Morris, M. J.; Westbrook, R. F. Cafeteria diet impairs expression of sensory-specific satiety and stimulus-outcome learning. *Front. Psychol.* **2014**, *5*, 852.

91. Jurdak, N.; Lichtenstein, A. H.; Kanarek, R. B. Diet-induced obesity and spatial cognition in young male rats. *Nutr. Neurosci.* **2008**, *11*, 48–54.

92. Warneke, W.; Klaus, S.; Fink, H.; Langley-Evans, S. C.; Voigt, J.-P. The impact of cafeteria diet feeding on physiology and anxiety-related behaviour in male and female Sprague-Dawley rats of different ages. *Pharmacol. Biochem. Behav.* **2014**, *116*, 45–54.

93. Sartorius, N.; Ustün, T. B.; Lecrubier, Y.; Wittchen, H. U. Depression comorbid with anxiety: results from the WHO study on psychological disorders in primary health care. *Br. J. Psychiatry. Suppl.* **1996**, 38–43.

94. Harmer, C. J.; Phillips, G. D. Enhanced conditioned inhibition following repeated pretreatment with d-amphetamine. *Psychopharmacology (Berl).* **1999**, *142*, 120–31.

95. Rada, P.; Avena, N. M.; Hoebel, B. G. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience* **2005**, *134*, 737–744.

96. Avena, N. M.; Rada, P.; Moise, N.; Hoebel, B. G. Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience* **2006**, *139*, 813–820.

97. Spangler, R.; Wittkowski, K. M.; Goddard, N. L.; Avena, N. M.; Hoebel, B. G.; Leibowitz, S. F. Opiate-like effects of sugar on gene expression in reward areas of the rat brain. *Brain Res. Mol. Brain Res.* **2004**, *124*, 134–42.

98. Foley, K. K. A.; Fudge, M. A. M.; Kavaliers, M.; Ossenkopp, K.-P. K. Quinpirole-induced behavioral sensitization is enhanced by prior scheduled exposure to sucrose: A multi-variable examination of locomotor activity. *Behav. Brain Res.* **2006**, *167*, 49–56.

99. Gosnell, B. A. Sucrose intake enhances behavioral sensitization produced by cocaine. *Brain Res.* **2005**, *1031*, 194–201.

100. Corwin, R. L.; Avena, N. M.; Boggiano, M. M. Feeding and reward: Perspectives from three rat models of binge eating. *Physiol. Behav.* **2011**, *104*, 87–97.

101. Wise, R. A. Dopamine, learning and motivation. *Nat. Rev. Neurosci.* **2004**, *5*, 483–94.

102. Schultz, W.; Hollerman, J. R. Dopamine neurons report an error in the temporal prediction of rewardduring learning. *Nat. Neurosci.* **1998**, *1*, 304–309.

103. Flagel, S. B.; Clark, J. J.; Robinson, T. E.; Mayo, L.; Czuj, A.; Willuhn, I.; Akers, C. A.; Clinton, S. M.; Phillips, P. E. M.; Akil, H. A selective role for dopamine in stimulus-reward

learning. *Nature* **2011**, *469*, 53–7.

104. Waelti, P.; Dickinson, A.; Schultz, W. Dopamine responses comply with basic assumptions of formal learning theory. *Nature* **2001**, *412*, 43–48.

105. Day, J. J.; Roitman, M. F.; Wightman, R. M.; Carelli, R. M. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci.* **2007**, *10*, 1020–8.

106. Rescorla, R. A. Associative changes with a random CS-US relationship. *Q. J. Exp. Psychol. Sect. B* **2000**, *53*, 325–340.

107. Holland, P. C. Stimuli associated with the cancellation of food and its cues enhance eating but display negative incentive value. *Learn. Behav.* **2014**, *42*, 365–82.

108. Brignell, C.; Griffiths, T.; Bradley, B. P.; Mogg, K. Attentional and approach biases for pictorial food cues. Influence of external eating. *Appetite* **2009**, *52*, 299–306.

109. Avena, N. M.; Long, K. A.; Hoebel, B. G. Sugar-dependent rats show enhanced responding for sugar after abstinence: evidence of a sugar deprivation effect. *Physiol. Behav.* **2005**, *84*, 359–62.

110. Colantuoni, C.; Rada, P.; McCarthy, J.; Patten, C.; Avena, N. M.; Chadeayne, A.; Hoebel, B. G. Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obes. Res.* **2002**, *10*, 478–88.

111. Schag, K.; Schönleber, J.; Teufel, M.; Zipfel, S.; Giel, K. E. Food-related impulsivity in obesity and binge eating disorder--a systematic review. *Obes. Rev.* **2013**, *14*, 477–95.

112. Rosval, L.; Steiger, H.; Bruce, K.; Israël, M.; Richardson, J.; Aubut, M. Impulsivity in women with eating disorders: problem of response inhibition, planning, or attention? *Int. J. Eat. Disord.* **2006**, *39*, 590–3.

113. Nasser, J. A.; Gluck, M. E.; Geliebter, A. Impulsivity and test meal intake in obese binge eating women. *Appetite* **2004**, *43*, 303–7.

114. Velázquez-Sánchez, C.; Ferragud, A.; Moore, C. F.; Everitt, B. J.; Sabino, V.; Cottone, P. High trait impulsivity predicts food addiction-like behavior in the rat. *Neuropsychopharmacology* **2014**, *39*, 2463–72.

115. Belin, D.; Mar, A. C.; Dalley, J. W.; Robbins, T. W.; Everitt, B. J. High impulsivity predicts the switch to compulsive cocaine-taking. *Science* **2008**, *320*, 1352–5.

116. de Wit, H. Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addict. Biol.* **2009**, *14*, 22–31.

117. Marwitz, S. E.; Woodie, L. N.; Blythe, S. N. Western-style diet induces insulin insensitivity and hyperactivity in adolescent male rats. *Physiol. Behav.* **2015**, *151*, 147–54.

118. Wu, T.; Deng, S.; Li, W.-G.; Yu, Y.; Li, F.; Mao, M. Maternal obesity caused by overnutrition exposure leads to reversal learning deficits and striatal disturbance in rats. *PLoS*

One **2013**, *8*, e78876.

119. Dawe, S.; Loxton, N. J. The role of impulsivity in the development of substance use and eating disorders. *Neurosci. Biobehav. Rev.* **2004**, *28*, 343–351.

120. Jentsch, J. D.; Taylor, J. R. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl).* **1999**, *146*, 373–90.

121. Wonderlich, S. A.; Connolly, K. M.; Stice, E. Impulsivity as a risk factor for eating disorder behavior: Assessment implications with adolescents. *Int. J. Eat. Disord.* **2004**, *36*, 172–182.

122. Lardeux, S.; Kim, J. J.; Nicola, S. M. Intermittent access to sweet high-fat liquid induces increased palatability and motivation to consume in a rat model of binge consumption. *Physiol. Behav.* **2013**, *114*, 21–31.

123. Gosnell, B. A.; Levine, A. S. Reward systems and food intake: role of opioids. *Int. J. Obes.* **2009**, *33*, S54–S58.

124. Mendez, I. A.; Ostlund, S. B.; Maidment, N. T.; Murphy, N. P. Involvement of Endogenous Enkephalins and β-Endorphin in Feeding and Diet-Induced Obesity. *Neuropsychopharmacology* **2015**, *40*, 2103–12.

125. Davis, C. A.; Levitan, R. D.; Reid, C.; Carter, J. C.; Kaplan, A. S.; Patte, K. A.; King, N.; Curtis, C.; Kennedy, J. L. Dopamine for "wanting" and opioids for "liking": a comparison of obese adults with and without binge eating. *Obesity (Silver Spring).* **2009**, *17*, 1220–5.

126. Drewnowski, A.; Darmon, N. The economics of obesity: dietary energy density and energy cost. *Am. J. Clin. Nutr.* **2005**, *82*, 265S–273S.

127. Bean, M. K.; Stewart, K.; Olbrisch, M. E. Obesity in America: Implications for Clinical and Health Psychologists. *J. Clin. Psychol. Med. Settings* **2008**, *15*, 214–224.

Supplementary Materials: Pattern of access determines influence of junk food diet on cue sensitivity and palatability.

Methods

Junk food diet composition

The junk food diet consisted of Cheetos, mild cheddar cheese, white bread, hot dogs, plain potato chips, Ritz crackers, Reese's peanut butter cups, plain strawberry Pop Tarts, sugar shredded wheat, Chips Ahoy cookies, Oreo cookies, and white chocolate.

Results

Initial behavioral training

Instrumental training: A univariate ANOVA (diet x duration) performed on data from the last three days of instrumental training (averaged into one score) showed no pre-existing differences in response rates between anticipated treatment groups (mean press rate per min 15.305 ± 0.580 SEM; **Fig. 1A**).

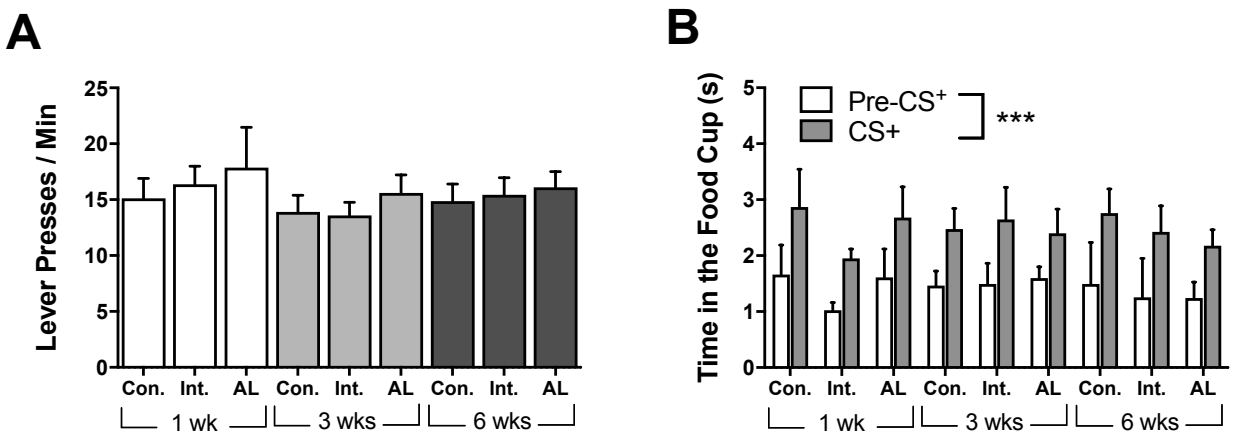

Figure 1. Initial Behavioral Training. (A) Lever presses per minute over the last three days of instrumental training (averaged) and (B) time in the food cup over the last three days of Pavlovian conditioning (averaged). All groups learned both tasks at an equal rate and to equal degree. *Con. = Control group; Int. = Intermittent group; AL = Ad Libitum group; CS^+ = reward-paired cue.*

Pavlovian conditioning: A univariate ANOVA (diet x duration) performed on the amount of time spent in the food cup before the cue (i.e., pre-CS period) over the last three days of Pavlovian conditioning revealed no significant differences between anticipated diet or duration groups (mean 1.538 sec ± 0.146 SEM; **Fig. 1B**). An rmANOVA (diet x duration) comparing pre-CS^+ versus CS^+ responding revealed a significant effect of cue ($F(1,70) = 24.72$, $p < 0.001$), whereby rats increased their time in the food cup during the CS^+ period (CS^+ and 30 seconds immediately proceeding the CS^+, divided by 2). There was no significant effect of diet or duration, or any interaction between these factors.

Changes in body composition

A multivariate ANOVA (diet x duration) was conducted for starting weight, final body weight, percent change in body weight (shown in the main paper) and abdominal adipose tissue content as a percentage of body weight. Diet and duration groups did not differ in their starting weights (all F's < 1; mean 299.68 g, ± 1.72 SEM).

Final body weights: There was a significant effect of duration on final body weights (**Fig. 2A**). Unsurprisingly, rats exposed to all diets for longer periods prior to euthanasia weighed more at the end of the experiment than those exposed for shorter durations. Specifically, 6-week rats weighed more than 1 or 3 week rats. (See **Table 1** for detailed statistics.)

Table 1. Body Composition Analysis
Diet x Duration

Final Body Weights	**df**	**F**	**Sig.**
Diet			ns
Duration	2	10.98	< 0.001
Diet x Duration			ns
Error	70		
Unpaired t-tests	**df**	**t**	**Sig.**
1 vs. 3 weeks			ns
1 vs. 6 weeks	49	4.55	< 0.001
3 vs. 6 weeks	53	3.09	< 0.03
Abdominal White Fat (%)	**df**	**F**	**Sig.**
Diet	2	17.34	< 0.001
Duration	2	8.50	< 0.001
Diet x Duration	4	5.42	< 0.01
Error	70		
Unpaired t-tests	**df**	**t**	**Sig.**
1 vs. 3 weeks	50	2.92	< 0.01
1 vs. 6 weeks	49	3.26	< 0.01
3 vs. 6 weeks			ns
Con. vs. Int.			ns
Con. vs. AL	49	3.94	< 0.001
Int. vs. AL	54	4.08	< 0.001

Abdominal adipose tissue: Despite no differences in final body weight across diet groups, there were main effects of diet and duration on abdominal white fat expressed as a percentage of body weight (**Fig. 2B**), and an interaction between these factors. Ad Libitum rats had significantly more white fat than Control or Intermittent rats, and 3- and 6-week rats had more than 1-week exposure groups. Notably, 3- and 6-week Ad Libitum rats had more white fat than any other diet or duration group. (See **Table 1** for detailed statistics.)

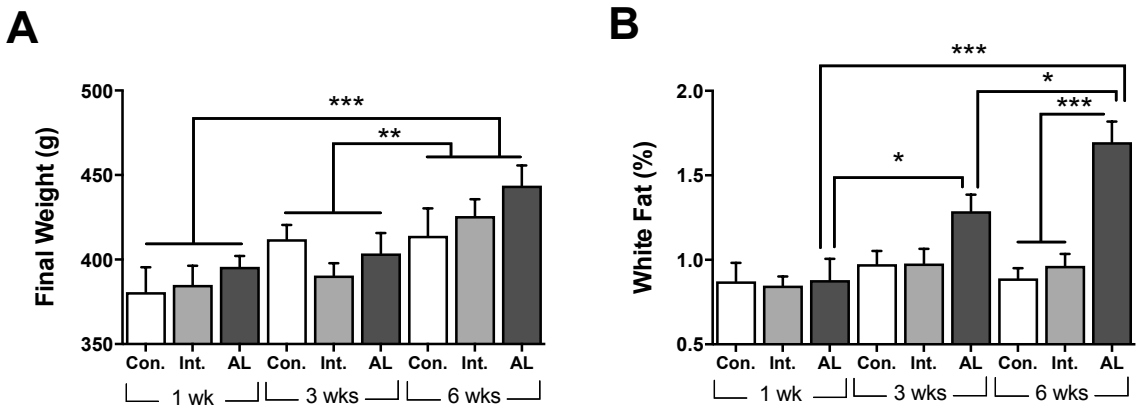

Figure 2. Changes in Body Composition. (A) Final weights (g) at the end of the experiment, where 6-week rats (i.e., older rats) gained the most weight, regardless of diet group. (B) Percentage of abdominal white adipose tissue at the end of the experiment, showing that Ad Libitum rats gained more white fat than other groups, becoming more pronounced with increasing diet duration. *Con. = Control group; Int. = Intermittent group; AL = Ad Libitum group.*

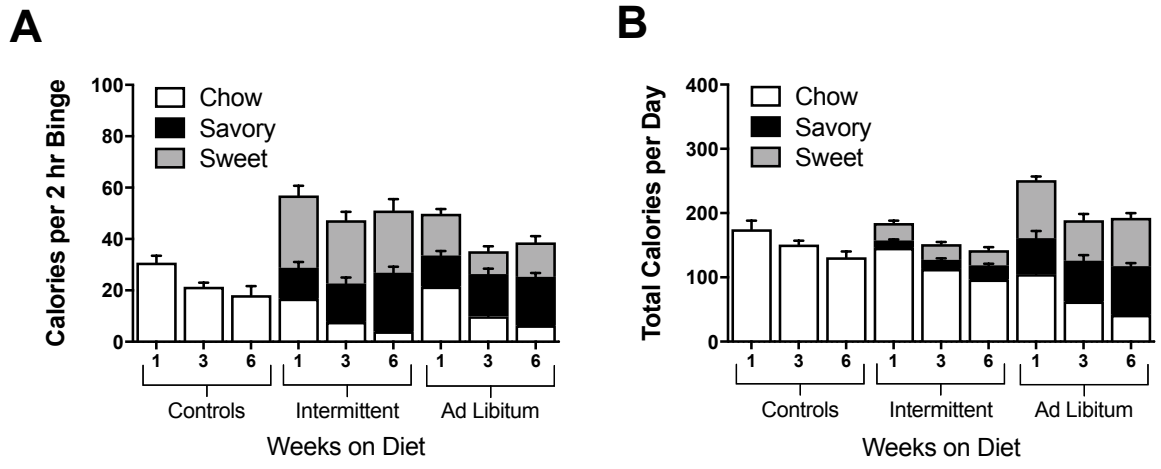

Figure 3. Junk Food Consumption Patterns. Calories consumed from home chow, "Sweet" foods, or "Savory" foods during (A) the daily 2 h binge period, or (B) over 24 hrs (inclusive of the binge period.).

Consumption patterns

Food consumption per cage (2 rats per cage) was measured every day both before and after the 2 h binge period. Junk foods were classified as savory (e.g., hot dogs, cheese, crackers) or sweet (e.g., cookies, chocolates, Pop Tarts). Means and SEM are presented in **Figure 3**.

Behavioral retraining

Instrumental retraining: After junk food exposure, rats underwent 3 days of instrumental retraining. A univariate ANOVA (diet x duration) performed on lever pressing activity revealed main effects of diet and duration, but no interactions (See **Table 2**). Post-hoc comparisons revealed that Ad Libitum rats responded less than Control or Intermittent rats (**Fig. 4A**), and that 6-week rats lever-pressed significantly less than 1- or 3-week rats (**Fig. 4B**).

Table 2. Behavioral Retraining Analysis
Diet x Duration

Instrumental Retraining	**df**	**F**	**Sig.**
Diet	2	4.69	< 0.05
Duration	2	7.77	< 0.01
Error	70		
Unpaired t-tests	**df**	**t**	**Sig.**
Con. vs. Int.			ns
Con. vs. AL	49	2.29	< 0.05
Int. vs. AL	54	2.58	< 0.05
1 vs. 3 weeks			ns
1 vs. 6 weeks	49	4.44	< 0.001
3 vs. 6 weeks	53	2.25	< 0.05
Pavlovian Retraining (Cue x Diet x Duration)	**df**	**F**	**Sig.**
Cue	1	556.28	< 0.001
Error	70		
Instrumental Extinction	**df**	**F**	**Sig.**
Diet	2	4.87	< 0.05
Error	70		
Unpaired t-tests	**df**	**t**	**Sig.**
Con. vs. Int.			ns
Con. vs. AL	49	2.67	< 0.05
Int. vs. AL	49	3.00	< 0.01

Pavlovian retraining: Instrumental training was immediately followed by 1 day of Pavlovian retraining, which consisted of two sessions separated by approximately 2 h. During the first session a novel auditory cue (CS^o) was presented in the exact same fashion as all CS^+ sessions, except no rewards were delivered. The second session was a normal CS^+ session, identical to all previous Pavlovian conditioning sessions. We compared the increase in conditioned responding (i.e., time in the food cup during the cue minus time before the cue) during the CS^+ and CS^o sessions. A rmANOVA (cue x diet x duration) revealed a significant main effect of cue, whereby rats spent more time in the food cup during the CS^+ than during

the CS^{o} presentations, but no significant main effects of, or interactions with, diet or duration (See **Table 2**; **Fig. 4C**).

Instrumental extinction: After Pavlovian retraining and before the PIT test, rats underwent 30 min of instrumental extinction, during which they had access to the lever, but no SCM was delivered. This served to lower response rates, facilitating detection of the PIT effect. A diet x duration ANOVA revealed a main effect of diet (**Fig. 4D**), but not duration, and no interaction between these factors (See **Table 2**). Post-hoc comparisons revealed that Ad Libitum rats exhibited lower response rates than Control and Intermittent rats.

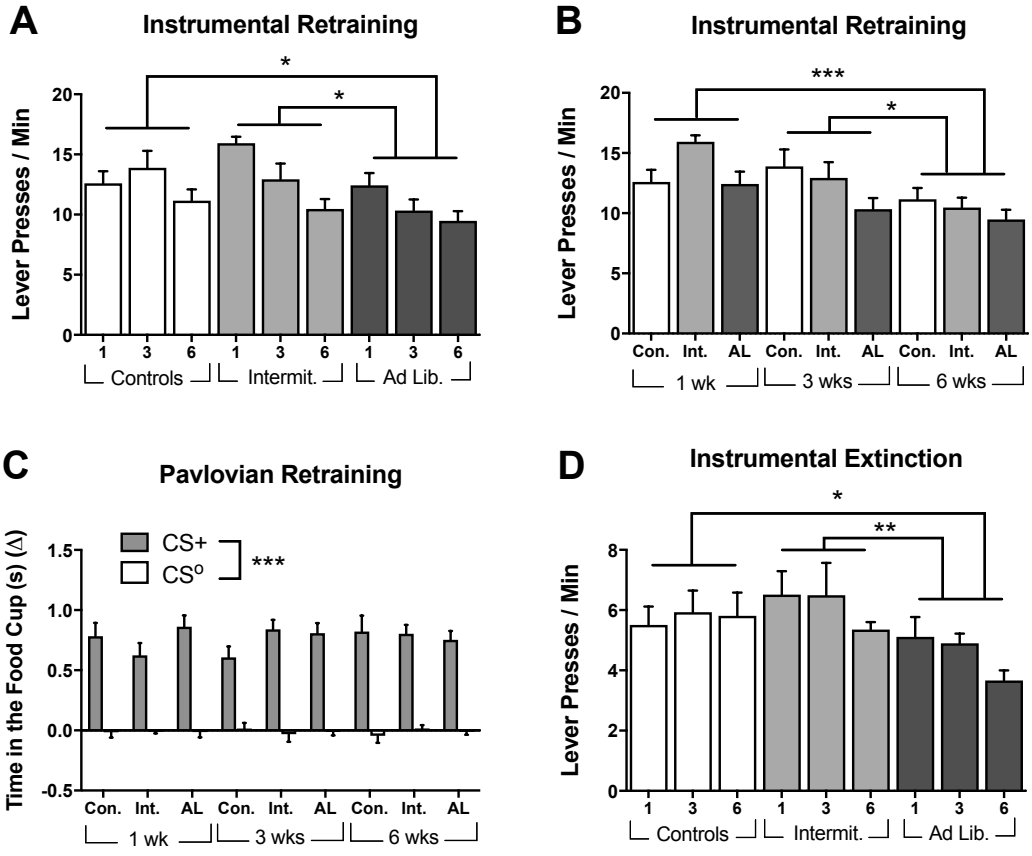

Figure 4. Behavioral Retraining. Behavioral retraining procedures occurring after junk food exposure and immediately prior to PIT testing. (A) and (B) Instrumental retraining. (C) Pavlovian retraining. (D) Instrumental extinction.

Food consumed prior to the sated PIT test

One hour prior to the PIT test, rats were singly-housed with ad libitum access to water. During this time, rats undergoing their "sated" PIT test were provided unrestricted access to lab chow. Order of sated versus hungry tests was counterbalanced across PIT tests. A univariate ANOVA (diet x duration) on chow consumed during this time resulted in a significant effect of diet ($F(2,70) = 3.27$, $p < 0.05$; **Fig. 5**), but no effect of duration and no interaction between these two factors. Post-hoc t-tests revealed that Ad Libitum rats consumed significantly fewer grams of chow than Controls ($t(49) = 2.65$, $p < 0.02$).

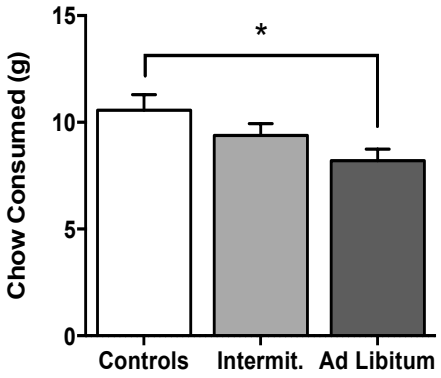

Figure 5. Chow Consumption. Chow consumed (g) during the chow access period immediately prior to the sated PIT test.

Hungry Pavlovian-to-instrumental (PIT) testing

To determine the impact of junk food exposure on cue-evoked reward seeking under routine, hungry, PIT conditions, we presented 18 h food-deprived rats with the CS^+ and CS^o, and recorded lever presses in the absence of reward deliveries. An ANOVA performed on lever-press activity with factors: cue, diet, duration, and weight gain (repeated measures for cue) revealed no significant effects for any of these factors. Results are presented here identically to the sated test for comparison (**Fig. 6**). An identical ANOVA performed on time spent in the food cup revealed a strong main effect of cue ($F(1,62) = 8.95$, $p < 0.01$), suggesting all rats learned the significance of the CS^+, while there was no increase in food cup entries during the CS^o.

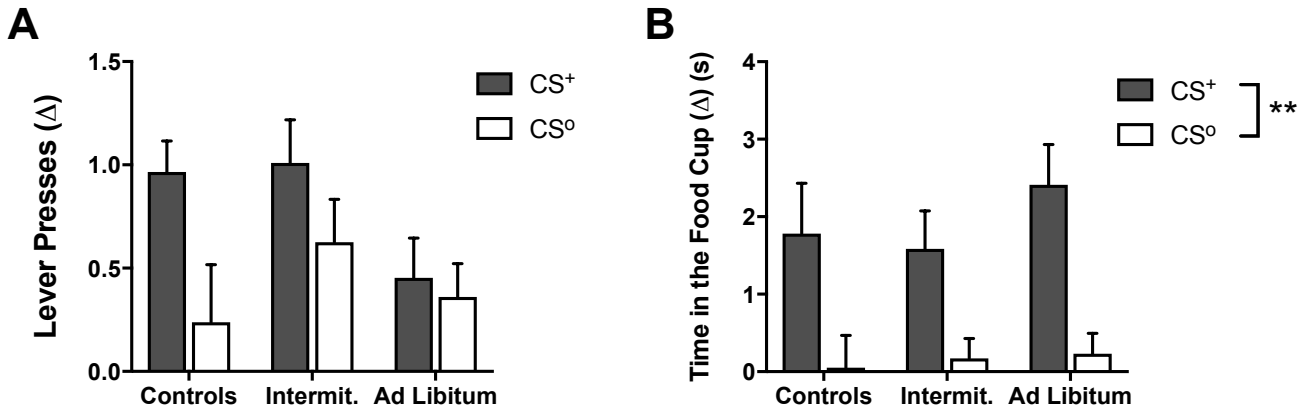

Figure 6. Hungry Pavlovian-to-Instrumental Transfer Test. (A) Increase in lever pressing from pre-cue (baseline) responding per 30 sec. (B) Increase in time spent in the food cup from baseline per 30 sec.

Hungry lick analysis

Immediately after the PIT test, rats were administered a 5-min SCM exposure test, during which all licks were recorded. When drinking palatable solutions, rodents take occasional pauses of varying lengths, resulting in distinct bouts of licking behavior [5]. The average bout length is considered to reflect the experienced palatability/hedonic impact of the solution, particularly during periods of short access, such that involvement of post-ingestive processes is precluded [6,7]. Thus, in addition to total number of licks, we also assessed the average bout length, where a bout is a series of licks in which each lick is separated by no more than 1 second. We conducted a multivariate (total licks and bout length) ANOVA with the factors diet, duration, and weight gain, on data shown in **Figure 7**. This analysis revealed no significant factors or interactions for total licks, but did reveal a significant effect of diet on bout length ($F(2,64) = 3.16$, $p < 0.05$). However, follow-up tests narrowly ($P = 0.07$) failed to support evidence of longer bouts in Intermediate rats relative to controls. These data are presented identically to the sated tests to facilitate comparison.

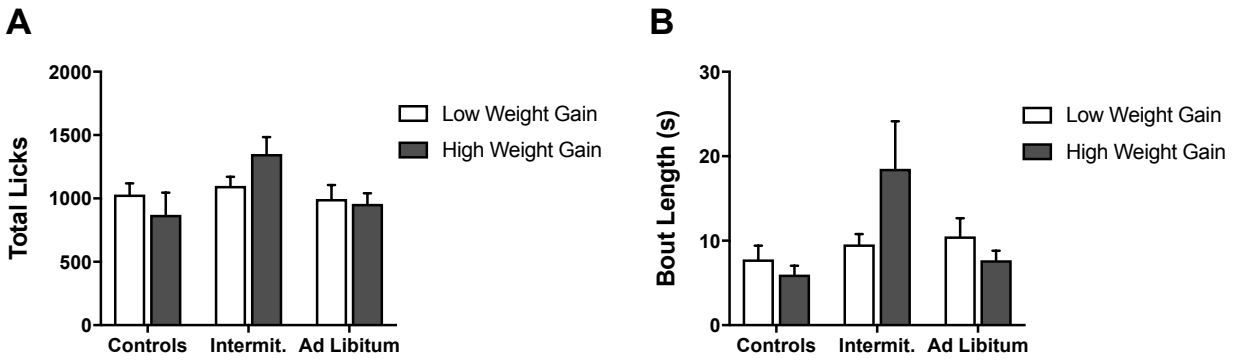

Figure 7. Hungry Licking Microstructure Analysis. (A) Total number of licks and (B) bout length (in seconds) during a 5-min hungry lick test for sweetened condensed milk immediately after the PIT test.

References

1. Ostlund, S. B.; Kosheleff, A. R.; Maidment, N. T. Differential Effects of Systemic Cholinergic Receptor Blockade on Pavlovian Incentive Motivation and Goal-Directed Action Selection. *Neuropsychopharmacology* **2013**, *39*, 1490–1497.

2. LeBlanc, K. H.; Maidment, N. T.; Ostlund, S. B. Repeated cocaine exposure facilitates the expression of incentive motivation and induces habitual control in rats. *PLoS One* **2013**, *8*, e61355.

3. Wyvell, C. L.; Berridge, K. C. Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. *J. Neurosci.* **2001**, *21*, 7831–40.

4. Ostlund, S. B.; Leblanc, K. H.; Kosheleff, A. R.; Wassum, K. M.; Maidment, N. T. Phasic mesolimbic dopamine signaling encodes the facilitation of incentive motivation produced by repeated cocaine exposure. *Neuropsychopharmacology* **2014**, *39*, 1–9.

5. Davis, J. D. The Microstructure of Ingestive Behavior. *Ann. N. Y. Acad. Sci.* **1989**, *575*, 106–121.

6. Ostlund, S. B.; Kosheleff, A. R.; Maidment, N. T.; Murphy, N. P. Decreased consumption of sweet fluids in mu opioid receptor knockout mice: a microstructural analysis of licking behavior. *Psychopharmacology (Berl).* **2013**, *229*, 105–113.

7. Higgs, S.; Cooper, S. J. Effects of benzodiazepine receptor ligands on the ingestion of sucrose, intralipid, and maltodextrin: an investigation using a microstructural analysis of licking behavior in a brief contact test. *Behav. Neurosci.* **1998**, *112*, 447–57.

Chapter 3

Junk Food Exposure Disrupts Outcome Devaluation And Outcome-Specific Pavlovian-To-Instrumental Transfer In Rats

Introduction

The global obesity epidemic remains a serious health concern driven by changes in the global food supply – more cheap and processed foods are readily available in developed and developing countries than ever before [reviewed in 1]. Many of these contemporary "convenience" and pre-packaged foods are not only extremely cheap to procure, they exploit our innate preferences for sugars, salts, and fats [2], potentiating cravings and continued consumption in vulnerable individuals, long past the point of satiety [3]. Given an environment promoting consumption of cheap, processed "junk" foods, the burden falls on individual responsibility to choose actions that limit intake of such foods. Recent studies examining the interoceptive cues (internal, innate physiological signals) regulating appetite have shown that overeating and obesity may result from either hypersensitivity to interoceptive hunger signals, or hyposensitivity to interoceptive satiety signals [4]. Consequently, food consumption may be initiated more readily, stopped more slowly, or both, resulting in caloric intake quickly overtaking actual caloric need.

External cues, such as the acquisition of Pavlovian associations between food and predictive stimuli in the environment (i.e., a logo or jingle) can also drive food seeking and consumption. Importantly, action *choice* as a result of these drives depends on two forms of incentive learning: instrumental incentive learning, whereby outcome value as a result of an action is encoded, and Pavlovian incentive learning, whereby the excitatory effects of a stimulus become associated with specific sensory components of reward [reviewed in 5,6]. Via these mechanisms, specific Pavlovian cues can, with repeated cue-reward pairings, come to elicit the representation of the outcome, and subsequently drive specific instrumental actions. The logic behind advertisements

exploits this learning phenomenon: reminding consumers of a specific product should drive its acquisition, and not that of an alternate product. In the laboratory, the outcome-specific Pavlovian-to-instrumental transfer (osPIT) paradigm has been used to probe the selective motivational components of reward-paired stimuli. While common-place in rodent studies, it is increasingly being applied to human subjects. Using rewards such as chocolate milk, soda, and juice, Bray et al. [7] showed that Pavlovian cues associated with each reward would increase the specific response known to procure that reward (i.e., the cue predictive of juice increased the likelihood of performing the action known to produce juice). Sensitivity to such food-paired cues may be increased in obesity [8], possibly contributing to non-homeostatic, reward-driven overeating.

Animal models have shown that poor diets (i.e., refined or high-sugar diets, etc.) may have consequences for behavior and cognition. A particularly interesting area of focus is whether a junk food diet, frequently the result of undisciplined consummatory behavior, might *exacerbate* the kind of poor decision making that leads to compulsive food seeking and craving, which may result in a harmful cycle of maladaptive overeating. Indeed, high fat diets have been shown to impair learning and memory [9–11], and palatable junk food diets have been found to increase impulsivity on a delay discounting task [12] and alter reward liking and craving [13–15], deficits that could potentially drive further consumption of junk foods. Other studies have shown that *how* the diet is consumed may also matter: sugar- and junk food-binging rats display addiction-like behaviors not seen in rats with ad libitum sugar access or control rats [16,17]. A recent study from our lab (Chapter 2) demonstrated that rats intermittently (2 hrs / day) exposed to junk food displayed greater reward liking than controls or rats continuously exposed (24 hrs / day). Further,

these intermittently-fed rats also demonstrated increased cue sensitivity, where they engaged in reward-seeking in response to not just a reward-paired cue, but a "neutral" cue as well, suggesting a hypersensitivity to generalizing the motivational effect of reward-paired cues to stimuli only loosely paired with reward. Therefore, pattern of access must be considered when evaluating how junk food might impact behavior.

An inability to make appropriate decisions about food consumption is a major factor in compulsive and non-homeostatic eating, i.e., we generally know which foods we *should* be eating (or whether we should be eating at all), but this is not always reflected in our actions. Interestingly, junk food diets have been shown to alter stimulus-outcome learning and incentive value [18], which could have downstream consequences for decision making about food choice. Here, we investigated whether a junk food diet could alter action selection guided by specific satiety and cues. We used a variety of junk foods (i.e., a cafeteria diet) to model contemporary food options, as food consumption and weight gain is enhanced when presented with a variety of flavors and textures (i.e., the "buffet effect"), versus just one food [19,20]. Because of the differences between continuous access and intermittent feeding [16,17], we used both ad libitum (24 h) and restricted, intermittent (1 h) daily access to junk food for 6 weeks. To probe whether a junk food diet impacts sensitivity to outcome value, we used selective satiety-based outcome devaluation, followed by an osPIT test to assess whether a junk food diet might impact the use of Pavlovian associative cues to guide instrumental actions. We hypothesized that intermittently-exposed rats would demonstrate greater disruptions in reward-seeking behavior, indicated by indiscriminate and increased lever pressing on both tasks, while ad libitum exposure would produce only moderate disruptions in satiety- and cue-guided instrumental actions.

Methods

Subjects and apparatus

Adult (7 weeks old) male Sprague-Dawley rats (n = 24) were pair-housed for the duration of the experiment. Rats were food restricted to 85% of their free-feeding body weight during initial behavioral training. All behavioral training took place in sound- and light-attenuating operant chambers (Med Associates, East Fairfield, VT). Each chamber was equipped with two retractable levers, a white noise generator, a clicker audio generator, a pellet dispenser which delivered chocolate flavored pellets (Bio-Serv, Frenchtown, NJ) and an infusion pump which delivered a 2 sec infusion of 50% sweetened condensed milk solution (SCM)/H_2O into a food cup. All experimental procedures were approved by the UCLA Institutional Animal Care and Use Committee and were in accord with the National Research Council Guide for the Care and Use of Laboratory Animals. The experimental timeline is summarized in **Table 1**, and described in detail below.

Pavlovian conditioning

Behavioral training was modified from protocols previously used in our lab [21,22]. Rats received 8 daily sessions of Pavlovian conditioning, where each of the two auditory cues was consistently paired with one of the outcomes. For half of the subjects, the clicker was paired with chocolate pellets, and the noise was paired with SCM, whereas the other half of the subjects received the opposite stimulus-outcome pairings. Each training session consisted of 8 total trials, during which each stimulus was presented 4 times. Each trial lasted 2 min, during which the

corresponding outcome was delivered on a random time (RT) 30 sec schedule. Each trial was separated by a variable 5-min intertrial interval (range = 4 – 6 min).

Phase	Duration	Procedure
Pavlovian Conditioning	8 d	CS_1 → $Outcome_1$ CS_2 → $Outcome_2$
Instrumental Training	11 d	$Response_1$ → $Outcome_1$ $Response_2$ → $Outcome_2$
Diet Exposure	6 weeks	Control, Intermittent or Ad libitum exposure
Mild Food Restriction	3 d	14 hrs chow per day
Outcome Devaluation Test 1	1 d	Sated on $Outcome_1$
Outcome Devaluation Test 2	1 d	Sated on $Outcome_2$
PIT Test	1 d	Both levers extended, CS_1 and CS_2 present, no outcomes

Table 1. Experimental Timeline

Instrumental training

Rats received 11 days of instrumental training, with two sessions conducted each day, separated by at least 20 min. In the first session, rats were trained that pressing either the left or right lever would result in either the pellet or SCM reward (counterbalanced), with the second session providing training with the other response-outcome contingency. Each day, the session order was reversed. During the first two days of training, each lever-press response was continuously reinforced with the appropriate food outcome. The reinforcement schedule was then changed to random ratio 5 (RR-5) for days 3-4, RR-10 for days 5-6, RR-15 for days 7-8, and RR-20 for days 9-11.

Junk food diet

Following training, rats were assigned to one of three diet groups: Controls, Intermittent, or Ad Libitum. During this time, all rats had continuous access to chow and water in their home cages, while the two treatment groups (Intermittent and Ad Libitum) also received access to two junk foods (one sweet, one savory) each day for either 1 hr only (Intermittent group; modeling restricted or binge eating) or for 24 hrs (Ad Libitum group; modeling chronic overeating) [23–25]. Each day, the junk foods were exchanged for different junk foods. Junk foods consisted of

Hershey's chocolates, Kit Kats, Chips Ahoy cookies, Oreo cookies, shelf-stable sugar-coated donuts, shelf-stable brownies, Ritz crackers, Cheetos, Doritos, bagels, hot dogs, and cheddar cheese. Diet exposure was continued for 6 weeks, after which point rats were maintained on 14 hrs access to standard laboratory chow only (i.e., 10 hrs food deprivation) to maintain mild food deprivation for behavioral testing. Importantly, because our intent was to assess the impact of junk food exposure on the expression of food-seeking behavior, and not on learning about such actions, we conducted these tests in extinction (no reinforcement), without any further post-diet exposure retraining.

Outcome devaluation testing

After 3 days of mild food restriction, rats were given two sessions of outcome devaluation testing, 48 hrs apart (devaluing one outcome for test 1, and the other for test 2). Here, we evaluated how diet exposure influenced rats' ability to adapt their choice between food-seeking actions after being selectively satiated on one of the two food rewards. Immediately preceding each test, rats were singly housed with water ad libitum, and given unrestricted access to either pellets or SCM for 1 hr. This sensory-specific satiety procedure is used to temporarily reduce the incentive value (i.e., devaluing) of the food, while leaving the incentive value of the alternate food unaffected. Immediately after this, rats were placed in the operant chambers for a 5-min choice extinction test during which both levers were extended, but no auditory cues were presented nor were any outcomes delivered. Forty-eight hours later, rats were given a second outcome devaluation test using the opposite outcome (i.e., if test 1 used pellets, test 2 used SCM).

Pavlovian-to-instrumental transfer testing

Forty-eight hours after the second devaluation test, rats were given an osPIT test in order to assess the effects of junk food exposure on the outcome-specific influence of food-paired cues on food seeking behavior. Both levers were inserted into the chamber for the duration of the session. Lever presses and food cup entries were continuously recorded, but no outcomes were delivered. During the PIT test, each cue was presented non-contingently (i.e., cue onset and offset occur regardless of lever pressing) 4 times for 2 min at a time. A pseudorandom (ABBA) trial order was used, with trials separated by a fixed 5-min interval.

Data analysis and statistics

Data were analyzed using SPSS (IBM, Armonk, NY). Diet effects (Control vs. Intermittent vs. Ad Libitum) were analyzed using repeated-measures analyses of variance (rmANOVA), with paired- and independent-sample post-hoc t-tests where appropriate. Effects were defined as statistically significant when $p < 0.05$, and post-hoc comparisons were corrected using Holm's sequential Bonferroni correction [26,27], e.g., in a family of 3 comparisons, at least one comparison must meet an alpha criterion of $p \leq 0.0167$ (i.e., 0.05/3), while a second comparison must meet an alpha criterion $p \leq 0.025$ (i.e., 0.05/2), while the last comparison must meet a criterion of $p \leq 0.05$ (0.05/1), in order for each effect to be considered significant. Where results were dependent on three significant digits, (i.e., for Holm's corrections), p values are expressed to the thousandths position, otherwise data are reported to the hundredths.

All data were tested for violations of normality using the Shapiro-Wilk test (with diet as a factor). For the devaluation test, 3 out of 6 groups were not normally distributed (devalued

action: Ad Libitum group; valued action: Ad Libitum and Controls; all p's < 0.05; all other groups: p's > 0.05). Therefore, these data were analyzed and reported as the log transformation. To control for individual differences in attention away from the lever (which would most likely be directed towards the food cup) during the devaluation test, total food cup entries were included as a covariate in the analysis.

A univariate ANOVA of pre-cue pressing during the osPIT test confirmed that diet groups did not differ on this measure ($p = 0.42$; mean rate of pre-cue lever pressing per 2 min: 3.49 ± 0.41 SEM), thus data are presented as differences scores from baseline responding, i.e., the average number of pre-cue lever presses were subtracted from the average number of lever presses during the cue. All data are expressed as means ± standard error of the mean (SEM).

Results

Initial behavioral training

Initial behavioral training occurred in two phases: Pavlovian conditioning and instrumental lever press training. Each phase was analyzed to ensure all rats learned the task and for potential pre-existing differences between future diet group assignments.

Pavlovian conditioning: A cue phase (pre-cue vs. cue) x diet rmANOVA of the last two days of Pavlovian conditioning (**Fig. 1A**) showed that rats learned to approach the food cup during the cue (main effect of cue phase: $F_{(1,21)} = 331.60$, $p < 0.001$), where approach behavior increased during the cue period. There was no effect of future diet group assignment (all p's > 0.30).

Instrumental training: To assess rats' response rates and food cup approach behavior going into the diet exposure phase, the last two days of instrumental training were averaged and analyzed (**Fig. 1B**). A multivariate response (press and food cup entry) x diet ANOVA failed to show an effect of future group assignment on lever press rate ($p = 0.17$) or time in the food cup ($p = 0.79$).

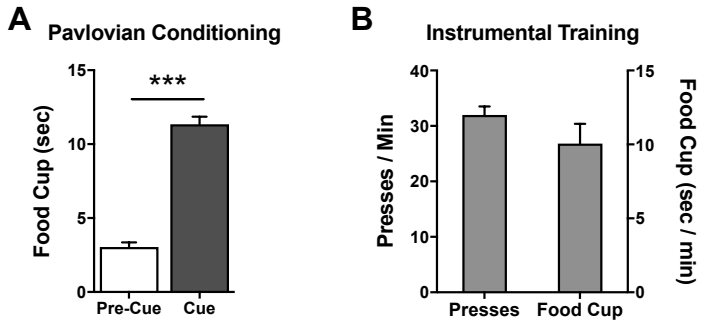

Figure 1. Pavlovian and Instrumental Training. All rats learned to check the food cup during the cue during Pavlovian conditioning (A), and the instrumental lever press response during instrumental training (B). No pre-existing differences were found between anticipated diet groups.

Food consumption during the consumption phase of the devaluation test

An outcome (pellets vs. SCM) x diet rmANOVA on outcomes eaten during the consumption phase of the devaluation test (**Fig. 2**) revealed a significant effect of outcome ($F_{1,21} = 945.10$, $p < 0.001$), where rats consumed more 50% SCM solution than pellets, a significant effect of diet ($F_{2,21} = 4.72$, $p = 0.02$), and a significant outcome x diet interaction ($F_{2,21} = 5.03$, $p = 0.02$). Holm-corrected independent samples t-tests revealed that Ad Libitum rats consumed fewer grams of the SCM solution than Intermittent rats ($t_{14} = 2.78$, $p < 0.015$) or Controls ($t_{14} = 3.18$, $p = 0.007$). Control rats did not differ from Intermittent rats on SCM consumption ($p = 0.56$). Diet groups did not differ on consumption of pellets: Controls versus Intermittent: $p = 0.22$; Controls versus Ad Libitum: $p = 0.48$, although

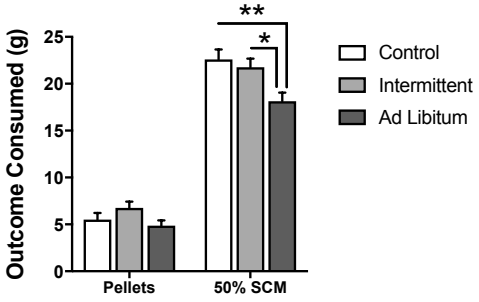

Figure 2. Outcomes Consumed During the Sating Phase of the Devaluation Test. Ad Libitum rats consumed significantly fewer grams of SCM solution than Intermittent or Control rats. All rats consumed more grams of SCM solution than pellets.

there was a trend for Ad Libitum rats to have consumed fewer grams than Intermittent rats ($p = 0.048$), but this trend did not reach Holm-corrected criterion for significance.

Outcome devaluation testing

We used satiety-based outcome devaluation to assess whether diet exposure could alter instrumental reward-seeking behavior based on expected outcome value. When successful at using expected outcome value to guide behavior, rats will press more on the non-devalued outcome lever versus the devalued outcome lever. Due to the effects of extinction on non-reinforced, sated instrumental actions over repeated tests, responding quickly decreases over time. Thus, we compared early (first half) versus late (second half) responding in an action (devalued vs. non-devalued pressing) x diet x time (first vs. second half) rmANOVA, which revealed a strong effect of time ($F_{1,16} = 18.02$, $p = 0.001$), but failed to reveal any other significant effects (all p's > 0.10). Given this strong effect, we suspected extinction effects due to repeated and sated testing might have obscured any dietary influences, thus we targeted our analysis to the first half of each devaluation test (i.e., the first 2.5 min). A diet x action (devalued vs. non-devalued pressing) rmANOVA on the first half of the devaluation test (**Fig. 3**) failed to show a significant main effect of action ($p = 0.15$) or diet ($p = 0.86$), but did reveal a significant diet x action interaction ($F_{2,17} = 3.69$, $p < 0.05$). Follow-up paired t-tests confirmed that Controls pressed more for the valued outcome than devalued outcome ($t_7 = 4.56$, $p = 0.004$), while Intermittent ($p = 0.35$) and Ad Libitum ($p = 0.28$) rats did not differ in their rate of pressing (**Fig. 3**).

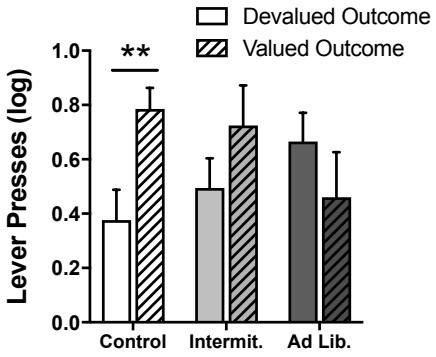

Figure 3. Outcome Devaluation Testing. Both groups of junk food-fed rats (i.e., Intermittent and Ad Libitum) failed to adjust instrumental responding away from a devalued outcome during the first half of the devaluation test.

Outcome-specific Pavlovian-to-instrumental transfer testing

After 1 day off, we conducted an osPIT test to assess whether diet exposure might alter rats' ability to use reward-paired cues to invigorate reward-seeking actions based on shared outcome representation (i.e., does a cue predictive of pellets motivate pressing for pellets, i.e., "Same" pressing, or the SCM, i.e., "Different" pressing). A multivariate analysis for the effects of diet on total food cup entries and total lever pressing, failed to reveal any effect of diet on any measure (food cup entries: $p = 0.87$; total presses: $p = 0.42$), suggesting all groups were equally responsive on both measures.

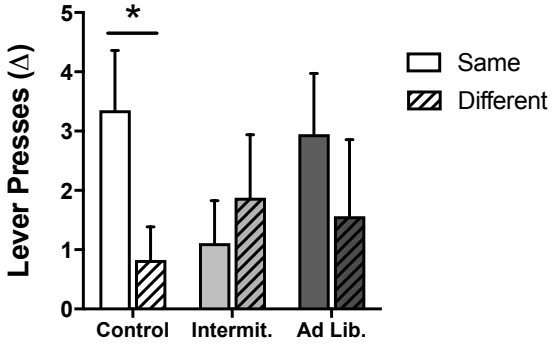

Figure 4. Outcome-Specific Pavlovian-to-Instrumental Transfer Test. Control rats successfully used stimulus-outcome and action-outcome associations to guide instrumental reward seeking, while both junk food-fed groups did not. Same = pressing for the lever associated with the same outcome predicted by the cue; Different = pressing for the lever associated with the outcome *not* predicted by the cue.

An action (Same cue-lever pair vs. Different cue-lever pair) x diet rmANOVA (**Fig. 4**) revealed a significant action x diet interaction ($F_{2,21} = 3.53$, $p < 0.05$), and a trend for a significant main effect of action ($F_{1,21} = 4.16$, $p = .054$), but failed to reveal a main effect of diet ($p = 0.81$). Holm-corrected paired t-tests revealed a significant difference for Same vs. Different pressing for only the Chow controls ($t_7 = 3.31$, $p = 0.013$), and a trend towards such an effect in the Ad Libitum group ($t_7 = 2.68$, $p = 0.032$), though this effect did not reach Holm-corrected significance of $p \leq 0.025$ (Intermittent group: $p = 0.55$). Independent-samples t-test between groups on Same and Different lever presses revealed no differences in the *magnitude* of lever pressing between groups (all p's > 0.10), suggesting any dietary effects on responding were restricted to action selection, not general activity or willingness to lever press.

These results suggest that, in the Controls, instrumental reward seeking is sensitive to Pavlovian cues, which are successfully able to bias action selection in an outcome-specific manner, as expected. However, Intermittent junk food diet exposure, and, to a lesser extent, Ad libitum exposure, disrupted this effect, rendering rats' instrumental actions insensitive to the specific motivational effects of Pavlovian cues.

Weight change

A univariate analysis of weight change as a percentage of initial body weights failed to reveal a difference among diet groups ($p = 0.187$; **Fig. 5**).

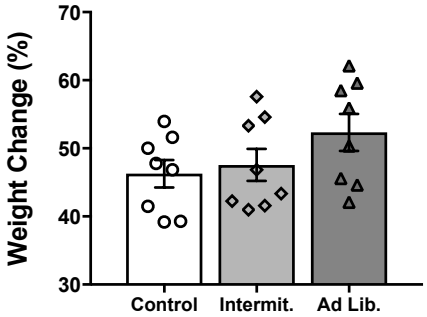

Figure 5. Change in Body Weight. There was no significant difference in weight gain as a percentage of initial body weight between diet groups.

Discussion

We report disruptions in appropriate reward seeking after junk food exposure in a test of outcome devaluation using specific satiety and an osPIT test. Specifically, we found that intermittent and ad libitum junk food exposure altered rats' initial ability to adjust instrumental responding in response to a diminution in expected outcome value (**Fig. 3**). Further, both junk food exposure groups were also impaired in using Pavlovian stimulus-outcome associations to guide instrumental actions (**Fig. 4**). In our control group, rats increased reward seeking only when a cue predicting that *same* reward was present, while actions for an alternate, non-paired reward were minimal. Both junk food groups failed to demonstrate this effect: they appeared to engage in indiscriminate reward-seeking inconsistent with prior stimulus-outcome and action-

outcome learning. The osPIT effect in control rats was not due to greater learning, or pre-existing differences in instrumental or Pavlovian goal approach behavior, but to an intact ability to use previously-acquired stimulus- and action-outcome learning to guide reward seeking. These deficits occurred despite no change in baseline rates of instrumental behavior or conditioned food cup approach behavior at test. Interestingly, ad libitum-fed rats consumed fewer grams of SCM solution (compared to intermittent-fed or control rats; **Fig. 2**) during the consumption phase of the devaluation test, though this did not translate into behavioral differences when compared to intermittent-fed rats on our behavioral measures. These data suggest that junk food exposure impairs the capacity to make adaptive decisions based on internal need state and to use external cues to guide reward-seeking behavior.

Junk food may promote impulsive actions

Our two junk food-fed groups displayed similar decision-making deficits in tests of outcome devaluation and cue-guided action selection, despite differences in the pattern of junk food consumption (i.e., intermittent versus ad libitum exposure). Although pattern of access does appear to matter across a variety of measures [16,17,23,28,29], it is possible that some assays, such as those reported here, are less sensitive to these difference. For instance, it is possible that both groups of junk food-fed rats experienced changes in dopamine function that rendered them more impulsive, a key factor in poor decision making, promoting indiscriminate lever pressing on both tasks without regard for future consequences.

Consistent with this hypothesis, recent evidence suggests that a junk food diet can increase impulsivity on a delay discounting task [12]. Further, reductions in mesostriatal D2 receptors are

well documented to occur after junk food exposure [12,30,31], and are associated with weight gain in both humans [32,33] and rats [34]. Interestingly, we found no difference in weight gain between our two junk food groups despite their different feeding patterns, suggesting that any effects of junk food on striatal D2 receptors would be due exclusively to junk food exposure, and would likely be similarly distributed between groups.

Importantly, dopamine neurons in the ventral tegmental area (VTA) project not only to mesolimbic structures, but also to regions of the prefrontal cortex involved in decision making [35], thus any junk food or weight gain-driven changes to D2 receptor function or availability on VTA dopamine neurons may be consequential for meso*cortical* dopamine signaling, also. Further, reductions in striatal D2 receptors, such as those seen after prolonged junk food exposure and weight gain, are associated with decreased metabolism in cortical regions associated with decision making [36–39], increased impulsivity [40], and decreased inhibitory GABAergic receptor binding in the striatum [41] and the frontal cortex [40,42], consistent with downregulation of inhibitory mechanisms in these regions. It is therefore possible that our junk food-exposed rats experienced increased impulsivity on tasks that might be less sensitive to the differential effects of restricted versus extended junk food access, as a result of altered mesolimbic and mesocortical dopamine signaling.

Junk food may disrupt memory retrieval

The inability to appropriately use stimulus-outcome associations to guide reward-seeking behavior (**Fig. 4**) may also have emerged as a result of associative memory impairment. In associative conditioning paradigms, the ability of a reward-paired cue to elicit conditioned

responses depends on the strength of the stimulus-outcome association, or how well the cue elicits the representation of the outcome, and how well this representation drives conditioned responding. If the cue fails to elicit a robust representation of the outcome, conditioned and instrumental responses may be disrupted. Given the amount of time between training and testing during the 6 week junk food exposure phase, it is possible that the specific stimulus-outcome or action-outcome associations required for our tasks were poorly represented in the junk food groups. While high-fat and/or refined carbohydrate diets have been shown to impair cognitive performance on a variety of tasks [9–11], the hippocampus appears preferentially vulnerable to the deleterious effects of poor quality diets, with reports of decreased neurogenesis [43], BDNF mRNA [44,45], and deficits in hippocampal-dependent learning [46–49] and memory functions [46,50–52]. Interestingly, in obese human subjects, associative learning was impaired when using a food reward, but not with a monetary reward [53,54], suggesting that our tasks, which required intact associations between a stimulus or action and a food outcome, might have been disproportionately difficult for our junk food rats. This may have manifested as an inability to correctly recall stimulus-outcome or action-outcome associations, therefore cue-evoked or choice instrumental responses in our osPIT and devaluation test, respectively, would appear indiscriminate.

Junk food may promote habitual reward seeking

Goal-directed and habitual reward seeking strategies differ in their sensitivity to action outcomes – goal-directed behavior is characterized by the ability to flexibly adapt responses to current needs and motivational states, while habitual behavior is insensitive to such changes [55]. When instrumental responding occurs using a goal-directed strategy, rats will avoid pressing for the

devalued reward, and instead engage in the lever associated with the valued outcome instead. If responding relies instead on a habitual strategy, rats may be more likely to engage indiscriminately with each lever. Because our junk food rats initially demonstrated indiscriminate lever pressing (**Fig. 3**), it is possible that junk food exposure may facilitate, but not drive, a shift towards habitual responding. Our results fit well with other reports of poor diets promoting a habitual strategy. For instance, restricted access (but not ad libitum access) promoted habitual responding during an outcome devaluation task using specific satiety [29,56]. In human subjects, obese men (who presumably are obese due to excess consumption of poor-quality foods) are less sensitive to outcome devaluation than their lean counterparts [57], and obese participants habituate more slowly to a food stimulus, resulting in greater caloric intake, than their lean peers [58].

Interestingly, Tantot et al. [59] recently reported that a high-fat diet can compromise behavioral sensitivity to outcome devaluation when trained using a random interval schedule, but training with a random ratio could "rescue" a goal-directed strategy. Here, we used a random ratio schedule of reinforcement, which might explain why rats' behavior appeared time sensitive (i.e., most abnormal early in the test, but normalizing by the end). Another recent study [60] found that simply offering standard lab chow in an intermittent pattern of exposure could promote a shift in responding from a goal-directed to habitual strategy, suggesting perhaps intermittently-exposed junk food rats might be particularly vulnerable to the acquisition of a habitual strategy (though we report no difference between junk food groups here).

Junk food may disrupt flavor-nutrient satiety learning: implications for devaluation and osPIT

Our junk food rats may have also experienced a disruption in "flavor-nutrient satiety learning" (i.e., conditioned satiety), that may have contributed to their initial indiscriminate lever pressing during the devaluation test and altered stimulus-outcome or action-outcome associations in our osPIT test (**Fig. 6**). In the ancestral environment, the orosensory components of foods (i.e., tastes, flavors, textures) were essential factors guiding the selection of highly nutritive and caloricly dense (and scarce) foods, for which preferences quickly develop. While these preferences are thought to be innate [61], postingestive, interoceptive and metabolic processes signaling the caloric and nutritive content of the consumed food, can, via Pavlovian conditioned associations, further strengthen the association between foods and their caloric or nutritive content, i.e., flavor-nutrient learning [62,63]. As a result, flavor nutrient learning can both promote increased food intake as a result of learned preferences (flavor-nutrient *hedonic* learning), and it can decrease food intake by promoting learned satiety responses (flavor-nutrient *satiety* learning), whereby animals learn associations between a food's orosensory components and its caloric density as determined by postingestive metabolic consequences. Flavor-nutrient learning can result in cues associated with nutritive and caloric foods reliably

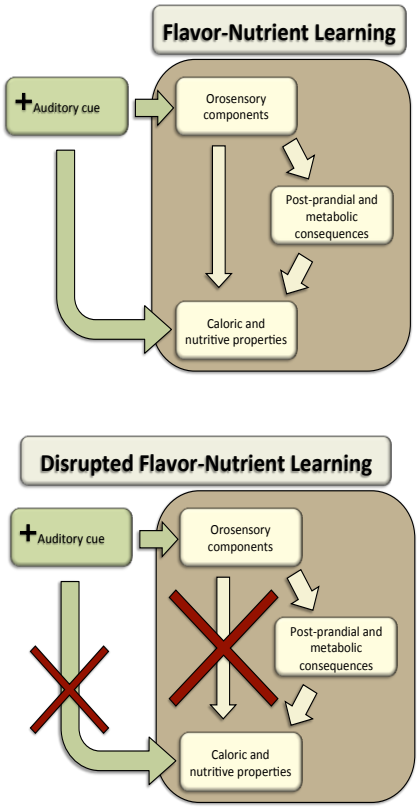

Figure 6. Potential Mechanism for Disrupted Action Selection. Disruptions in flavor-nutrient learning may result in an inability to use internal or external cues to guide action selection based on anticipated outcomes.

eliciting cephalic responses such as salivation and insulin secretion even before the food is consumed [64–66], and can appropriately restrain consumption of high-density foods due to the expected calories they provide [67,62]. Thus, via Pavlovian associative conditioning, the orosensory cues of specific foods can evoke neural and hormonal metabolic conditioned reactions capable of modulating energy utilization and behaviors (i.e., cessation of food consumption) [33,34].

In the present environment, where foods are highly processed, refined and frequently altered (i.e., using non-nutritive sweeteners to be "low-sugar", or removing fats to be "low-fat"), tastes, flavors, and textures are increasingly unreliable indicators of a food's nutritive or caloric postingestive consequences. There is some evidence that components of the contemporary processed diet may contribute to a deficits in flavor-nutrient satiety learning [70–73] (conversely, [74,75]). Proponents of the flavor confusion hypothesis suggest this increasing inconsistency between a food's orosensory components and its nutritive content (and thus postingestive metabolic effects) may disrupt Pavlovian associations and learning about the postingestive consequences of various foods, and inhibit flavor-nutrient satiety learning [67,62]. This dissociation may weaken a food's ability to evoke appropriate physiological responses involved in signaling satiety and regulating food intake, which may lead to a decreased reliance on interoceptive cues to guide food choices [71,76–78]. Indeed, the consumption of highly refined, high carbohydrate, and artificial foods is associated with an inability to regulate later caloric intake, promoting overeating, and suggesting an inability to predict a food's caloric content or a lack of reliance on interoceptive cues to appropriately guide behavior [reviewed in 35–37]. The variety of refined and processed foods our junk food rats experienced may have contributed to

modest deficits in flavor-nutrient satiety learning, where they initially failed to use interoceptive cues about specific satiety to adjust reward seeking appropriately.

Further, in our training context, rats are given opportunities to learn that a specific cue predicts a specific food reward, which would have consistent post-prandial and metabolic consequences (i.e., auditory cue → post-prandial and caloric effects conditioning). If contemporary Westernized diets disrupt associative learning between a food and it's metabolic consequences, perhaps these rats failed to accurately maintain the anterograde association between a predictive auditory cue and the food outcome, as measured in our osPIT test. Because our diet manipulation occurred after training (and we did not employ any post-diet retraining), it is unlikely that any of the reported changes occurred as a result of deficits in *encoding*. Instead, it is more likely that exposure to each outcome during the devaluation testing phase may have allowed opportunities for new learning where each outcome was "newly" experienced in the context of disrupted flavor-nutrient learning. This may have manifested in our junk food rats as an ambiguity of the cue-outcome or action-outcome associations, where cue-driven lever pressing appears indiscriminate with respects to the cue-outcome and lever-outcome relationships.

Junk food may disrupt the use of satiety as a negative feature stimulus

A recent hypothesis suggests that satiety signals may exert inhibitory control over appetitive and consummatory behavior by acting as negative feature stimuli [46]. Appropriate energy regulation depends on increasing consummatory behaviors when in an energy deficit (and thus when interoceptive hunger signals would be prominent), and decreasing such behaviors when in a surplus (i.e., when satiety signals would dominate). When hungry, food intake is associated with

appetitive and rewarding postingestive effects (e.g., activation of the mesolimbic reward system), but such effects are minimized when sated [reviewed in 41]. In normal animals, energy regulation might then depend on the successful use of negative feature stimuli (i.e., satiety) to signal that food intake will not be followed by rewarding postingestive outcomes. In our junk food rats, initial indiscriminate responding may result as a failure to use satiety as a signal that the devalued outcome will not be rewarding. Rats with selective lesions of the hippocampus are impaired on tasks requiring the use of a learned negative features stimulus [81], as are rats fed on a Western diet (interestingly, hippocampal lesions or a Western diet do not impair learning tasks requiring a *positive* feature stimulus) [46]. Further, hippocampal lesions impair rats' ability to use interoceptive cues about current satiety state to limit appetitive behavior [82]. These results are particularly interesting in light of evidence that the hippocampus appears preferentially vulnerable to the deleterious effects of poor quality diets [43–45,52].

Does the method of devaluation matter?

Importantly, while a specific satiety manipulation holds more face validity for probing overeating (i.e., eating beyond interoceptive signals of satiety as a major contributor to obesity), many paradigms have also employed the use of the emetic agent lithium chloride (LiCl) as a method of devaluation. While not directly compared, Nelson & Killcross [83] report devaluation after specific satiety and LiCl pairing, with a greater overall effect of devaluation after LiCl pairing. In many diet-manipulation studies, it is possible that the aversion is simply not of sufficient magnitude to restore a goal-directed action strategy, and not that animals are incapable of it. While general eating beyond satiety may be a crucial component of maladaptive eating behavior, it would be interesting to know if junk food-exposed or obese animals are also less

sensitive to conditioned taste aversion – i.e., food poisoning – as well, a question beyond the scope of this paper.

Summary

What emerges as strange is that we found no statistical differences between rats exposed to junk food on an intermittent versus ad libitum schedule on either measure, despite differences between these two groups on SCM solution consumption immediately prior to the devaluation test (**Fig. 2**), and on behavior in other studies (i.e., intermittent exposure tends to produce a behavioral phenotype akin to addiction, while ad libitum, 24-hour exposure does not) [16,17,23,28,29]. This suggests that perhaps the causal factor for our reported deficits is access to junk food itself, as both patterns of exposure provide a complex sensory experience, engaging gustatory, olfactory and somatosensory systems to integrate taste, smell and texture. However, to our knowledge, there are few within-study comparisons between intermittent and ad libitum exposure, thus perhaps differences between patterns of consumption cited across the literature are actually due to other methodological differences, and direct comparisons between exposure patterns across studies should be approached with caution. Admittedly, it is possible that all junk food rats experienced a "binge" effect of sorts during daily food changes, when old foods were removed and fresh foods were administered, creating a pseudo-binge-like phenotype in even the ad libitum-exposed rats, minimizing expected differences between each group. Importantly, we hypothesize our results are due to either deficits in behavioral control (i.e., increased impulsivity) or in retrieval of stimulus-outcome or action-outcome associations, and not due to deficits in

encoding such relationships, as our junk food exposure occurred after initial training and immediately prior to any testing (without any retraining).

Comparisons across the literature are fraught with difficulty due to the wide variety (in content and execution) of junk food diets: studies report a range of variety versus monodiet paradigms, high-fat versus high-carbohydrate food substances, highly refined foods versus simply fat- or sugar-enriched, binge or intermittent versus extended or ad libitum access, and duration of exposure (e.g., from 3 weeks [84] to 6 months [85]). Despite this, our results showing modest disruption in outcome devaluation and deficits in using outcome representations to guide reward seeking behavior after junk food exposure fit well with previous reports. What remains unclear is whether, in both tasks, junk food rats would have normalized their behavior had they been reinforced, which would have allowed rats to update their representation of the reward or action-outcome association.

References

1. Swinburn, B. A.; Sacks, G.; Hall, K. D.; McPherson, K.; Finegood, D. T.; Moodie, M. L.; Gortmaker, S. L. The global obesity pandemic: shaped by global drivers and local environments. *Lancet (London, England)* **2011**, *378*, 804–14.

2. Krebs, J. R. The gourmet ape: evolution and human food preferences. *Am. J. Clin. Nutr.* **2009**, *90*, 707S–711S.

3. Johnson, F.; Wardle, J. Variety, palatability, and obesity. *Adv. Nutr.* **2014**, *5*, 851–9.

4. Simmons, W. K.; DeVille, D. C. Interoceptive contributions to healthy eating and obesity. *Curr. Opin. Psychol.* **2017**, *17*, 106–112.

5. Balleine, B. Incentive Processes in Instrumental Conditioning. In; 2001.

6. Dickinson, A.; Balleine, B. The role of learning in the operation of motivational systems. In *Steven's Handbook of Experimental Psychology*; Pashler, H.; Gallistel, R., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2002; pp. 497–533.

7. Bray, S.; Rangel, A.; Shimojo, S.; Balleine, B.; O'Doherty, J. P. The Neural Mechanisms Underlying the Influence of Pavlovian Cues on Human Decision Making. *J. Neurosci.* **2008**, *28*, 5861–5866.

8. Stice, E.; Spoor, S.; Bohon, C.; Small, D. M. Relation Between Obesity and Blunted Striatal Response to Food Is Moderated by TaqIA A1 Allele. *Science (80-.).* **2008**, *322*.

9. Greenwood, C. E.; Winocur, G. Learning and memory impairment in rats fed a high saturated fat diet. *Behav. Neural Biol.* **1990**, *53*, 74–87.

10. Greenwood, C. E.; Winocur, G. Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty-acid intake. *Behav. Neurosci.* **1996**, *110*, 451–9.

11. Winocur, G.; Greenwood, C. E. The effects of high fat diets and environmental influences on cognitive performance in rats. *Behav. Brain Res.* **1999**, *101*, 153–61.

12. Robertson, S. H.; Rasmussen, E. B. Effects of a cafeteria diet on delay discounting in adolescent and adult rats: Alterations on dopaminergic sensitivity. *J. Psychopharmacol.* **2017**, 26988111773575.

13. Vendruscolo, L. F.; Gueye, A. B.; Vendruscolo, J. C. M.; Clemens, K. J.; Mormède, P.; Darnaudéry, M.; Cador, M. Reduced alcohol drinking in adult rats exposed to sucrose during adolescence. *Neuropharmacology* **2010**, *59*, 388–394.

14. Shin, A. C.; Townsend, R. L.; Patterson, L. M.; Berthoud, H.-R. "Liking" and "wanting" of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R1267-80.

15. Lesser, E. N.; Arroyo-Ramirez, A.; Mi, S. J.; Robinson, M. J. F. The impact of a junk-food diet during development on "wanting" and "liking." *Behav. Brain Res.* **2017**, *317*, 163–178.

16. Avena, N. M.; Hoebel, B. G. A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience* **2003**, *122*, 17–20.

17. Buda-Levin, A.; Wojnicki, F. H. E. E.; Corwin, R. L. Baclofen reduces fat intake under binge-type conditions. *Physiol. Behav.* **2005**, *86*, 176–84.

18. Reichelt, A. C.; Morris, M. J.; Westbrook, R. F. Cafeteria diet impairs expression of sensory-specific satiety and stimulus-outcome learning. *Front. Psychol.* **2014**, *5*, 852.

19. Rolls, B. J.; Rowe, E. A.; Rolls, E. T.; Kingston, B.; Megson, A.; Gunary, R. Variety in a meal enhances food intake in man. *Physiol. Behav.* **1981**, *26*, 215–221.

20. Rolls, B. J.; Rolls, E. T.; Rowe, E. A.; Sweeney, K. Sensory specific satiety in man. *Physiol. Behav.* **1981**, *27*, 137–142.

21. Ostlund, S. B.; Balleine, B. W. Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. *J. Neurosci.* **2008**, *28*, 4398–405.

22. Ostlund, S. B.; Kosheleff, A. R.; Maidment, N. T. Differential Effects of Systemic Cholinergic Receptor Blockade on Pavlovian Incentive Motivation and Goal-Directed Action Selection. *Neuropsychopharmacology* **2013**, *39*, 1490–1497.

23. Lardeux, S.; Kim, J. J.; Nicola, S. M. Intermittent access to sweet high-fat liquid induces increased palatability and motivation to consume in a rat model of binge consumption. *Physiol. Behav.* **2013**, *114*, 21–31.

24. Robinson, M. J.; Burghardt, P. R.; Patterson, C. M.; Nobile, C. W.; Akil, H.; Watson, S. J.; Berridge, K. C.; Ferrario, C. R. Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology* **2015**.

25. Johnson, P. M.; Kenny, P. J. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* **2010**, *13*, 635–41.

26. Holm, S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand. J. Stat.* **1979**, *6*, 65–70.

27. Blakesley, R. E.; Mazumdar, S.; Dew, M. A.; Houck, P. R.; Tang, G.; Reynolds, C. F.; Butters, M. A. Comparisons of methods for multiple hypothesis testing in neuropsychological research. *Neuropsychology* **2009**, *23*, 255–64.

28. Puhl, M. D.; Cason, A. M.; Wojnicki, F. H. E.; Corwin, R. L.; Grigson, P. S. A history of bingeing on fat enhances cocaine seeking and taking. *Behav. Neurosci.* **2011**, *125*, 930–42.

29. Furlong, T. M.; Jayaweera, H. K.; Balleine, B. W.; Corbit, L. H. Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *J. Neurosci.* **2014**, *34*, 5012–22.

30. Alsiö, J.; Olszewski, P. K.; Norbäck, A. H.; Gunnarsson, Z. E. A.; Levine, A. S.; Pickering, C.; Schiöth, H. B. Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity

phenotype in rats. *Neuroscience* **2010**, *171*, 779–787.

31. Alsiö, J.; Olszewski, P. K.; Levine, A. S.; Schiöth, H. B. Feed-forward mechanisms: addiction-like behavioral and molecular adaptations in overeating. *Front. Neuroendocrinol.* **2012**, *33*, 127–39.

32. Volkow, N. D.; Wang, G.-J.; Telang, F.; Fowler, J. S.; Thanos, P. K.; Logan, J.; Alexoff, D.; Ding, Y.-S.; Wong, C.; Ma, Y.; Pradhan, K. Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. *Neuroimage* **2008**, *42*, 1537–43.

33. Wang, G.-J.; Volkow, N. D.; Logan, J.; Pappas, N. R.; Wong, C. T.; Zhu, W.; Netusll, N.; Fowler, J. S. Brain dopamine and obesity. *Lancet* **2001**, *357*, 354–357.

34. Hajnal, A.; Margas, W. M.; Covasa, M. Altered dopamine D2 receptor function and binding in obese OLETF rat. *Brain Res. Bull.* **2008**, *75*, 70–6.

35. Carr, D. B.; Sesack, S. R. GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. *Synapse* **2000**, *38*, 114–123.

36. Volkow, N. D.; Fowler, J. S.; Wang, G.-J.; Hitzemann, R.; Logan, J.; Schlyer, D. J.; Dewey, S. L.; Wolf, A. P. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse* **1993**, *14*, 169–177.

37. Volkow, N. D.; Chang, L.; Wang, G. J.; Fowler, J. S.; Ding, Y. S.; Sedler, M.; Logan, J.; Franceschi, D.; Gatley, J.; Hitzemann, R.; Gifford, a; Wong, C.; Pappas, N. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *Am. J. Psychiatry* **2001**, *158*, 2015–21.

38. Volkow, N. D.; Wang, G.-J.; Telang, F.; Fowler, J. S.; Logan, J.; Jayne, M.; Ma, Y.; Pradhan, K.; Wong, C. Profound Decreases in Dopamine Release in Striatum in Detoxified Alcoholics: Possible Orbitofrontal Involvement. *J. Neurosci.* **2007**, *27*, 12700–12706.

39. Volkow, N. D.; Fowler, J. S. Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cereb. Cortex* **2000**, *10*, 318–25.

40. Jupp, B.; Caprioli, D.; Saigal, N.; Reverte, I.; Shrestha, S.; Cumming, P.; Everitt, B. J.; Robbins, T. W.; Dalley, J. W. Dopaminergic and GABA-ergic markers of impulsivity in rats: evidence for anatomical localisation in ventral striatum and prefrontal cortex. *Eur. J. Neurosci.* **2013**, *37*, 1519–1528.

41. Caprioli, D.; Sawiak, S. J.; Merlo, E.; Theobald, D. E. H.; Spoelder, M.; Jupp, B.; Voon, V.; Carpenter, T. A.; Everitt, B. J.; Robbins, T. W.; Dalley, J. W. Gamma aminobutyric acidergic and neuronal structural markers in the nucleus accumbens core underlie trait-like impulsive behavior. *Biol. Psychiatry* **2014**, *75*, 115–23.

42. Sandoval-Salazar, C.; Ramírez-Emiliano, J.; Trejo-Bahena, A.; Oviedo-Solís, C. I.; Solís-Ortiz, M. S. A high-fat diet decreases GABA concentration in the frontal cortex and hippocampus of rats. *Biol. Res.* **2016**, *49*, 15.

43. Lindqvist, A.; Mohapel, P.; Bouter, B.; Frielingsdorf, H.; Pizzo, D.; Brundin, P.; Erlanson-Albertsson, C. High-fat diet impairs hippocampal neurogenesis in male rats. *Eur. J. Neurol.* **2006**, *13*, 1385–1388.

44. Molteni, R.; Barnard, R. J.; Ying, Z.; Roberts, C. K.; Gómez-Pinilla, F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* **2002**, *112*, 803–14.

45. Martire, S. I.; Maniam, J.; South, T.; Holmes, N.; Westbrook, R. F.; Morris, M. J. Extended exposure to a palatable cafeteria diet alters gene expression in brain regions implicated in reward, and withdrawal from this diet alters gene expression in brain regions associated with stress. *Behav. Brain Res.* **2014**, *265*, 132–41.

46. Kanoski, S. E.; Davidson, T. L. Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. *Physiol. Behav.* **2011**, *103*, 59–68.

47. Jurdak, N.; Lichtenstein, A. H.; Kanarek, R. B. Diet-induced obesity and spatial cognition in young male rats. *Nutr. Neurosci.* **2008**, *11*, 48–54.

48. Jurdak, N.; Kanarek, R. B. Sucrose-induced obesity impairs novel object recognition learning in young rats. *Physiol. Behav.* **2009**, *96*, 1–5.

49. Kanoski, S. E.; Zhang, Y.; Zheng, W.; Davidson, T. L. The Effects of a High-Energy Diet on Hippocampal Function and Blood-Brain Barrier Integrity in the Rat. *J. Alzheimer's Dis.* **2010**, *21*, 207–219.

50. Yu, H.; Bi, Y.; Ma, W.; He, L.; Yuan, L.; Feng, J.; Xiao, R. Long-term effects of high lipid and high energy diet on serum lipid, brain fatty acid composition, and memory and learning ability in mice. *Int. J. Dev. Neurosci.* **2010**, *28*, 271–276.

51. Beilharz, J. E.; Maniam, J.; Morris, M. J. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain. Behav. Immun.* **2014**, *37*, 134–41.

52. Francis, H. M.; Stevenson, R. J. Higher reported saturated fat and refined sugar intake is associated with reduced hippocampal-dependent memory and sensitivity to interoceptive signals. *Behav. Neurosci.* **2011**, *125*, 943–955.

53. Zhang, Z.; Manson, K. F.; Schiller, D.; Levy, I. Impaired associative learning with food rewards in obese women. *Curr. Biol.* **2014**, *24*, 1731–6.

54. Coppin, G.; Nolan-Poupart, S.; Jones-Gotman, M.; Small, D. M. Working memory and reward association learning impairments in obesity. *Neuropsychologia* **2014**, *65*, 146–155.

55. Balleine, B. W.; Dickinson, A. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. *Neuropharmacology* **1998**, *37*, 407–419.

56. Ahn, S.; Phillips, A. G. Repeated cycles of restricted food intake and binge feeding disrupt sensory-specific satiety in the rat. *Behav. Brain Res.* **2012**, *231*, 279–285.

57. Horstmann, A.; Dietrich, A.; Mathar, D.; Pössel, M.; Villringer, A.; Neumann, J. Slave to habit? Obesity is associated with decreased behavioural sensitivity to reward devaluation. *Appetite* **2015**, *87*, 175–183.

58. Epstein, L. H.; Robinson, J. L.; Roemmich, J. N.; Marusewski, A. Slow rates of habituation predict greater zBMI gains over 12 months in lean children. *Eat. Behav.* **2011**, *12*, 214–8.

59. Tantot, F.; Parkes, S. L.; Marchand, A. R.; Boitard, C.; Naneix, F.; Layé, S.; Trifilieff, P.; Coutureau, E.; Ferreira, G. The effect of high-fat diet consumption on appetitive instrumental behavior in rats. *Appetite* **2017**, *108*, 203–211.

60. Parkes, S. L.; Furlong, T. M.; Black, A. D.; Balleine, B. W. Intermittent feeding alters sensitivity to changes in reward value. *Appetite* **2017**, *113*, 1–6.

61. Breslin, P. A. S. An Evolutionary Perspective on Food and Human Taste. *Curr. Biol.* **2013**, *23*, R409–R418.

62. Sclafani, A. Learned controls of ingestive behaviour. *Appetite* **1997**, *29*, 153–158.

63. Touzani, K.; Sclafani, A. Conditioned flavor preference and aversion: role of the lateral hypothalamus. *Behav. Neurosci.* **2001**, *115*, 84–93.

64. Woods, S. C.; Vasselli, J. R.; Kaestner, E.; Szakmary, G. A.; Milburn, P.; Vitiello, M. V Conditioned insulin secretion and meal feeding in rats. *J. Comp. Physiol. Psychol.* **1977**, *91*, 128–33.

65. Johnson, W. G.; Wildman, H. E. Influence of external and covert food stimuli on insulin secretion in obese and normal persons. *Behav. Neurosci.* **1983**, *97*, 1025–8.

66. Simon, C.; Schlienger, J. L.; Sapin, R.; Imler, M. Cephalic phase insulin secretion in relation to food presentation in normal and overweight subjects. *Physiol. Behav.* **1986**, *36*, 465–469.

67. Martin, A. A. Why can't we control our food intake? The downside of dietary variety on learned satiety responses. *Physiol. Behav.* **2016**, *162*, 120–129.

68. Mattes, R. D. Physiologic Responses to Sensory Stimulation by Food: Nutritional Implications. *J. Am. Diet. Assoc.* **1997**, *97*, 406–413.

69. Woods, S. C.; Ramsay, D. S. Pavlovian influences over food and drug intake. *Behav. Brain Res.* **2000**, *110*, 175–182.

70. Davidson, T. L.; Swithers, S. E. Food Viscosity Influences Caloric Intake Compensation and Body Weight in Rats. *Obes. Res.* **2005**, *13*, 537–544.

71. Swithers, S. E.; Davidson, T. L. A role for sweet taste: Calorie predictive relations in energy regulation by rats. *Behav. Neurosci.* **2008**, *122*, 161–173.

72. Swithers, S. E.; Ogden, S. B.; Davidson, T. L. Fat substitutes promote weight gain in rats consuming high-fat diets. *Behav. Neurosci.* **2011**, *125*, 512–518.

73. Warwick, Z. S.; Schiffman, S. S. Flavor-calorie relationships: effect on weight gain in rats.

Physiol. Behav. **1991**, *50*, 465–70.

74. Palframan, K. M.; Myers, K. P. Modern "junk food" and minimally-processed "natural food" cafeteria diets alter the response to sweet taste but do not impair flavor-nutrient learning in rats. *Physiol. Behav.* **2016**, *157*, 146–157.

75. Perez, C.; Fanizza, L. J.; Sclafani, A. Flavor Preferences Conditioned by Intragastric Nutrient Infusions in Rats fed Chow or a Cafeteria Diet. *Appetite* **1999**, *32*, 155–170.

76. Swithers, S. E.; Davidson, T. L. Obesity: outwitting the wisdom of the body? *Curr. Neurol. Neurosci. Rep.* **2005**, *5*, 159–62.

77. Swithers, S. E.; Baker, C. R.; Davidson, T. L. General and persistent effects of high-intensity sweeteners on body weight gain and caloric compensation in rats. *Behav. Neurosci.* **2009**, *123*, 772–780.

78. Swithers, S. E.; Martin, A. A.; Davidson, T. L. High-intensity sweeteners and energy balance. *Physiol. Behav.* **2010**, *100*, 55–62.

79. Davidson, T. L.; Swithers, S. E. A Pavlovian approach to the problem of obesity. *Int. J. Obes.* **2004**, *28*, 933–935.

80. Berthoud, H.-R. The neurobiology of food intake in an obesogenic environment. *Proc. Nutr. Soc.* **2012**, *71*, 478–487.

81. Holland, P. C.; Lamoureux, J. A.; Han, J.-S.; Gallagher, M. Hippocampal lesions interfere with Pavlovian negative occasion setting. *Hippocampus* **1999**, *9*, 143–157.

82. Davidson, T. L.; Kanoski, S. E.; Chan, K.; Clegg, D. J.; Benoit, S. C.; Jarrard, L. E. Hippocampal lesions impair retention of discriminative responding based on energy state cues. *Behav. Neurosci.* **2010**, *124*, 97–105.

83. Nelson, A.; Killcross, S. Amphetamine exposure enhances habit formation. *J. Neurosci.* **2006**, *26*, 3805–12.

84. la Fleur, S. E.; Vanderschuren, L. J. M. J.; Luijendijk, M. C.; Kloeze, B. M.; Tiesjema, B.; Adan, R. A. H. A reciprocal interaction between food-motivated behavior and diet-induced obesity. *Int. J. Obes. (Lond).* **2007**, *31*, 1286–94.

85. Blaisdell, A. P.; Lau, Y. L. M.; Telminova, E.; Lim, H. C.; Fan, B.; Fast, C. D.; Garlick, D.; Pendergrass, D. C. Food quality and motivation: a refined low-fat diet induces obesity and impairs performance on a progressive ratio schedule of instrumental lever pressing in rats. *Physiol. Behav.* **2014**, *128*, 220–5.

Chapter 4

A High-Fructose Diet Disrupts Insulin Signaling, Dopamine Reuptake, And Incentive Motivation

Introduction

Diet-induced insulin resistance is quickly becoming a worldwide health concern as diets "westernize": i.e., the prevalence of dietary refined carbohydrates and simple sugars increases as the consumption of nutritive foods decreases [1,2]. Diet-induced insulin dysfunction, characterized by a slow progression from insulin insensitivity, to insulin resistance, to type 2 diabetes, typically develops over several years, resulting in heart disease, stroke, blindness, lower-limb amputations, and cognitive impairment [3,4], and is an important risk factor in dementia and Alzheimer's disease [5]. While peripheral insulin signaling has been readily evaluated by simple blood tests for many years [6], neuronal insulin signaling has only recently come under investigation. Such diagnoses are more difficult to make, though imaging and post-mortem analyses of diabetics [7,8], and animal models of insulin resistance [9] have shown altered levels and activation of effectors in the brain insulin signaling pathway [10,11]. The specific contribution of neuronal insulin dysmetabolism to cognitive impairment remain unclear, though the widespread distribution of insulin receptors in the brain suggest possibly broad implications [12,13].

Insulin receptors, though widespread, are densely localized in the ventral midbrain [14]. A growing body of evidence shows neuronal insulin to be a significant modulator of the dopamine transporter (DAT): specifically, increasing insulin in the brain increases DAT mRNA [15] and facilitates DAT activity and translocation to the synaptic membrane, thereby promoting dopamine clearance [16–20]. Conversely, decreasing insulin levels via fasting or inducing diabetes reduces DAT expression and dopamine clearance [21–23]. Because extracellular dopamine is quickly and predominantly cleared by the DAT [24], altered DAT function has significant consequences for dopamine signaling, which is heavily implicated in reward seeking behavior [25,26].

Pleasant rewards (e.g., recreational drugs, enjoyable foods) stimulate dopamine release in the ventral striatum from neurons originating in the ventral tegmental area (VTA). Initially, dopamine release occurs as a result of the reward itself, but, with repeated pairings, shifts to the onset of earlier and earlier predictors, or "cues" [27,28]. Reward-paired cues (e.g., drug paraphernalia, food advertising) are key precipitators of reward seeking, as they frequently take on their own motivating properties. The incentive sensitization theory of addiction [29] proposes that this occurs as a result of rewards repeatedly engaging the mesolimbic dopamine system in the presence of such cues. Via interactions with the hippocampus [30], these repeated pairings "sensitize" the dopamine system to be increasingly attuned to these cues, and the neural system gradually comes to attribute excessive salience to them; i.e., these cues become attractive and "wanted." The sensitization of incentive salience is believed to be responsible for the transformation of reward "wanting" into excessive cravings. As a result, these cues acquire their own motivational value and subsequently drive reward-seeking behavior. Despite the popularity of this model, the molecular mechanisms underlying the proposed hyper-reactivity of dopamine transmission in response to reward-paired cues remain to be fully elucidated.

Given the role of neuronal insulin as a modulator of the DAT, and the DAT's role in terminating dopamine signaling, whether and how neuronal insulin signaling can influence cue-invigorated reward seeking is unclear. We hypothesize that chronic access to an insulin-disrupting diet capable of inducing insulin resistance will decrease insulin signaling in the ventral tegmental area, downregulate DAT function, and enhance the excitatory effects of reward-paired cues, increasing incentive motivation for food reward. Here, we investigated whether an insulin-disrupting diet would: 1) decrease insulin signaling in the ventral midbrain, 2) decrease DAT surface expression in the nucleus accumbens, 3) disrupt DAT-mediated reuptake, 4) disrupt incentive motivation for a food reward, and 5) alter dopamine release during a test of incentive motivation. To this end, we used the

Pavlovian-to-instrumental transfer (PIT) paradigm, which is uniquely able to measure the capacity of reward-paired cues to acquire motivational salience, thereby driving instrumental behaviors to procure reward [31,32]. By training rats with the reward-paired cue and instrumental response in separate phases of the experiment it is possible to prevent any direct association from forming between these two events. Consequently, any cue-triggered instrumental responding observed at test can be attributed to the motivational (response-invigorating) properties of that cue [31,32], making the PIT paradigm an ideal assay of cue-evoked incentive motivation. We also tested whether administration of the diabetes-treatment drug pioglitazone (PGZ) would normalize dopamine reuptake and behavior during a PIT test.

Experiment 1. A high-fructose diet produces insulin resistance in the brain

Methods

Subjects

Forty-eight male Sprague-Dawley rats approximately 10 weeks old, weighing approximately 360.08 g ± 3.06 SEM at the start of the experiment, were housed in a climate-controlled vivarium with water provided ad libitum in the homecage throughout the experiment. Body weight and diet consumption were measured every other day. All procedures in this and subsequent experiments were approved by the UCLA IACUC.

Diet

Rats were divided into two diet groups: Fructose rats were fed a 60% fructose diet (TD. 89247; Envigo, Madison, WI) with water available ad libitum, and control rats were given the control diet (TD. 98394; Envigo), formulated to be otherwise nutritionally and calorically comparable to the

experimental fructose diet. Fructose was used due to it's ubiquity within the modern Western diet and it's ability to rapidly produce insulin resistance [33]. Within these diet groups, rats were divided into 3 diet exposure duration groups: 1, 3, and 6 week exposure durations. This dietary regimen was chosen as previous studies have shown this diet to be capable of rapidly producing insulin resistance in the periphery with minimal weight gain (which can present a substantial confound) [33–36], as well as for its content validity as a model of human insulin resistance resulting from chronic access to poor quality, diabetogenic foods [37,38] (versus other models such as streptozotocin-induced insulin dysfunction [39]).

Biochemical analysis

Trunk blood was collected for analysis of peripheral insulin resistance. At the end of diet exposure, rats were fasted overnight (~ 12 hrs) from all food, and euthanized in the morning. Rats were briefly anesthetized with isoflurane and decapitated. Trunk blood (400 µl) was immediately collected and mixed with 60 µl EDTA (10 mg/ml) for 1- and 3-week duration samples. The 6-week exposure group samples were mixed with 400 µl EDTA, thus precluding a direct comparison with the 1- and 3-week groups. Samples were centrifuged at 2000 rpm for 20 min at 4° C. Samples were analyzed by an outside chemistry service for peripheral glucose and triglyceride concentrations.

Dissections

Blunt dissections of the ventral midbrain were collected for analysis of neuronal insulin function. Immediately after decapitation, brains were quickly excised, cooled on dry ice, placed in a brain matrix placed on an inverted petri dish on wet ice, then cut with two blades at approximately 5.0 – 6.0 mm posterior to bregma. This section was removed, placed flat on an inverted petri dish, and the ventral midbrain (VTA and substantia nigra) dissected by hand (to produce approximately 10-20 mg tissue), and stored at -80° C for future Western analysis.

Western analysis

Brain tissue was homogenized in lysis buffer (137 mM NaCl, 20 mM Tris-HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 0.1 mM benzothonium, 0.5 mM sodium vanadate). After centrifugation at 12,500 g for 20 min, the supernatant was collected and immediately processed for total protein concentration determination according to the Micro BCA procedure (Pierce, Rockford, IL), using bovine serum albumin as standard. A total of 25 μg of protein from each sample was used. Protein samples were separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to a PVDF membrane. After 5% non-fat milk blocking, the membranes were incubated with a primary antibody overnight at 4° C, followed by a secondary antibody for 1 hr at room temperature. The following primary antibodies were used: phospho-insulin receptor (1:1000, Abcam, Cambridge, MA), phospho-IRS1 (1:1000, EMD Millipore, Billerica, MA), insulin receptor (1:1000, Santa Cruz Biotechnology Inc., Santa Cruz, CA), IRS1 (1:1000, EMD Millipore, Billerica, MA) and actin (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA). The secondary antibodies used were anti-goat or anti-rabbit IgGHRP (1:10,000, Santa Cruz Biotechnology Inc., Santa Cruz, CA). After rinsing with buffer (0.1% Tween-20 in PBS), the immunocomplexes were visualized by chemiluminescence using the Amersham ECL Plus Western Blotting Detection kit (GE Healthcare Bio-Sciences, Piscataway, NJ) according to the manufacturer's instructions.

Data analysis and statistics

Data were analyzed by univariate and multivariate ANOVA where appropriate with SPSS (IBM, Armonk, NY). Independent samples t-tests were used after significant overall effects were detected. Effects were considered significant when $p < 0.05$. When post-hoc comparisons consisted of 3 or more comparisons per family, Holm's sequential Bonferroni correction [40,41] was used to control

for Type I errors, e.g., in a family of 3 comparisons, at least one comparison must meet an alpha criterion of $p \leq 0.0167$ (i.e., 0.05/3), while a second comparison must meet an alpha criterion $p \leq 0.025$ (i.e., 0.05/2), etc. The TyG Index was used to quantify insulin resistance, which compares triglyceride and glucose concentrations using the formula (Ln(glucose x triglycerides / 2)), and is a standard assay of insulin resistance [42–44]. Adiposity is expressed as the percentage of the final body weights, and body weights are expressed as the percentage change from their starting weights. All data, unless otherwise noted, are presented as means ± SEM.

Results

Neuronal insulin resistance

We examined tissue from the ventral midbrain to assess neuronal insulin resistance. A multivariate ANOVA (diet x duration x protein) on the phosphorylation state of IRS-1 and Akt revealed a main effect of diet and duration for both proteins (**Fig. 1**). There was also a significant diet x duration interaction for IRS-1, and a trend toward a significant interaction for Akt. Post-hoc comparisons revealed that Fructose rats showed decreased Akt phosphorylation as quickly as 1 week after beginning diet exposure, and a significant difference emerged at 3 weeks for IRS-1. (See **Table 1** for detailed statistics.)

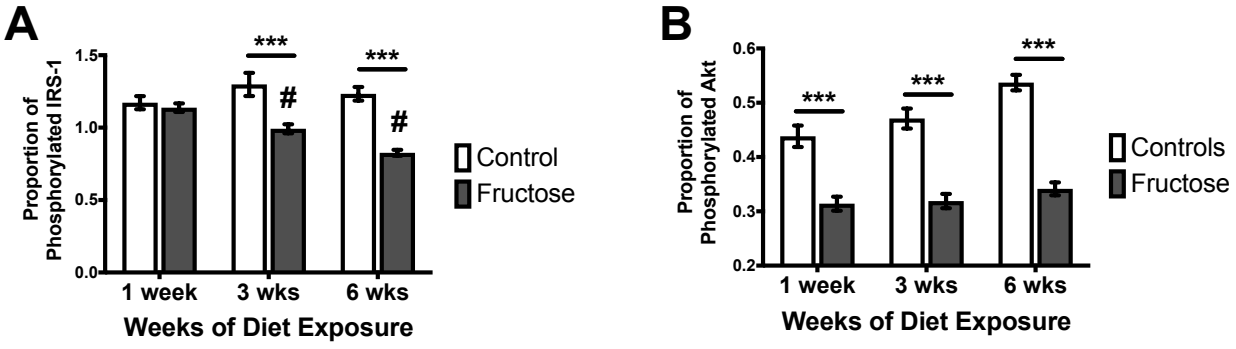

Figure 1. Markers of Insulin Resistance in the Ventral Midbrain. Proportion of phosphorylated IRS-1 (A) and Akt (B), effectors in the insulin signaling cascade. Insulin signaling is decreased in Fructose rats relative to Controls, suggesting increased insulin resistance.

Peripheral insulin resistance

We examined trunk blood to assess changes in peripheral insulin resistance. Plasma samples from the 6-week group were diluted with an overabundance of the anticoagulant EDTA (400 µl versus 60 µl for the 1- and 3-week groups, all samples mixed with 400 µl blood). As a result, all samples are corrected to their respective control group (i.e., 1-week Fructose samples are presented normalized to 1-week Control samples).

Table 1. Neuronal Insulin Resistance Analysis
Diet x Duration x Protein

IRS-1		**df**	**F**	**Sig.**
	Diet	1	43.21	< 0.001
	Duration	2	4.50	0.02
	Diet x Duration	2	8.63	< 0.01
	Error	*42*		
Akt		**df**	**F**	**Sig.**
	Diet	1	157.37	< .001
	Duration	2	8.90	< 0.01
	Diet x Duration			ns
	Error	*42*		
Independent samples t-tests		**df**	**t**	**Sig.**
IRS-1				
	Fructose vs. Controls			
	1 week			ns
	3 weeks	14	3.57	0.003
	6 weeks	14	7.95	< 0.001
	Fructose			
	1 vs. 3 weeks	14	3.38	0.005
	1 vs. 6 weeks	14	8.64	< 0.001
	3 vs. 6 weeks	14	4.45	0.001
Akt				
	Fructose vs. Controls			
	1 week	14	5.26	< 0.001
	3 weeks	14	6.72	< 0.001
	6 weeks	14	10.53	< 0.001
	Fructose			
	All comparisons ns			

A univariate ANOVA on the TyG Index (**Fig. 2A**) revealed a main effect of diet ($F_{1,49} = 6.06$, $p < 0.02$), a main effect of duration ($F_{2,49} = 5.51$, $p < 0.01$), and a significant diet x duration interaction ($F_{2,49} = 5.52$, $p < 0.01$). Independent samples t-tests failed to reveal a difference between Fructose and Controls after 1 week of exposure ($p = 0.45$), but did reveal diet differences after 3 weeks ($t_{14} = 4.05$, $p < 0.01$), and 6 weeks ($t_{14} = 3.17$, $p < 0.01$). Further comparisons across durations for Fructose rats revealed a significant difference between 1- and 3-week rats ($t_{16} = 3.02$, $p = 0.008$), where 3-week Fructose rats scored higher on the TyG Index than 1-week Fructose rats, indicating a greater degree of insulin resistance relative to their respective controls. Comparisons between 6-week Fructose rats and the 1- or 3-week Fructose rats were not conducted due to the differences in sample dilution.

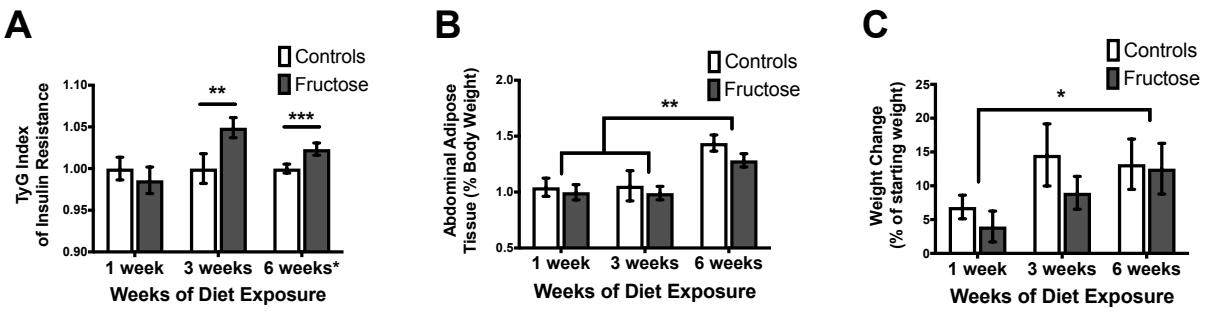

Figure 2. Peripheral Insulin Resistance and Body Weight. (A) The TyG Index of Insulin Resistance shows that after 3 weeks of fructose exposure, rats were significantly more insulin resistant than Controls. *6 week samples were differentially diluted, thus preclude direct comparison with 3- and 1-week samples. (B) Abdominal adipose tissue as a percentage of the final body weights reveal that, while 6-week rats had more abdominal white fat, there were no differences between diet groups. (C) Changes in body weight as a percentage of starting weights suggest that 6-week rats gained more weight than 1-week rats, but that diet had no impact on body weight.

Body weight and abdominal adiposity

A multivariate ANOVA on the percent of abdominal adipose tissue (weight of adipose tissue / final body weight; **Fig. 2B**) and the percentage change in body weight ((final weight – start weight)/ start weight; **Fig. 2C**) failed to reveal a main effect of diet (adiposity: $p = 0.20$; weights: $p = 0.25$), nor a diet x duration interaction (adiposity: $p = 0.78$; weights: $p = 0.75$), but did reveal a significant main effect of duration (adiposity: $F_{2,49} = 10.94$, $p < 0.001$; weights: $F_{2,49} = 3.27$, $p < 0.05$). Independent t-tests on the percentage of abdominal white fat revealed no difference between 1- and 3-week groups ($p = 0.95$), but did reveal a difference between 3- and 6-week groups ($t_{30} = 3.86$, $p = 0.001$), and 1- and 6-week groups ($t_{32} = 4.79$, $p < 0.001$), where longer exposures had a greater percentage of abdominal adipose tissue. Independent t-tests on the change in body weight failed to reveal a difference (when corrected for multiple comparisons) between 1- and 3-week groups ($t_{32} = 2.23$, $p = 0.03$), nor a difference between 3- and 6-week groups ($p = 0.77$), but did reveal a differences between 1- and 6-week groups ($t_{32} = 2.64$, $p = 0.013$), where 6-week exposed rats, unsurprisingly, gained more weight than 3-week exposed rats.

Experiment 2. A high-fructose diet decreases membrane-associated DAT in the NAc

Methods

Subjects and diet

Subjects were 20 male Sprague-Dawley rats, aged 6 weeks and weighing approximately 238.56 g ± 1.53 SEM at the start of diet exposure. Diets were identical to those described above in Experiment 1 (TD. 89247; 60% fructose and TD.98394 control diet, Envigo). Rats were maintained on their diets ad libitum for 5 weeks. After 5 weeks, rats were briefly anesthetized with isoflurane, decapitated, and brains rapidly removed, flash frozen in liquid nitrogen and stored at -80° C.

Dissections

At a cryostat, a 200 μm-thick slice was taken around the striatum (beginning approximately 1.60-1.70 mm anterior to bregma), laid flat, and the nucleus accumbens dissected with a scalpel. Samples were again frozen at -80° C until subcellular fractionation.

Subcellular fractionation

Methods were based on the "Basic Protocol" outlined in Hallett et al. [45]. In brief, brains were homogenized for 12-15 sec in 500 μl ice-cold TEVP buffer (10mM Tris HCl, 5mM NaF, 1mM Na_3VO_4, 1mM EDTA, 1mM EGTA, pH adjusted to 7.4) + 320 mM sucrose. Samples were centrifuged at 800 g for 10 min at 4° C, producing a supernatant (S1) and pellet (P1; discarded). Supernatant (S1) was removed, placed in a new tube, and centrifuged at 9,200 g for 15 min at 4^o C, producing a supernatant (S2) and pellet (P2; containing crude synaptosomal membranes). The supernatant (S2) was removed and placed in a specialty centrifuge tube (Beckman-Coulter, Brea, CA). Approximately 4.5 ml TEVP buffer + 35.6 mM sucrose buffer was added to the tube (until

full), and samples were centrifuged in an ultracentrifuge at 165,000 g for 2 hrs at 4° C, generating a supernatant (discarded) and pellet (P3; light membranes and recycling endosomes). The P3 pellet was rinsed in 50 µl TEVP, resuspended in 100 µl TEVP, vortexed for 5-10 sec to break up the pellet, and saved for polyacrylamide gel electrophoresis. The P2 pellet was rinsed in 50 µl TEVP + 35.6 mM sucrose, resuspended in 100 µl TEVP + 35.6 mM sucrose, and vortexed 5-10 sec to break up the pellet. P2 fractions were kept on ice for 40 min to hypo-osmotically lyse the samples, then centrifuged at 25,000 g for 20 min at 4° C. The resulting supernatant was discarded; the pellet (LP1; synaptosomal membranes) was rinsed once in 50 µl TEVP, resuspended in 100 µl TEVP, vortexed 5-10 sec, and stored at -80° C until polyacrylamide gel electrophoresis. Two samples were lost during processing (1 from each diet group).

Gel electrophoresis & Western blotting

Protein content was determined using the Bio-Rad DC Protein Assay kit (Bio-Rad, Hercules, CA). Sufficient sample for 13 µg protein were loaded into new sample tubes, along with 7.5 µl NuPAGE LDS 4X (lithium dodecyl sulfate) sample buffer (Invitrogen, Carlsbad, CA), 3 µl 1M dithiothreitol, and diluted with H_2O for a total volume of 30 µl. Samples were briefly vortexed prior to heating at 70° C for 10 min to denature proteins, then placed on ice for a minimum of 2 min prior to loading. Samples were loaded into Bio-Rad Mini Protein TGX 10% gels for separation by gel electrophoresis. Proteins were subsequently transferred to polyvinylidene fluoride membrane (PVDF) (Millipore, Burlington, MA). Nonspecific binding sites were blocked for 1 hr at room temperature in blocking buffer (5% nonfat dry milk in PBS). Blots were then incubated overnight at 35° C in primary antibody (1:2000 mouse monoclonal anti-α-tubulin [#05–829, Millipore], 1:2000 rabbit anti-DAT [#AB-2231, Millipore]. The next morning blots were rinsed in PBS + 0.1% tween-20 3 times, 10 min each, and left on a shaker at room temperature between rinses. Blots were incubated in secondary antibody (PBS, 0.1% tween-20, 2.5% milk, 1:10,000 each for Stabilized Peroxidase Conjugated Goat

Anti-Rabbit and Stabilized Peroxidase Conjugated Goat Anti-Mouse) for 1 hr at room temperature. After incubation, blots were rinsed 3 times as previously described. PageRuler Plus Prestained Protein Ladder (Thermo Fisher, Waltham, MA) was used for molecular weight estimation. Blots were cut at 35 and 130 kDa. Immunoblots were incubated in SuperSignal West Pico Stable Peroxide & Luminol/Enhancer (Thermo Fisher, Waltham, MA) for 5 min to permit chemiluminescent detection. Immunocomplexes were visualized using Bio-Rad ChemiDoc Touch Imagery.

Data analysis and statistics

Data were analyzed using GraphPad Prism (GraphPad Prism, La Jolla, CA). T-tests were used to compare Fructose rats versus Controls on body weights and DAT measures. Body weights are expressed as the percentage change in weight from their starting weight. DAT values were adjusted to internal tubulin controls, and relative density calculated with NIH ImageJ software (NIH, Rockville, MD). DAT values are expressed as the ratio of synaptosomal membrane DAT to synaptosomal membrane + endosomal DAT (i.e., synaptosomal DAT / (synaptosomal + endosomal DAT)), then normalized to control values.

Results

Body weight

A t-test was conducted on the change in body weight ((final weight – start weight) / start weight; **Fig. 3A**), which revealed a significant effect of diet ($t_{16} = 2.90$, $p = 0.01$), where Controls weighed more than Fructose rats.

DAT localization

A t-test was conducted on the proportion of DAT at the neuronal membrane (versus internalized) (**Fig. 3B**), which revealed a significant effect of diet ($t_{16} = 2.34$, $p = 0.03$), with Fructose rats having a significantly lower proportion of membrane-bound DAT than Controls.

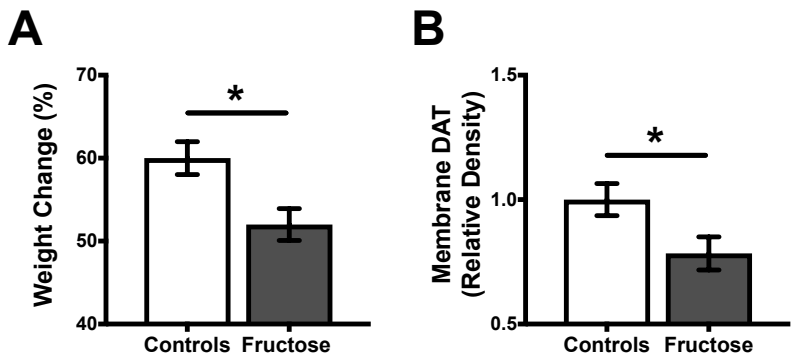

Figure 3. Changes in Body Weight and DAT Localization. (A) Fructose rats gained less weight than Controls, and (B) had proportionately fewer DATs at the synaptosomal membrane than Controls.

Experiment 3. A high-fructose diet disrupts dopamine reuptake

Methods

Subjects and diet

Subjects were 50 male Sprague-Dawley rats, aged 6 weeks and weighing approximately 200.29 g ± 2.27 SEM at the start of diet exposure. Diets were identical to those previously described, with the addition of two new pioglitazone-fortified diets. Pioglitazone (PGZ) is a peroxisome proliferator-activated receptor (PPAR) agonist that improves insulin sensitivity, and is a commonly used drug in the treatment of type 2 diabetes [46,47]. PGZ was ordered from Sigma-Aldrich (St. Louis, MO) and supplemented to the high-fructose and control diets by Envigo at a concentration of 200 ppm (approximately 10 mg/kg/day) [48,49], resulting in two additional groups: Fruc-PGZ and Con-PGZ. Rats were allowed ad libitum exposure to their diets for 5 weeks.

Carbon fiber electrode manufacture

Carbon fiber recording electrodes were constructed in-house by inserting a single carbon fiber (grade 34-700; Goodfellow Corp, Coraopolis, PA) into a glass tube (0.5 mm ID, 1.0 mm OD; AM Systems, Sequim, WA), and pulled to a fine tip around the fiber with a puller (Narishige, Tokyo, Japan). A silver wire was then coated in conductive silver epoxy (MG Chemicals, B.C. Canada) and inserted into the open end, and sealed with clear epoxy, leaving a short protruding end to allow connection to recording equipment. Prior to calibration, the exposed carbon fiber was trimmed to 100-200 μm. Electrodes were calibrated in a custom-made flow cell with 0.5, 1, and 5 μM dopamine standards in phosphate-buffered saline. Electrodes were soaked in 70% EtOH for 30-45 min prior to implantation.

Fast scan cyclic voltammetry

Fast scan cyclic voltammetry was used to identify changes in dopamine signaling in the NAc. After 5 weeks of diet exposure, rats were anesthetized with isoflurane, placed in a stereotaxic frame, and surgically implanted with a carbon fiber recording electrode (targeted at the NAc core: AP: +1.8 mm, ML: +1.4 mm, DV: -7.0 mm), a bipolar stimulating electrode (AP: -5.1 mm, ML: +1.0 mm, DV: between -7.0 and -9.0 mm, optimized as described below; Plastics One, Roanoke, VA), and an Ag/AgCl reference electrode (Basi, West Lafayette, IN) in the contralateral hemisphere. Recording equipment, consisting of a computer with HDCV software (University of North Carolina), potentiostat and current amplifier (Keithley Instruments, Solon, OH), was used to apply a triangular waveform (scanning from -0.4 to +1.3 to -0.4) at 400 V/s, at an initial scan rate of 60 Hz for 15-20 min (allowing the electrode to equilibrate), then 10 Hz for all recordings.

Dopamine was electrically evoked using a stimulus isolator (made in-house) to deliver a series of biphasic, 2 ms, 60 Hz pulses. Stimulations were administered as a series of 2 sec pulse trains along a range of 100 – 500 μA. The stimulating electrode was initially lowered to -7.0 mm below dura, at

which point the position was slowly optimized by producing brief stimulations (200 μA for 2 sec; 5 min apart), and lowering by 100-200 μm increments until a robust DA signal was identified. Once optimal placements were achieved, all stimulations were conducted at 5 min intervals. After a series of stimulations ranging from 100-500 μA at 2 sec durations, cocaine HCl (NIDA) dissolved in sterile saline was administered (10 mg/kg, i.p.), and, after 15 min, stimulations were repeated.

After all stimulations were completed, rats were immediately decapitated, brains quickly excised and flash frozen in liquid nitrogen, and stored at -80° C. Carbon fiber electrodes were immediately soaked in 70% EtOH and post-calibrated as previously described when possible, and individual calibration factors were used to convert DA currents to dopamine concentration estimates.

Data analysis and statistics

Reuptake kinetics were analyzed using GraphPad Prism (GraphPad Prism, La Jolla, CA). We modeled reuptake kinetics of the DAT by fitting a plateau followed by one-phase decay model (*Y = IF(X<X0, Y0, Plateau+(Y0-Plateau)*exp(-K*(X-X0)))*) to the falling phase of the curve (peak until 3 sec after stimulation) after subtracting from moment-of-stimulation baselines (thus all data are deltas from baseline). The one-phase decay model generates the time constant tau, which is a reliable and recommended measure of DA uptake [50]. Due to dramatic differences in amplitude between pre- and post-cocaine stimulations, pre- and post-cocaine stimulations were analyzed separately but otherwise identically. Because the rate of reuptake is dependent on the amplitude of the evoked signal, we employed a range of stimulation intensities (from 100-500 μA) in order to maximize the likelihood of finding similar peak amplitudes across all samples. Using the stimulation parameters that generated the most similar peak amplitudes, we further controlled for variation in peak amplitude by constraining the peak (Y_o) to be shared across all groups, allowing us to isolate differences in decay. High individual variability precluded fitting the function directly to individual data points,

thus it was fit to group means, with no weighting. Goodness-of-fit was confirmed using R square (all groups ≥ 0.99), and normality of residuals assessed with D'Agostino & Pearson omnibus K2 normality test (all groups passed) [51].

To evaluate DAT-mediated reuptake kinetics, we used nonlinear curve fitting on pre- and post-cocaine traces to generate the time constant tau, reported as means ± 99% confidence intervals. Confidence intervals describe the probability that the true value of interest is within a given range [52]. Values above or below the confidence interval's upper and lower limit (respectively) are not excluded, but improbable, with the degree of improbability increasing proportionally to distance from the mean. Non-overlapping confidence intervals represent two significantly different samples, while overlapping confidence intervals are generally considered to represent samples that may not be significantly different [52].

We then conducted ANOVAs (diet x drug) for pre-cocaine peak amplitude, weight change, and food consumption. Analyses were deemed significant when $p < 0.05$. Where significant effects were identified, independent samples t-tests were performed [53,54]. All data, unless otherwise noted, are presented as means ± SEM.

Results

Dopamine signal decay

As described above (in *Data Analysis and Statistics*), dopamine traces from the peak amplitude after stimulation to 3 sec after peak were fit to a non-linear model using a plateau followed by one phase decay exponential function. These data are descriptively shown in **Fig. 4**.

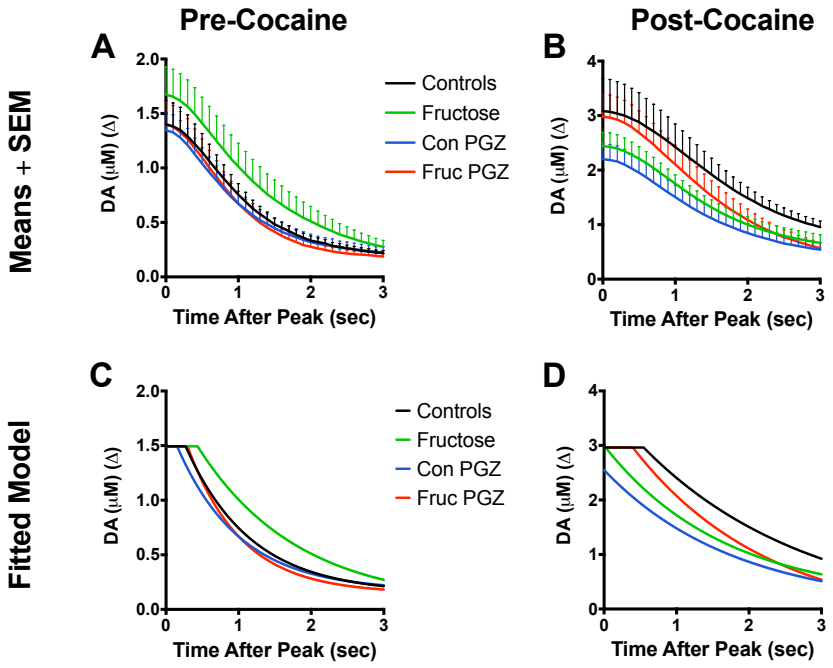

Figure 4. Dopamine From Peak Amplitude After Stimulation to 3 sec After Peak.

A & B. Dopamine concentration estimates (μM; normalized to the moment of stimulation), before (A) and after (B) 10 mg/kg (i.p.) cocaine (Means + SEM).

C & D. Nonlinear curves representing the fitted decay model before (C) and after (D) i.p. cocaine.

Tau: Quantifying rate of decay

To evaluate DAT-mediated reuptake kinetics, we used nonlinear curve fitting on pre- and post-cocaine traces to generate the time constant tau, reported as means ± 99% confidence intervals (**Fig. 5B** and **5C**). Prior to cocaine administration, Fructose rats demonstrated significantly slower

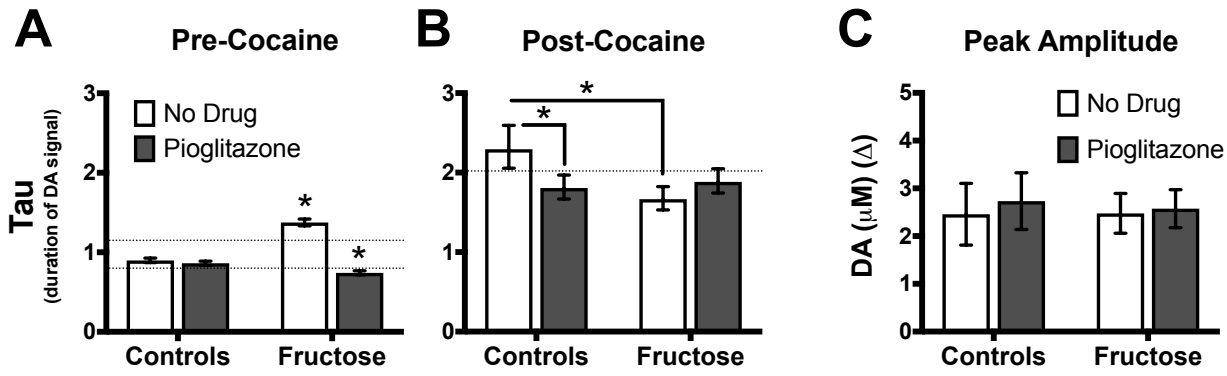

Figure 5. Changes in DAT-Mediated Reuptake and Dopamine Release. A & B. Tau is a time constant (in sec) generated from nonlinear modeling representing the rate of signal decay, where larger values represent slower decay (i.e., prolonged reuptake). (A) Prior to cocaine administration, Fructose rats showed the slowest dopamine reuptake, while Fruc-PGZ rats displayed the fastest. *denotes statistically significant effects from all other groups. (B) After cocaine administration, Controls showed the slowest dopamine reuptake. (C) Peak amplitude after 2 sec, 200 μA stimulation. Groups did not differ in peak evoked dopamine after equal stimulation parameters. (A & B: Means ± 99% CI; C: Means ± SEM)

dopamine reuptake than Controls or either pioglitazone-treated group, while Fruc-PGZ rats displayed the most rapid dopamine reuptake, with significantly smaller tau values than any other group (**Fig. 5A**). After 10 mg/kg cocaine, Control rats displayed significantly slower dopamine reuptake than Fructose or Con-PGZ rats (**Fig. 5B**).

These data suggest that Fructose rats displayed prolonged DAT-mediated dopamine reuptake, consistent with a disruption in DAT function or expression. Rats fed the same fructose diet supplemented with pioglitazone (group Fruc-PGZ) did not show this same deficit in dopamine reuptake, and displayed, unexpectedly, *more* efficient reuptake than Controls. Further, Controls were most affected by a cocaine challenge, displaying the most prolonged dopamine reuptake. Fructose and Con-PGZ rats displayed faster dopamine reuptake than Controls.

Peak amplitude

We evaluated peak amplitude after a 2 sec, 200 μA stimulation for changes in evoked dopamine release (**Fig. 5C**). An ANOVA on peak amplitude (diet x drug) failed to reveal a significant effect of diet ($p = 0.89$), drug ($p = 0.71$), nor an interaction between these factors ($p = 0.86$), suggesting any diet or drug effects were isolated to dopamine reuptake, not release.

Changes in body weight

We evaluated differences in weight change and food consumption (**Fig. 6**). An ANOVA (diet x drug) of the

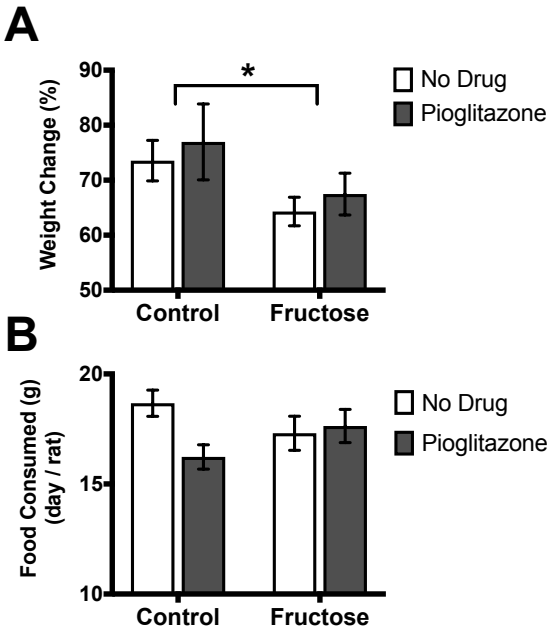

Figure 6. Changes in Body Weight and Food Consumption. (A) Rats on the control diets increased their body weight more than rats on the fructose diet. (B) There was no difference in average daily food consumption. (Means ± SEM)

weight gained (expressed as a percentage of initial weight; **Fig. 6A**) revealed a significant effect of diet ($F_{1,50}$ = 4.73, p = 0.03), where Fructose rats gained less weight than Controls. There was no effect of drug (p = 0.45), and no diet x drug interaction (p = 0.98). An ANOVA (diet x drug; **Fig. 6B**) of average food consumed per day failed to reveal an effect of diet (p = 0.978), or drug (p = 0.159), but did suggest a (nonsignificant) trend towards an interaction between these factors (p = 0.070).

Experiment 4. A high-fructose diet disrupts incentive motivation for food rewards

Methods

Subjects and apparatus

Subjects were 40 male Sprague-Dawley rats approximately 8 weeks old, weighing approximately 262.48 g ± 1.41 SEM at the start of the experiment. During pre-training, rats were food restricted to 85% of their free-feeding body weight. Body weight and diet consumption were measured every other day. All behavioral training and testing occurred in identical sound- and light-attenuating operant chambers (Med Associates, St. Albans, VT). Each chamber was equipped with a house light, retractable lever, clicker, white noise generator, and food cup delivering food pellets (Bioserv, Flemington, NJ).

Behavioral training

Rats were given 10 days of instrumental training with the levers continuously extended, where each session lasted 30 min, or until 30 food pellets were earned. Reinforcement schedules were gradually shifted over days from continuous reinforcement (CRF; each press = 1 food pellet) to random-interval (RI) 45 sec, where each press after 45 sec earned a food pellet (CRF = 3 days, RI 5 sec = 1 day, RI 10 sec = 1 day, RI 20 sec = 1 day, RI 30 = 1 day, RI 45 sec = 3 days). After this, rats were

then given 8 days of Pavlovian conditioning. Each session consisted of the presentation of one of two auditory cues (CS^+; either click or whitenoise, counterbalanced) for 2 min, during which they would receive food pellets delivered on a variable 30 sec interval schedule. Each session consisted of 10 trials, and each trial was separated by a variable 3 min inter-trial interval (ITI).

Diet

Following Pavlovian training, rats were divided as in Experiment 3 into either: Control, Fructose, Control + pioglitazone (Con-PGZ), and Fructose + pioglitazone (Fruc-PGZ) and fed the assigned diet ad libitum for 4 weeks. Diets were identical to those described in Experiment 3. After the diet exposure phase, rats were returned to moderate food restriction, where they were given ad libitum access to their experimental diets for only 2 hrs per day. This protocol was used to ensure motivation to work for food.

Retraining and PIT testing

Rats were given two days of behavioral retraining, consisting of 1 day of instrumental retraining using a RI 45 sec schedule of reinforcement (identical to the last day of instrumental training) and 1 day of Pavlovian conditioning. The Pavlovian conditioning retraining session was conducted in two sessions separated by approximately 2 hrs. The first session was identical to the regular initial Pavlovian conditioning sessions, except rats were presented with a novel auditory sound (CS^o; click or whitenoise) not previously used, which would *not* be paired with any pellet delivery. The second session was identical to the initial conditioning sessions, i.e., the same CS^+ stimulus was used and paired with reward.

Rats were given one PIT test, conducted in extinction (i.e., no food pellets were delivered to avoid any new learning). The PIT test began with 15 minutes of instrumental extinction, in order to

suppress response rates, thereby facilitating detection of the PIT effect. After this, rats began the cued phase of the test, where rats were presented with each of the auditory cues (CS^+ and CS^o) 4 times, presented in an ABBA design. Cues were presented for a fixed 2 min duration, and separated by a fixed 6 min ITI.

Biochemical analysis

At the end of the experiment rats, rats were food deprived 8-10 hours, briefly anesthetized with isoflurane and decapitated. Brains were rapidly removed, flash frozen in liquid nitrogen and stored at -80° C for future analysis. Trunk blood was collected (400 µl) and mixed with 60 µl 10% EDTA and centrifuged at 2000 rpm for 20 min at 4° C. Samples were processed by an outside laboratory for glucose and triglyceride content. Data are normalized to Control values.

Data analysis and statistics

Data were analyzed with SPSS (IBM, Armonk, NY). Lever presses and food cup entries were converted to elevation scores, where baseline was considered the 1 min period immediately preceding the cue period. Because each cue was presented for 2 min, scores were halved to get a per minute average (to account for the use of a 1 min baseline). Scores were then converted to the ratio of responding during the CS^+ versus the CS^o (CS / (CS^+ + CS^o). Food cup entries were transformed identically. Differences in CS responding were analyzed via rmANOVA (cue x diet x drug), and total food cup entries were included as a covariate in the analysis of lever presses, and vice versa, to control for response competition. Effects were considered significant when $p < 0.05$. Independent and paired samples t-tests were performed for select a priori planned comparisons, using the Holm correction procedure for multiple comparisons [53,54]. All data are presented as means ± SEM.

Results

PIT testing

Lever pressing: To determine the impact of fructose and pioglitazone exposure on incentive motivation for a food reward, we examined lever-pressing during the PIT test. A rmANOVA (cue x diet x drug) on lever presses revealed a significant main effect of the cue ($F_{1,35} = 7.01$, $p = 0.01$), a significant cue x diet interaction ($F_{1,35} = 5.37$, $p = 0.13$), a significant 3-way interaction ($F_{1,35} = 4.22$, $p = 0.047$), but failed to reveal a significant cue x drug interaction ($p = 0.23$) (**Fig. 7A**). There were no significant main effects of diet or drug (all p's > 0.25). Paired t-tests (CS^+ versus CS^o) revealed a significant increase in lever pressing in response to the CS^+ versus the CS^o in Controls ($t_9 = 5.46$, $p < 0.001$), Con-PGZ ($t_9 = 3.93$, $p = 0.003$), and Fruc-PGZ ($t_9 = 6.40$, $p < 0.001$), but not in Fructose rats ($p = 0.34$).

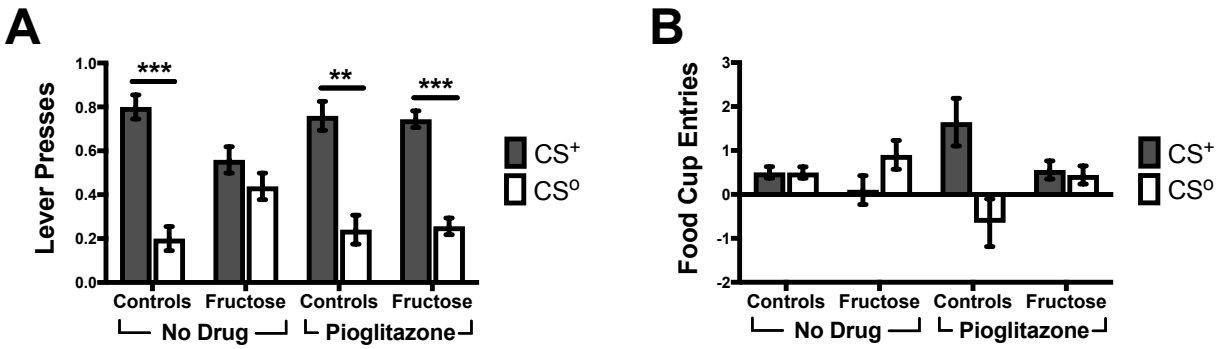

Figure 7. Pavlovian-to-Instrumental Transfer Test. Lever presses (A) and food cup entries (B) during the CS^+ and CS^o, expressed as the ratio between CS^+ and CS^o responding. Fructose rats increased lever pressing in response to both cues.

Food cup entries: An identical analysis of food cup entries failed to reveal a significant effect of cue ($p = 0.97$), but did reveal a significant cue x diet interaction ($F_{1,35} = 4.82$, $p = 0.04$), a significant cue x drug interaction ($F_{1,35} = 5.51$, $p = 0.03$), but failed to reveal a three-way interaction ($p = 0.33$) (**Fig. 7B**). Paired t-tests failed to reveal a significant increase in food cup entries in response to the CS^+

versus the CS^o in any group (Controls: $p = 0.99$; Fructose: $p = 0.25$; Con-PGZ: $p = 0.06$; Fruc-PGZ: $p = 0.79$), though there was a trend towards significance in the Con-PGZ group.

Biochemical analysis

To determine whether pioglitazone treatment would restore insulin sensitivity in fructose-exposed rats, we analyzed blood glucose and triglycerides using the TyG index of insulin resistance. A two-way ANOVA (diet x drug) of insulin resistance revealed significant main effects of diet ($F_{1,36} = 22.89$, $p < 0.001$) and drug ($F_{1,36} = 7.52$, $p < 0.01$), but no significant interaction ($p = 0.169$) (**Fig. 8**). We conducted a priori planned comparisons between Controls versus Fructose, Controls versus Fruc-PGZ, and Fructose versus Fruc-PGZ, therefore used Holm-corrected alpha levels for a family of three comparisons. These t-tests revealed that Fructose rats were more insulin resistant than Controls ($t_{18} = 4.57$, $p < 0.001$), while simultaneous treatment with pioglitazone reduced the severity of insulin resistance ($t_{18} = 3.00$, $p < 0.01$). However, simultaneous treatment of Fructose rats with pioglitazone was not sufficient to fully restore insulin levels to those seen in Controls ($t_{18} = 2.20$, $p = 0.04$).

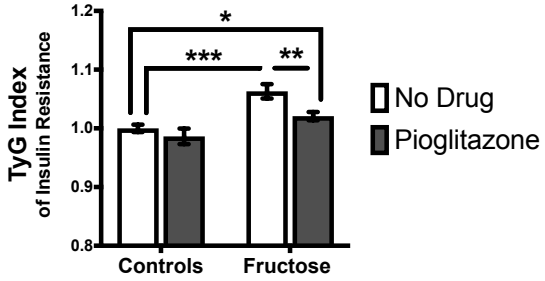

Figure 8. Peripheral Insulin Resistance. Fructose rats were more insulin resistant than Controls or Fruc-PGZ rats. Fruc-PGZ rats remained moderately more insulin resistant than Controls.

Experiment 5. A high-fructose diet increases dopamine release during a PIT test

Methods

All behavioral procedures in Exp. 4 were identical to those described in Exp. 3 with exceptions noted below.

Subjects and apparatus

Subjects were male Sprague-Dawley rats (n = 29, controls = 14, fructose = 15) approximately 6 weeks old, weighing approximately 194.82 g ± 1.77 SEM at the start of the experiment.

Behavioral training

Behavioral training was identical to Exp. 3, with the exception that Pavlovian conditioning was conducted first, followed by the instrumental training phase. Instrumental training was conducted in 8 days, with CRF only on Day 1 before gradually increasing the response contingency each day until RI 45 sec, where they remained for the last three days, as in Experiment 4.

Electrode fabrication

Carbon fiber microelectrodes were manufactured and pre-calibrated in house. A single carbon fiber (grade 34-700; Goodfellow Corp, Coraopolis, PA) was inserted into a fused silica tubing (12 mm long, 90 μm wide; Polymicro Technologies). On one end of the electrode, the carbon fiber was pulled through Devcon two-component epoxy (ITW Performance Polymers, Glenview, IL), sealing one end of the silica tubing while leaving a short length of fiber protruding. Conductive silver epoxy (MG Chemicals, B.C. Canada) was used at the other end of the electrode to connect the carbon fiber to a connector pin to permit future recordings. After curing, this silver epoxy was sealed with Devcon two-component epoxy to insulate and secure the connections. Prior to calibration, the exposed carbon fiber was trimmed to 100-200 μm. Electrodes were calibrated in a custom-made flow cell with 0.25, 0.5, 1, and 5 μm dopamine standards in phosphate-buffered saline.

Reference electrodes were constructed by trimming a silver wire to 0.5 cm and securing it to a connector pin with conductive silver epoxy (MG Chemicals, B.C. Canada). After curing, the connection was insulated with Devcon two-component epoxy (ITW Performance Polymers,

Glenview, IL). Prior to surgery, reference electrodes were soaked in 6% bleach for 10-20 hrs. Immediately prior to surgery, reference electrodes were rinsed with water, dipped in 2% nafion solution, and allowed to dry before implantation.

Diet and surgery

After the last day of instrumental training, rats were divided into either Fructose or Control groups, and fed their assigned diet ad libitum for 4 weeks. The day after beginning the ad libitum phase, electrodes were surgically implanted to allow in-vivo dopamine monitoring. Rats were anesthetized with isoflurane (5% induction, 1.5-2.5% maintenance) and prepared for aseptic surgery, during which they were chronically implanted with a precalibrated carbon fiber microelectrode aimed at the nucleus accumbens core (AP: +1.8 mm, ML: +1.4 mm, relative to bregma, and 6.5 mm below dura) and an Ag/AgCl reference electrode in the contralateral cortex. Both electrodes were affixed to the skull with dental cement. Rats were singly housed following surgery.

Behavioral retraining

Following the diet exposure phase, rats were returned to moderate food restriction, where they were given 15 g of their respective diets per day. After 1 day of moderate food restriction, rats began behavioral retraining in operant boxes equipped with fast-scan cyclic voltammetry (FSCV) monitoring equipment, which ensured habituation to the final testing environment. Retraining consisted of 3 days of instrumental retraining using a RI 45 sec schedule of reinforcement (identical to the last day of instrumental training), where in the first session rats were untethered, but in all subsequent retraining and testing sessions rats were tethered to the voltammetric recording equipment. The Pavlovian conditioning sessions were conducted in two sessions in one day (separated by 2-3 hrs): session 1 consisted of a novel auditory sound (CS^o; click or whitenoise) not

previously used, that would *not* be paired with any pellet delivery. Session 2 was conducted identically to initial CS^+ conditioning sessions during initial conditioning.

Dopamine detection with fast-scan cyclic voltammetry

On the day of the PIT test, rats were tethered to FSCV recording equipment via an electrical swivel (Crist Instruments, Hagerstown, MD). A custom-made voltammetric potentiostat was used to apply a triangular waveform (−0.4 V to +1.3 V at 400 V/s; scan rate of 10 Hz) to the carbon fiber microelectrode through a head-mounted amplifier. Dopamine at the electrode surface undergoes oxidation and reduction reactions at approximately +0.65 V and −0.2 V, respectively. Using background subtraction, these changes in current reveal the distinct dopamine cyclic voltammogram signature that characterizes dopamine detection. Individual day-of-testing reference offsets were used to account for reference electrode degradation over time, and to bring the dopamine oxidation reaction to occur between +0.65 – +0.75. Random, unpredicted delivery of 3 food pellets was used to confirm appropriate offsets and dopamine detection prior to beginning testing. If dopamine detection was unsuccessful or unclear, rats were returned to their homecages and retested 2-3 days later. Principal component regression analysis (Tar Heel CV software) of the voltammetric data was used to distinguish DA currents from those arising from other electroactive species. When dopamine was successfully detected in response to a pellet probe, behavioral testing was initiated after allowing the background current to stabilize (~30–60 min).

Pavlovian-to-instrumental transfer testing

All PIT tests were conducted between 35 and 42 days post surgery. On test day, rats were tethered and the electrode allowed to equilibrate for 30-60 min. The reference electrode offset was adjusted

such that dopamine oxidation in response to a unsignaled pellet delivery occurred between +0.65 – +0.75. After this, rats were given one PIT test identical to that described in Experiment 4.

Electrode placement verification

After behavioral testing, rats were deeply anesthetized with isoflurane until unconscious. A custom-made lesion maker was used to apply a 30-45 sec current to the working electrode in order to provide a lesion, facilitating electrode placement verification. Rats were subsequently decapitated, brains excised and placed in fixing solution (10% formalin, 30% sucrose), stored at 4° C until slicing at 50 μm thickness. Slices were mounted on glass slides and stained with cresyl violet. Light microscopy was used to examine electrode placement in the nucleus accumbens.

Data analysis and statistics

Behavioral analysis: Lever presses and food cup entries were analyzed with SPSS (IBM, Armonk, NY), as elevations ratios from the average baseline responding (CS / (pre-CS + CS), where baseline was considered the 1 min period immediately preceding the cue period. Because each cue was presented for 2 min, scores were halved to get a per-minute average (to account for the use of a 1 min baseline). Responding during the CS^{+} and CS^{o} was analyzed via rmANOVA. Due to the extensive equilibration required on test day (30-60 min), responding when tethered extinguishes quickly, thus we analyzed only the first two presentations of each CS. All data, unless otherwise noted, are presented as means + SEM.

Neurochemical analysis: To quantify changes in oxidation current over time, we used background subtraction from 1 sec before each cue presentation and 4 sec before each lever press. Chemometric analysis using Tar Heel CV software was used to isolate changes in current as a result of dopamine redox reactions from those resulting from other electroactive species. The training set used for

chemometric analysis included cycling voltammograms representing various intensities of in vivo dopamine, pH currents, and background drift. Peri-event (± 5 sec) current data were converted to dopamine concentration estimates using individual pre-implant calibration factors (average calibration factor: Controls, 33.17 nM/nA ± 4.49 SEM; Fructose, 31.38 nM/nA ± 4.27 SEM), and normalized to the moment of CS onset and 3 sec before lever presses. Because of the long time course involved in testing (i.e., 30-60 min of pre-test calibration, and a 1 hr test), subject engagement with the task was greater in the beginning of the PIT test. Therefore, we restricted our neurochemical analysis to the first two presentations of each CS (averaged). Dopamine traces were then plotted in GraphPad Prism, from which area under the curve (AUC) and maximum (peak) amplitude were calculated. AUC was calculated from the moment of CS onset to 4 sec post-onset, or 3 seconds before a lever press to 4 sec after a lever press. To identify meaningful increases in dopamine from random fluctuations, peaks defined by fewer than 4 adjacent points were ignored, as were peaks less than 5% of the distance from the minimum to maximum peak. AUC and peak dopamine concentration were calculated for each individual trace. For each of these two measures, each variable (CS onset and lever presses during each CS) was analyzed as an rmANOVA, and, if significant, followed by a priori CS^+ versus CS^o paired t-tests for each diet group.

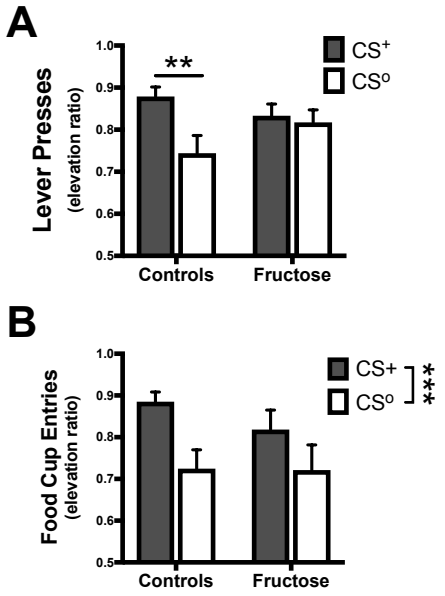

Figure 9. Pavlovian-to-Instrumental Transfer Test. Lever presses (A) and food cup entries (B) during the CS^+ and CS^o, expressed as the elevation ratio from baseline. Fructose rats increased lever pressing in response to both cues.

Results

PIT testing

To determine the impact of fructose exposure on incentive motivation for a food reward, we examined lever presses during

the first four cue presentations of the PIT test (two trials each of the CS^+ and CS^o). A rmANOVA (cue x diet) on lever presses (**Fig. 9A**) revealed a significant main effect of the cue ($F_{1,27} = 9.30$, $p < 0.01$), and a significant cue x diet interaction ($F_{1,27} = 5.80$, $p = 0.02$), but no significant effect of diet ($p = 0.71$). Paired t-tests revealed a significant increase in lever pressing for the CS^+ versus the CS^o only in Controls ($t_{13} = 4.25$, $p < 0.01$), but not in Fructose rats ($p = 0.68$). An identical analysis of food cup entries (**Fig. 9B**) revealed a significant main effect of cue ($F_{1,27} = 38.92$, $p < 0.001$), but no interaction with diet ($p = 0.14$), nor a main effect of diet ($p = 0.58$).

Neurochemical analysis

To determine the impact of fructose exposure on dopamine release in the nucleus accumbens, we plotted traces around CS onsets and lever presses during the CS^+ and CS^o (**Fig. 10**). We then examined area under the curve (AUC) and peak amplitude for these two variables (CS onsets and

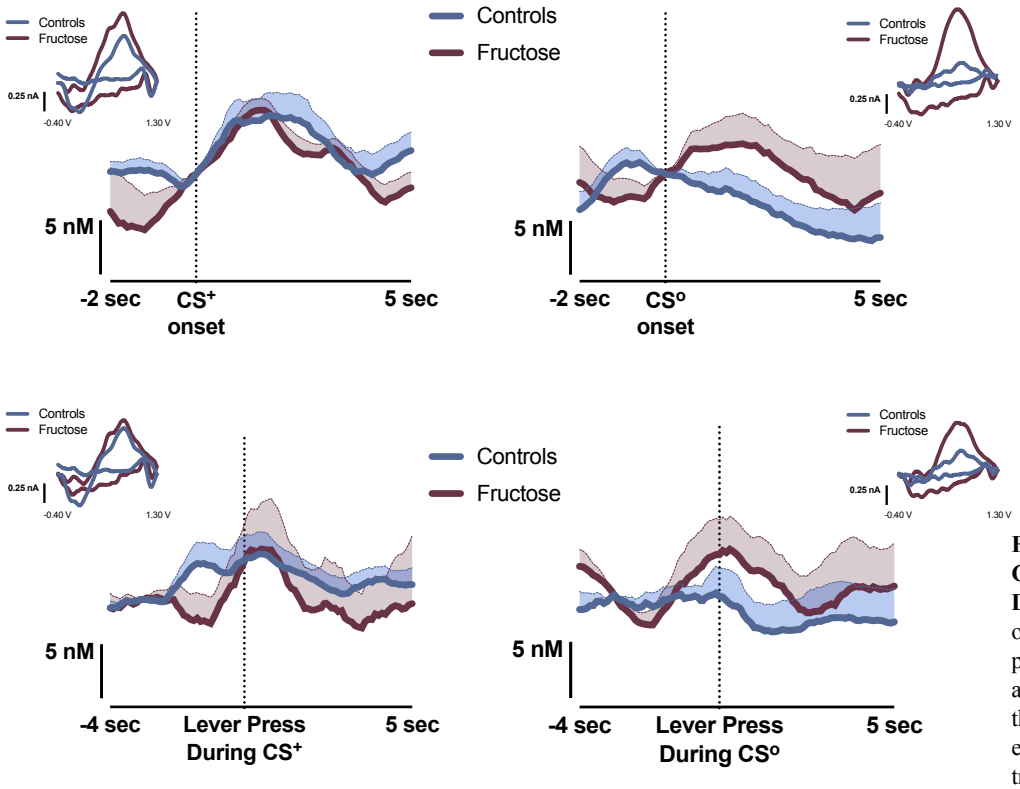

Figure 10. Dopamine Concentration Traces During a PIT Test. CS onset includes the first two presentations of each trial, averaged. Lever presses are the first two presses during each of the first two CS trials (averaged). Means + SEM (shaded area).

lever presses during the CSs) during the first four cue presentations of the PIT test (two trials each of the CS^+ and CS^o). See **Table 2** for statistics.

Table 2. Neurochemical Analysis

CS Onset	**df**	**F**	**Sig.**
AUC (rmANOVA)			
Cue	27	7.85	< 0.01
Diet			*0.50 ns*
Cue x Diet	27	4.67	< 0.04
Peak Amplitude (rmANOVA)			
Cue	27	8.06	< 0.01
Diet			*0.36 ns*
Cue x Diet	27	4.70	< 0.04
Paired t-tests	**df**	**t**	**Sig.**
AUC			
Controls	27	3.45	0.0019
Fructose			*0.65 ns*
Peak Amplitude			
Controls	27	3.48	0.0017
Fructose			*0.63 ns*
Lever Presses During CSs	**df**	**F**	**Sig.**
AUC (rmANOVA)			
Cue	27	6.87	< 0.05
Diet			*0.31 ns*
Cue x Diet			*0.55 ns*
Peak Amplitude (rmANOVA)			
Cue			*0.26 ns*
Diet			*0.38 ns*
Cue x Diet			*0.68 ns*
Paired t-tests	**df**	**t**	**Sig.**
AUC			
Controls			*0.03 ns*
Fructose			*0.16 ns*
Peak Amplitude			
Controls			*0.29 ns*
Fructose			*0.60 ns*

Non-significant comparisons are italicized and presented for reader benefit.

CS onset: A (cue x diet) rmANOVA of AUC (**Fig. 11A**) revealed a significant effect of cue ($F_{1, 27} = 7.85$, $p < 0.01$), a significant interaction ($F_{1, 27} = 4.67$, $p < 0.04$), but failed to reveal an effect of diet ($p = 0.50$). A priori paired t-tests (CS^+ versus CS^o) for each diet group revealed a significant difference in Controls ($t_{27} = 3.45$, $p < 0.01$), but not in Fructose rats ($p = 0.65$). An identical analysis for peak amplitude (**Fig. 11B**) revealed a significant effect of cue ($F_{1, 27} = 8.06$, $p < 0.01$), a significant interaction ($F_{1, 27} = 4.70$, $p < 0.04$), but failed to reveal an effect of diet ($p = 0.36$). A priori paired t-tests (CS^+ versus CS^o) for each diet group revealed a significant difference in Controls ($t_{27} = 3.48$, $p < 0.01$), but not in Fructose rats ($p = 0.63$).

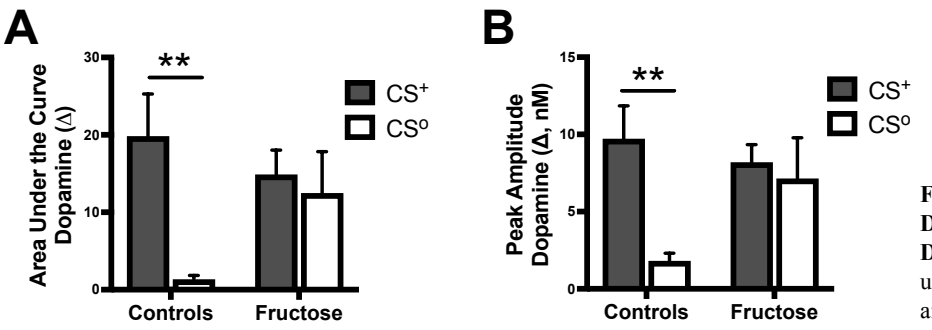

Figure 11. Change in Dopamine After CS Onset During a PIT Test. (A) Area under the curve, and (B) peak amplitude (nM).

Lever presses during each CS: Identical analyses were conducted for lever presses during the CSs. A (cue x diet) rmANOVA of AUC (**Fig. 12A**) revealed a significant effect of cue ($F_{1, 27} = 6.87$, $p < 0.05$), but failed to reveal an effect of diet ($p = 0.50$) or interaction ($p = 0.55$). An identical analysis for peak amplitude (**Fig. 12B**) failed to reveal an effect of cue ($p = 0.26$), diet ($p = 0.38$), nor an interaction ($p = 0.68$).

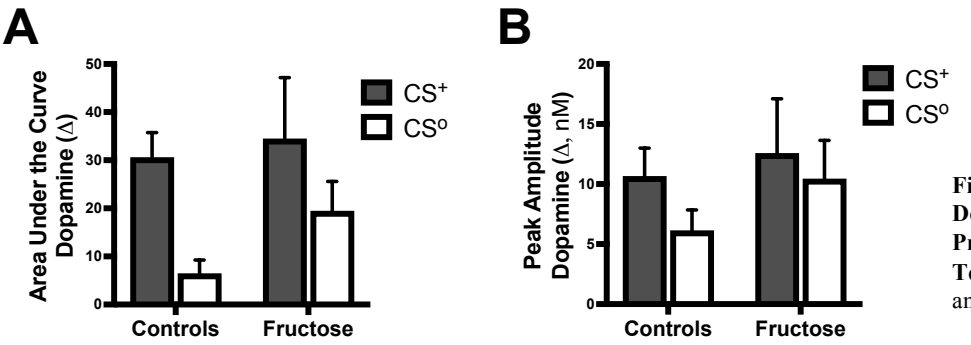

Figure 12. Change in Dopamine While Lever Pressing During CSs in a PIT Test. (A) Area under the curve, and (B) peak amplitude (nM).

Placement verification

Electrode placements were verified by light microscopy. Placements falling outside the nucleus accumbens resulted in removal from the experiment (n = 3) (**Fig. 13**).

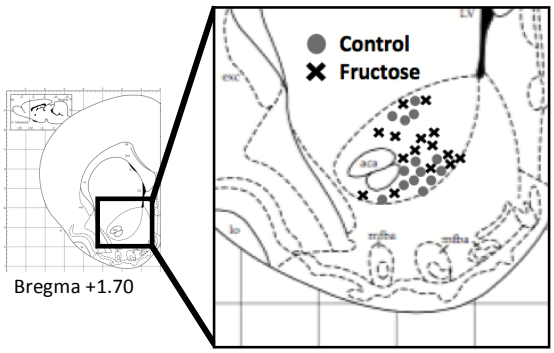

Figure 13. Electrode Placements for Individual Rats. All placements are between +2.00 and +1.40 mm AP from bregma. Reprint from Paxinos & Watson [115].

Discussion

We report that a high fructose diet can increase reward-seeking and potentiate phasic dopamine release in response to reward-paired and neutral environmental cues. Further, this diet also resulted in decreased insulin signaling in the ventral midbrain, decreased membrane-bound DAT in the nucleus accumbens, and prolonged dopamine signaling in the ventral striatum. Our results suggest that

disrupted insulin sensitivity may potentiate maladaptive reward-seeking behavior via dysregulation of the DAT. Notably, dopamine reuptake and reward seeking were normalized with simultaneous pioglitazone administration, further implicating insulin dysfunction in our reported deficits. Moreover, we report these changes in the absence of obesity, a frequent confound in studies examining the effects of diet on neurochemistry and behavior.

A high-fructose diet drives indiscriminate salience attribution

Previous reports that neuronal insulin is positively associated with DAT translocation to the membrane and function led to the prediction that neuronal insulin resistance would downregulate DAT activity, increase extracellular dopamine, and potentiate cue-invigorated reward-seeking as a result of increased dopamine sensitization during exposure to reward-paired cues. We initially expected greater magnitude of CS^{+}-invigorated reward-seeking and phasic dopamine release in our fructose-exposed rats, but instead report such events in response to both reward-paired and otherwise "neutral" cues. Indeed, our fructose-exposed rats demonstrated increased behavioral and dopaminergic responsivity to "neutral" cues. Their indiscriminate lever pressing suggests an increased susceptibility to the excitatory and motivational effects of environmental cues, including generalizing to those that are similar to, but distinct from, those previously paired with reward. While the neutral cue was never explicitly paired with a reward, it was still presented in a similar environment: the CS^{o} session occurred in identical operant boxes under the same handling procedures to the CS^{+}. Importantly, in our last experiment, the fructose group checked the food cup more during the reward-paired cue than during the neutral cue, suggesting an intact capacity to *distinguishing* between the cues.

Our fructose-exposed rats appeared to overgeneralize the excitatory response-invigorating effects of the CS^{+} to the seemingly neutral CS^{o} stimulus. Although the CS^{o} was never directly paired with

reward, it may have acquired (or been attributed) latent motivational properties due to it perceptual similarity to the CS^{+}, or through its second-order relationship with reward, in that it was presented in a context strongly associated with food reward. Regardless, it is not uncommon for "neutral" or ambiguous cues to acquire incentive motivational properties. For instance, previous studies have shown that cues that are presented in a random fashion with respect to food reward can still acquire the ability to stimulate food-seeking behavior [56]. Similarly, cues that signal the cancelation of food access acquire the ability to potentiate feeding [57], even though such a relationship might be expected to support inhibitory rather than excitatory learning. Although the CS^{o} stimulus used in the current experiment did not elicit an excessive motivational influence over reward seeking in our control rats, fructose exposure appeared to expose this underlying motivational influence, either through over-attribution of incentive salience to the CS^{o}, or through a nonspecific reduction in the motivational threshold for the instigation of reward-seeking behavior.

Dopamine sensitization drives incentive salience for "neutral" cues

The maladaptive CS^{o}-invigorated reward seeking seen in our fructose-exposed rats may result from sensitized mesolimbic dopamine transmission arising from increased dopamine signaling due to deficits in dopamine reuptake. The capacity of environmental cues to become meaningful, salient and "wanted" is thought to rely on sensitization of the mesolimbic dopamine region [26,58,59]. The theory of incentive motivation [29] has been championed primarily as an explanation for how psychostimulant drugs come to readily promote craving and relapse, an explanation which maps well onto "food addiction." Psychostimulants and food rewards share the ability to promote striatal dopamine release, the repeated synaptic and extracellular presence of which is thought to expedite associative learning between drug effects and environmental cues (e.g., drug paraphernalia, the smells and sight of favorite foods). Over time, "incentive salience" is attributed to the mental representations of such cues associated with activation of the dopamine system, causing them to

become attractive and "wanted." While our fructose-exposed rats did not have multiple experiences with the CS^{o} over days (as they did with the CS^{+}), one session after the development of insulin resistance may have been sufficient to cause the attribution of salience to a stimulus only loosely associated with reward (i.e., presented in a context known to provide food rewards). Conditions that support robust PIT effects, such as the optimal PIT paradigm employed here or psychostimulant sensitization [26], are associated with large dopamine release events time locked to salient events such as bouts of enthusiastic lever pressing and cue onsets [25,26]. That our fructose-exposed rats demonstrated this same effect in response to our neutral cue (Fig. 11A) indicates they may have attributed excessive salience and meaning to this stimulus. Interestingly, dopamine neurons will fire in response to stimuli known to the subject to explicitly *not* predict a reward, but to a lesser degree than firing in response to cues that do, suggesting dopamine neurons may support stimulus generalization [60–63]. Indeed, this is consistent with our in-vivo FSCV data suggesting fructose-exposed rats may have generalized reward-paired representations of the CS^{+} to the CS^{o}.

As discussed above, striatal dopamine is thought to promote associative learning between rewards and the stimuli that predict them. In our fructose rats, increased mesolimbic dopamine transmission as a result of downregulated reuptake may have promoted associative learning about the distal events that predict food reward in the operant chamber. Recent evidence from in-vivo voltammetry suggests that, with learning, dopamine concentrations begin to rise increasingly early in an action-sequence task, backpropogating away from reward delivery to the most distal cue [64]. Our fructose-exposed rats may have experienced such an effect, associating the chamber itself, and any auditory cue therein, with reward. Our results in Figures 9A and 11A fit well with such data: while our fructose-exposed rats do not show increased CS^{+}-evoked dopamine and reward seeking above and beyond that of controls, the indiscriminant nature of these effects is consistent with a hyperactive associative learning system, attributing salience to cues in the environment only loosely paired with food.

Indeed, access to sucrose (of which fructose is a component), above and beyond "regular" foods, has been shown to activate the mesolimbic reward system: sucrose consumption will repeatedly release dopamine in the nucleus accumbens shell [65,66], alter the expression [67] and availability [68] of dopamine receptors, and facilitate behavioral and locomotor sensitization to a dopamine agonist [69–71] (and vice versa [72]), effects thought to result from increases in extracellular dopamine in the nucleus accumbens [65,66,73]. A recent study also found that insulin, released in response to a caloric load such as sucrose, potentiates striatal dopamine release and can function independently as a reward signal [74]. This further supports our hypothesis that such dietary interventions may impact the dopaminergic systems involved in learning about and responding to reward-paired cues [75–78]. Importantly, dopamine sensitization as a result of repeated sucrose-induced dopamine release may occur acutely as a result of the tastant's orosensory components, not due to post-ingestive, metabolic processes, as even sucrose sham feeding promotes dopamine release [66,79]. To our knowledge, these effects have not been tested using fructose (one of two saccharides in sucrose), but if the effects are due to the orosensory component of sweetness [66,79], we would anticipate similar results, as fructose has been shown to be similarly palatable [80], and both fructose and sucrose are capable of eliciting consummatory behavior based on their orosensory components alone, independent of their postingestive effects [81].

Insulin resistance alters DAT's ability to promote dopamine reuptake

We report fructose-induced disruptions in DAT-mediated reuptake, likely due to decreased DAT availability at the membrane. Extracellular dopamine transmission is terminated by rapid dopamine reuptake into presynaptic terminals by the DAT [82,83]. Indeed, the powerful reinforcing properties of psychostimulants come largely from their ability to block the DAT, thereby indirectly functioning as dopamine agonists [84,85]. Recently, insulin has been indicted as a positive regulator of the DAT,

increasing DAT mRNA [15,21], DAT's presence at the membrane [16,19], and functional activity [17,86,87]. Conversely, decreasing insulin by fasting [21,22] downregulates DAT activity. Here, we show that our high-fructose diet not only induces neuronal insulin resistance in the ventral midbrain (Fig. 1), but also decreases the proportion of DAT at the synaptosomal membrane-associated fraction relative to that measured in the endosomal fraction (Fig. 3B). Our predicted downstream consequence is that reported in Figure 5A: a decrease in DAT's functional effects, as evidenced by slower dopamine reuptake. While other mechanisms also contribute to extracellular dopamine clearance (i.e., enzyme degradation), the DAT is overwhelmingly the primary mechanism responsible for terminating dopamine signaling after phasic release events [82,83], such as those during reward seeking or the presentation of reward-paired cues. Insulin-resistance-driven internalization of the DAT thus appeared to result in slower reuptake in the ventral striatum, where dopamine signaling encodes the salience of reward paired cues and reward associated actions [26]. Further, administration of cocaine, a powerful DAT blocker, dramatically increased dopamine availability and delayed dopamine reuptake in control rats, but had a modest effect prolonging dopamine reuptake in fructose-exposed rats, suggesting decreased availability of DATs on which cocaine could act. This is consistent with reports that a high-fat diet can reduce the cocaine-potentiating effects on dopamine [88], though whether or not this effect occurred as a result of insulin resistance was not tested.

Notably, the simultaneous administration of the diabetes-treatment drug pioglitazone, an insulin receptor sensitizer [46], rescued this deficit, normalizing dopamine reuptake similar to that seen in controls, and partially restoring cocaine's ability to potentiate and prolong extracellular dopamine transmission. Specifically, pioglitazone administration in the fructose-exposed rats appeared to initially prolong dopamine transmission after cocaine exposure, suggesting increased DAT availability on which cocaine could act, but with a rapid recovery of transporter function, i.e., DAT

appeared to be initially impaired, but dopamine levels quickly decreased to below those seen in controls. Interestingly, administration of pioglitazone alone (i.e., in the absence of the high-fructose diet) resulted in minimal changes to reuptake after cocaine, suggesting either decreased DAT availability altogether or, more likely, increased DAT availability and functionality after cocaine. It is possible that pioglitazone administration in the absence of insulin resistance upregulated DAT expression and function beyond normal homeostatic levels, thus "supercharging" reuptake capacity of the DAT.

Pioglitazone is a PPARγ agonist in the family of thiazolidinediones, which are known to increase sensitivity of the insulin receptor and restore neuronal insulin signaling in the presence of insulin resistance [89]. Thiazolidinediones have been shown to interact with the nigrostriatal dopamine system, mitigating the cognitive impairments induced by MPTP models of Parkinson's disease [90,91]. Importantly, insulin dysfunction is also implicated in Parkinson's disease [92,93], offering further evidence for insulin-dopamine interaction, but also obfuscating whether thiazolidinediones act primarily via insulin or another mechanism. More generally, PPARγ agonists have been shown to block the expression of locomotor sensitization to methamphetamine [94], reduce reinstatement of alcohol-seeking [95] and decrease nicotine self-administration [96] in animal models. However, whether these reports or our effects of pioglitazone on dopamine reuptake occur primarily via insulin's effect on DAT or an insulin-independent mechanism remains unclear, and is beyond the scope of the present studies.

Fructose causes insulin resistance independent of weight gain

Our fructose exposure produced quantifiable deficits in insulin signaling in as little as 3 weeks (Fig. 1), consistent with other reports [33]. While diet-induced peripheral insulin resistance is well documented to occur as a result of diets high in either fat [97,98], carbohydrates [99] or both [100],

increasing attention has investigated such effects in the brain; indeed, neuronal insulin resistance has been shown after a high-fat [98] and high-fructose [9] diet, consistent with our data. Neuronal insulin resistance, a relatively novel subject of investigation, has been implicated in cognitive impairments [97], especially on hippocampal-dependent memory tasks [101], but, to our knowledge, these data are the first to report its effects on reward seeking.

Diets capable of inducing insulin resistance frequently result in other metabolic changes, such as weight gain and obesity [102,103], factors associated with cognitive changes such as deficits in executive function [104], spatial learning [105] and memory [106]. Recent reports suggest that weight gain also impacts the dopamine system: weight gain is inversely related to striatal D2 receptor levels in rats [107] and availability in humans [108], a deficit rescued after weight loss from gastric bypass surgery [109]. An advantage of our model is the induction of insulin resistance in the absence of any weight gain, allowing us to isolate diet-induced insulin resistance as a driving factor. Importantly, recent work has demonstrated the development of type 2 diabetes in human populations to be largely a product of weight gain, as obesogenic diets can rapidly decrease insulin receptor sensitivity [74,110], resulting in the term "diabesity" to emphasize their comorbidity [102]. Given their interconnected nature, fully dissecting the effects of diet, obesity and insulin resistance on neurochemistry and behavior have proved difficult. Importantly, our fructose rats tended to gain significantly less weight than their control-fed counterparts (Fig. 3A & 6A), thus we cannot rule out other potential downstream consequences of inadequate weight gain, or their interactions with our measures.

Importantly, the mechanisms of fructose-induced insulin resistance are thought to differ from that experienced in typical diet-induced type 2 diabetes: typically, over-consumption of refined, high-carbohydrate diets induce repeated insulin release, eventually causing a downregulation of insulin

receptor sensitivity [111]. Because fructose is metabolized overwhelmingly by the liver, it has a minimal impact on acute peripheral insulin release, and instead increases lipogenesis and triglyceride synthesis [37]. It is through increased triglyceride synthesis and lipogenesis that fructose is thought to result in insulin resistance, though precise mechanisms are unclear. We hypothesize that our results would generalize to insulin resistance generated by other means, such as a Western diet or streptozotocin treatment (which destroys insulin-producing Beta cells of the pancreas), and are not isolated to fructose exposure alone. Further, the effects of dopamine sensitization as a result of the hedonic effects of fructose on the orosensory feeding system cannot be completely discounted, and may have independently played a role in sensitizing dopamine signaling.

Summary

In conclusion, our findings outline a potential mechanism for diet-induced insulin dysregulation in promoting maladaptive reward seeking. Such a mechanism would be consistent with many reports that obesity (a condition highly comorbid with insulin resistance) [88,112,113] and decreased insulin signaling [22,23] result in downregulated DAT-mediated reuptake. We report that insulin resistance appears to invigorate reward-seeking in response to environmental stimuli, which may contribute to a vicious cycle: insulin resistance might promote more reward seeking and taking (i.e., food consumption), which may further contribute to insulin resistance, and so on. Given preliminary evidence that PPARγ agonists can reduce drug seeking and self-administration in animal models [94,114], such drugs may be useful in reducing cue-driven overeating, even in the absence of type 2 diabetes.

References

1. Qi, L.; Cornelis, M. C.; Zhang, C.; van Dam, R. M.; Hu, F. B. Genetic predisposition, Western dietary pattern, and the risk of type 2 diabetes in men. *Am. J. Clin. Nutr.* **2009**, *89*, 1453–1458.

2. Chen, L.; Magliano, D. J.; Zimmet, P. Z. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. *Nat. Rev. Endocrinol.* **2011**, *8*, 228–236.

3. Biessels, G.-J.; ter Braak, E. W.; Erkelens, D. W.; Hijman, R. Cognitive function in patients with type 2 diabetes mellitus. *Neurosci. Res. Commun.* **2001**, *28*, 11–22.

4. Bjørgaas, M.; Gimse, R.; Vik, T.; Sand, T. Cognitive function in Type 1 diabetic children with and without episodes of severe hypoglycaemia. *Acta Paediatr.* **1997**, *86*, 148–153.

5. Barbagallo, M.; Dominguez, L. J. Type 2 diabetes mellitus and Alzheimer's disease. *World J. Diabetes* **2014**, *5*, 889.

6. Monzillo, L. U.; Hamdy, O. Evaluation of insulin sensitivity in clinical practice and in research settings. *Nutr. Rev.* **2003**, *61*, 397–412.

7. Liu, Y.; Liu, F.; Grundke-Iqbal, I.; Iqbal, K.; Gong, C.-X. Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. *J. Pathol.* **2011**, *225*, 54–62.

8. Anthony, K.; Reed, L. J.; Dunn, J. T.; Bingham, E.; Hopkins, D.; Marsden, P. K.; Amiel, S. A. Attenuation of insulin-evoked responses in brain networks controlling appetite and reward in insulin resistance: the cerebral basis for impaired control of food intake in metabolic syndrome? *Diabetes* **2006**, *55*, 2986–92.

9. Agrawal, R.; Gomez-Pinilla, F. "Metabolic syndrome" in the brain: deficiency in omega-3 fatty acid exacerbates dysfunctions in insulin receptor signalling and cognition. *J. Physiol.* **2012**, *590*, 2485–99.

10. Chen, Y.; Deng, Y.; Zhang, B.; Gong, C.-X. Deregulation of brain insulin signaling in Alzheimer's disease. *Neurosci. Bull.* **2014**, *30*, 282–294.

11. Mayer, C. M.; Belsham, D. D. Central Insulin Signaling Is Attenuated by Long-Term Insulin Exposure via Insulin Receptor Substrate-1 Serine Phosphorylation, Proteasomal Degradation, and Lysosomal Insulin Receptor Degradation. *Endocrinology* **2010**, *151*, 75–84.

12. Hill, J. M.; Lesniak, M. A.; Pert, C. B.; Roth, J. Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience* **1986**, *17*, 1127–38.

13. Schulingkamp, R. J.; Pagano, T. C.; Hung, D.; Raffa, R. B. Insulin receptors and insulin action in the brain: review and clinical implications. *Neurosci. Biobehav. Rev.* **2000**, *24*, 855–72.

14. Figlewicz, D. P. D. .; Evans, S. . B.; Murphy, J.; Hoen, M.; Baskin, D. . G. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res.* **2003**, *964*, 107–115.

15. Figlewicz, D. P.; Szot, P.; Chavez, M.; Woods, S. C.; Veith, R. C. Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res.* **1994**, *644*, 331–334.

16. Garcia, B. G.; Wei, Y.; Moron, J. A.; Lin, R. Z.; Javitch, J. A.; Galli, A. Akt is essential for insulin modulation of amphetamine-induced human dopamine transporter cell-surface redistribution. *Mol. Pharmacol.* **2005**, *68*, 102–9.

17. Mebel, D. M.; Wong, J. C. Y.; Dong, Y. J.; Borgland, S. L. Insulin in the ventral tegmental area reduces hedonic feeding and suppresses dopamine concentration via increased reuptake. *Eur. J. Neurosci.* **2012**, *36*, 2336–46.

18. Speed, N. K. The Role of Insulin Signaling on Dopamine Transporter Trafficking. *Dissertation* **2010**.

19. Speed, N. K.; Matthies, H. J. G.; Kennedy, J. P.; Vaughan, R. A.; Javitch, J. A.; Russo, S. J.; Lindsley, C. W.; Niswender, K.; Galli, A. Akt-dependent and isoform-specific regulation of dopamine transporter cell surface expression. *ACS Chem. Neurosci.* **2010**, *1*, 476–81.

20. Speed, N.; Saunders, C.; Davis, A. R.; Owens, W. A.; Matthies, H. J. G.; Saadat, S.; Kennedy, J. P.; Vaughan, R. A.; Neve, R. L.; Lindsley, C. W.; Russo, S. J.; Daws, L. C.; Niswender, K. D.; Galli, A. Impaired striatal Akt signaling disrupts dopamine homeostasis and increases feeding. *PLoS One* **2011**, *6*, e25169.

21. Patterson, T. A.; Brot, M. D.; Zavosh, A.; Schenk, J. O.; Szot, P.; Figlewicz, D. P. Food deprivation decreases mRNA and activity of the rat dopamine transporter. *Neuroendocrinology* **1998**, *68*, 11–20.

22. Owens, W. A.; Sevak, R. J.; Galici, R.; Chang, X.; Javors, M. A.; Galli, A.; France, C. P.; Daws, L. C. Deficits in dopamine clearance and locomotion in hypoinsulinemic rats unmask novel modulation of dopamine transporters by amphetamine. *J. Neurochem.* **2005**, *94*, 1402–1410.

23. Figlewicz, D. P.; Brot, M. D.; McCall, A. L.; Szot, P. Diabetes causes differential changes in CNS noradrenergic and dopaminergic neurons in the rat: a molecular study. *Brain Res.* **1996**, *736*, 54–60.

24. Carboni, E.; Tanda, G. L.; Frau, R.; Di Chiara, G. Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. *J. Neurochem.* **1990**, *55*, 1067–70.

25. Wassum, K. M.; Ostlund, S. B.; Loewinger, G. C.; Maidment, N. T. Phasic mesolimbic dopamine release tracks reward seeking during expression of Pavlovian-to-instrumental transfer. *Biol. Psychiatry* **2013**, *73*, 747–755.

26. Ostlund, S. B.; Leblanc, K. H.; Kosheleff, A. R.; Wassum, K. M.; Maidment, N. T. Phasic mesolimbic dopamine signaling encodes the facilitation of incentive motivation produced by repeated cocaine exposure. *Neuropsychopharmacology* **2014**, *39*, 1–9.

27. Schultz, W. Responses of midbrain dopamine neurons to behavioral trigger stimuli in the

monkey. *J. Neurophysiol.* **1986**, *56*, 1439–61.

28. Schultz, W.; Dickinson, A. Neuronal coding of prediction errors. *Annu. Rev. Neurosci.* **2000**, *23*, 473–500.

29. Robinson, T.; Berridge, K. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.* **1993**, *18*, 247–291.

30. Lodge, D. J.; Grace, A. A. Amphetamine Activation of Hippocampal Drive of Mesolimbic Dopamine Neurons: A Mechanism of Behavioral Sensitization. *J. Neurosci.* **2008**, *28*, 7876–7882.

31. Ostlund, S. B.; Balleine, B. W. On habits and addiction: An associative analysis of compulsive drug seeking. *Drug Discov. Today Dis. Model.* **2008**, *5*, 235–245.

32. Dickinson, A.; Balleine, B. Motivational control of goal-directed action. *Anim. Learn. Behav.* **1994**, *22*, 1–18.

33. Hwang, I. S.; Ho, H.; Hoffman, B. B.; Reaven, G. M. Fructose-induced insulin resistance and hypertension in rats. *Hypertension* **1987**, *10*, 512–516.

34. Lee, M. K.; Miles, P. D.; Khoursheed, M.; Gao, K. M.; Moossa, A. R.; Olefsky, J. M. Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. *Diabetes* **1994**, *43*, 1435–9.

35. Shih, C.-C.; Lin, C.-H.; Lin, W.-L.; Wu, J.-B. Momordica charantia extract on insulin resistance and the skeletal muscle GLUT4 protein in fructose-fed rats. *J. Ethnopharmacol.* **2009**, *123*, 82–90.

36. D'Angelo, G.; Elmarakby, A. A.; Pollock, D. M.; Stepp, D. W. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. *Hypertension* **2005**, *46*, 806–11.

37. Basciano, H.; Federico, L.; Adeli, K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr. Metab. (Lond).* **2005**, *2*, 5.

38. Elliott, S. S.; Keim, N. L.; Stern, J. S.; Teff, K.; Havel, P. J. Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr* **2002**, *76*, 911–22.

39. Wei, M.; Ong, L.; Smith, M. T.; Ross, F. B.; Schmid, K.; Hoey, A. J.; Burstow, D.; Brown, L. The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. *Hear. Lung Circ.* **2003**, *12*, 44–50.

40. Holm, S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand. J. Stat.* **1979**, *6*, 65–70.

41. Blakesley, R. E.; Mazumdar, S.; Dew, M. A.; Houck, P. R.; Tang, G.; Reynolds, C. F.; Butters, M. A. Comparisons of methods for multiple hypothesis testing in neuropsychological research. *Neuropsychology* **2009**, *23*, 255–64.

42. Unger, G.; Benozzi, S. F.; Perruzza, F.; Pennacchiotti, G. L. Triglycerides and glucose index:

A useful indicator of insulin resistance. *Endocrinol. y Nutr. (English Ed.* **2014**, *61*, 533–540.

43. Guerrero-Romero, F.; Simental-Mendía, L. E.; González-Ortiz, M.; Martínez-Abundis, E.; Ramos-Zavala, M. G.; Hernández-González, S. O.; Jacques-Camarena, O.; Rodríguez-Morán, M. The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 3347–51.

44. Simental-Mendía, L. E.; Rodríguez-Morán, M.; Guerrero-Romero, F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab. Syndr. Relat. Disord.* **2008**, *6*, 299–304.

45. Hallett, P. J.; Collins, T. L.; Standaert, D. G.; Dunah, A. W. Biochemical fractionation of brain tissue for studies of receptor distribution and trafficking. *Curr. Protoc. Neurosci.* **2008**, *Chapter 1*, Unit 1.16.

46. Smith, U. Pioglitazone: mechanism of action. *Int. J. Clin. Pract. Suppl.* **2001**, 13–8.

47. Kahn, C. R.; Chen, L.; Cohen, S. E. Unraveling the mechanism of action of thiazolidinediones. *J. Clin. Invest.* **2000**, *106*, 1305–7.

48. Suzuki, M.; Nomura, C.; Odaka, H.; Ikeda, H. Effect of an Insulin Sensitizer, Pioglitazone, on Hypertension in Fructose-Drinking Rats. *Jpn. J. Pharmacol.* **1997**, *74*, 297–302.

49. Liu, X.; Luo, D.; Zheng, M.; Hao, Y.; Hou, L.; Zhang, S. Effect of pioglitazone on insulin resistance in fructose-drinking rats correlates with AGEs/RAGE inhibition and block of NAPDH oxidase and NF kappa B activation. *Eur. J. Pharmacol.* **2010**, *629*, 153–158.

50. Yorgason, J. T.; España, R. A.; Jones, S. R. Demon Voltammetry and Analysis software: Analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. *J. Neurosci. Methods* **2011**, *202*, 158–164.

51. D'Agostino, R. Tests for Normal Distribution. In *Goodness-Of-Fit Techniques*; D'Agostino, R.; Stephens, M., Eds.; Macel Dekker, 1986.

52. du Prel, J.-B.; Hommel, G.; Röhrig, B.; Blettner, M. Confidence interval or p-value?: part 4 of a series on evaluation of scientific publications. *Dtsch. Arztebl. Int.* **2009**, *106*, 335–9.

53. Perneger, T. V What's wrong with Bonferroni adjustments. *BMJ* **1998**, *316*, 1236–8.

54. Ludbrook, J. On making multiple comparisons in clinical and experimental phamacology and physiology. *Clin. Exp. Pharmacol. Physiol.* **1991**, *18*, 379–392.

55. Wilcox, R. R. New Designs in Analysis of Variance. *Annu. Rev. Psychol.* **1987**, *38*, 29–60.

56. Rescorla, R. A. Associative changes with a random CS-US relationship. *Q. J. Exp. Psychol. Sect. B* **2000**, *53*, 325–340.

57. Holland, P. C. Stimuli associated with the cancellation of food and its cues enhance eating but display negative incentive value. *Learn. Behav.* **2014**, *42*, 365–82.

58. Wyvell, C. L.; Berridge, K. C. Incentive sensitization by previous amphetamine exposure:

increased cue-triggered “wanting” for sucrose reward. *J. Neurosci.* **2001**, *21*, 7831–40.

59. Harmer, C. J.; Phillips, G. D. Enhanced conditioned inhibition following repeated pretreatment with d-amphetamine. *Psychopharmacology (Berl).* **1999**, *142*, 120–31.

60. Kakade, S.; Dayan, P. Dopamine: generalization and bonuses. *Neural Networks* **2002**, *15*, 549–559.

61. Hollerman, J. R.; Schultz, W. Dopamine neurons report an error in the temporal prediction of reward during learning. *Nat. Neurosci.* **1998**, *1*, 304–309.

62. Waelti, P.; Dickinson, A.; Schultz, W. Dopamine responses comply with basic assumptions of formal learning theory. *Nature* **2001**, *412*, 43–48.

63. Day, J. J.; Roitman, M. F.; Wightman, R. M.; Carelli, R. M. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci.* **2007**, *10*, 1020–8.

64. Collins, A. L.; Greenfield, V. Y.; Bye, J. K.; Linker, K. E.; Wang, A. S.; Wassum, K. M. Dynamic mesolimbic dopamine signaling during action sequence learning and expectation violation. *Sci. Rep.* **2016**, *6*, 20231.

65. Rada, P.; Avena, N. M.; Hoebel, B. G. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience* **2005**, *134*, 737–744.

66. Avena, N. M.; Rada, P.; Moise, N.; Hoebel, B. G. Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience* **2006**, *139*, 813–820.

67. Spangler, R.; Wittkowski, K. M.; Goddard, N. L.; Avena, N. M.; Hoebel, B. G.; Leibowitz, S. F. Opiate-like effects of sugar on gene expression in reward areas of the rat brain. *Brain Res. Mol. Brain Res.* **2004**, *124*, 134–42.

68. Colantuoni, C.; Schwenker, J.; McCarthy, J.; Rada, P.; Ladenheim, B.; Cadet, J. L.; Schwartz, G. J.; Moran, T. H.; Hoebel, B. G. Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport* **2001**, *12*, 3549–52.

69. Foley, K. K. A.; Fudge, M. A. M.; Kavaliers, M.; Ossenkopp, K.-P. K. Quinpirole-induced behavioral sensitization is enhanced by prior scheduled exposure to sucrose: A multi-variable examination of locomotor activity. *Behav. Brain Res.* **2006**, *167*, 49–56.

70. Avena, N. M.; Hoebel, B. G. A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience* **2003**, *122*, 17–20.

71. Gosnell, B. A. Sucrose intake enhances behavioral sensitization produced by cocaine. *Brain Res.* **2005**, *1031*, 194–201.

72. Avena, N. M.; Hoebel, B. G. Amphetamine-sensitized rats show sugar-induced hyperactivity (cross-sensitization) and sugar hyperphagia. *Pharmacol. Biochem. Behav.* **2003**, *74*, 635–9.

73. Hajnal, A.; Norgren, R. Taste pathways that mediate accumbens dopamine release by sapid

sucrose. *Physiol. Behav.* **2005**, *84*, 363–9.

74. Stouffer, M. A.; Woods, C. A.; Patel, J. C.; Lee, C. R.; Witkovsky, P.; Bao, L.; Machold, R. P.; Jones, K. T.; de Vaca, S. C.; Reith, M. E. A.; Carr, K. D.; Rice, M. E. Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. *Nat. Commun.* **2015**, *6*, 8543.

75. Corwin, R. L.; Avena, N. M.; Boggiano, M. M. Feeding and reward: Perspectives from three rat models of binge eating. *Physiol. Behav.* **2011**, *104*, 87–97.

76. Wise, R. A. Dopamine, learning and motivation. *Nat. Rev. Neurosci.* **2004**, *5*, 483–94.

77. Schultz, W.; Hollerman, J. R. Dopamine neurons report an error in the temporal prediction of rewardduring learning. *Nat. Neurosci.* **1998**, *1*, 304–309.

78. Flagel, S. B.; Clark, J. J.; Robinson, T. E.; Mayo, L.; Czuj, A.; Willuhn, I.; Akers, C. A.; Clinton, S. M.; Phillips, P. E. M.; Akil, H. A selective role for dopamine in stimulus-reward learning. *Nature* **2011**, *469*, 53–7.

79. Hajnal, A.; Smith, G. P.; Norgren, R. Oral sucrose stimulation increases accumbens dopamine in the rat. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **2004**, *286*, 31–37.

80. Sclafani, A.; Mann, S. Carbohydrate taste preferences in rats: glucose, sucrose, maltose, fructose and polycose compared. *Physiol. Behav.* **1987**, *40*, 563–8.

81. Sclafani, A.; Nissenbaum, J. W. Oral versus postingestive origin of polysaccharide appetite in the rat. *Neurosci. Biobehav. Rev.* **1987**, *11*, 169–72.

82. Giros, B.; Caron, M. G. Molecular characterization of the dopamine transporter. *Trends Pharmacol. Sci.* **1993**, *14*, 43–9.

83. Jaber, M.; Jones, S.; Giros, B.; Caron, M. G. The dopamine transporter: A crucial component regulating dopamine transmission. *Mov. Disord.* **1997**, *12*, 629–633.

84. Volkow, N. D.; Wang, G. J.; Fowler, J. S.; Gatley, S. J.; Ding, Y. S.; Logan, J.; Dewey, S. L.; Hitzemann, R.; Lieberman, J. Relationship between psychostimulant-induced "high" and dopamine transporter occupancy. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 10388–92.

85. Pierce, R. C.; Kumaresan, V. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci. Biobehav. Rev.* **2006**, *30*, 215–38.

86. Lute, B. J.; Khoshbouei, H.; Saunders, C.; Sen, N.; Lin, R. Z.; Javitch, J. A.; Galli, A. PI3K signaling supports amphetamine-induced dopamine efflux. *Biochem. Biophys. Res. Commun.* **2008**, *372*, 656–61.

87. Schoffelmeer, A. N. M.; Drukarch, B.; De Vries, T. J.; Hogenboom, F.; Schetters, D.; Pattij, T. Insulin modulates cocaine-sensitive monoamine transporter function and impulsive behavior. *J. Neurosci.* **2011**, *31*, 1284–91.

88. Cone, J. J.; Chartoff, E. H.; Potter, D. N.; Ebner, S. R.; Roitman, M. F. Prolonged high fat diet reduces dopamine reuptake without altering DAT gene expression. *PLoS One* **2013**, *8*,

e58251.

89. Pipatpiboon, N.; Pratchayasakul, W.; Chattipakorn, N.; Chattipakorn, S. C. PPARγ Agonist Improves Neuronal Insulin Receptor Function in Hippocampus and Brain Mitochondria Function in Rats with Insulin Resistance Induced by Long Term High-Fat Diets. *Endocrinology* **2012**, *153*, 329–338.

90. Kumar, P.; Kaundal, R. K.; More, S.; Sharma, S. S. Beneficial effects of pioglitazone on cognitive impairment in MPTP model of Parkinson's disease. *Behav. Brain Res.* **2009**, *197*, 398–403.

91. Quinn, L. P.; Crook, B.; Hows, M. E.; Vidgeon-Hart, M.; Chapman, H.; Upton, N.; Medhurst, A. D.; Virley, D. J. The PPARγ agonist pioglitazone is effective in the MPTP mouse model of Parkinson's disease through inhibition of monoamine oxidase B. *Br. J. Pharmacol.* **2008**, *154*, 226–233.

92. Aviles-Olmos, I.; Limousin, P.; Lees, A.; Foltynie, T. Parkinson's disease, insulin resistance and novel agents of neuroprotection. *Brain* **2013**, *136*, 374–384.

93. Morris, J. K.; Bomhoff, G. L.; Gorres, B. K.; Davis, V. A.; Kim, J.; Lee, P.-P.; Brooks, W. M.; Gerhardt, G. A.; Geiger, P. C.; Stanford, J. A. Insulin resistance impairs nigrostriatal dopamine function. *Exp. Neurol.* **2011**, *231*, 171–180.

94. Maeda, T.; Kiguchi, N.; Fukazawa, Y.; Yamamoto, A.; Ozaki, M.; Kishioka, S. Peroxisome Proliferator-Activated Receptor Gamma Activation Relieves Expression of Behavioral Sensitization to Methamphetamine in Mice. *Neuropsychopharmacology* **2007**, *32*, 1133–1140.

95. Stopponi, S.; Somaini, L.; Cippitelli, A.; Cannella, N.; Braconi, S.; Kallupi, M.; Ruggeri, B.; Heilig, M.; Demopulos, G.; Gaitanaris, G.; Massi, M.; Ciccocioppo, R. Activation of Nuclear PPARγ Receptors by the Antidiabetic Agent Pioglitazone Suppresses Alcohol Drinking and Relapse to Alcohol Seeking. *Biol. Psychiatry* **2011**, *69*, 642–649.

96. Panlilio, L. V; Justinova, Z.; Mascia, P.; Pistis, M.; Luchicchi, A.; Lecca, S.; Barnes, C.; Redhi, G. H.; Adair, J.; Heishman, S. J.; Yasar, S.; Aliczki, M.; Haller, J.; Goldberg, S. R. Novel Use of a Lipid-Lowering Fibrate Medication to Prevent Nicotine Reward and Relapse: Preclinical Findings. *Neuropsychopharmacology* **2012**, *37*, 1838–1847.

97. Greenwood, C. E.; Winocur, G. High-fat diets, insulin resistance and declining cognitive function. *Neurobiol. Aging* **2005**, *26*, 42–45.

98. Pratchayasakul, W.; Kerdphoo, S.; Petsophonsakul, P.; Pongchaidecha, A.; Chattipakorn, N.; Chattipakorn, S. C. Effects of high-fat diet on insulin receptor function in rat hippocampus and the level of neuronal corticosterone. *Life Sci.* **2011**, *88*, 619–627.

99. Wright, D. W.; Hansen, R. I.; Mondon, C. E.; Reaven, G. M. Sucrose-induced insulin resistance in the rat: modulation by exercise and diet. *Am J Clin Nutr* **1983**, *38*, 879–883.

100. Stranahan, A. M.; Norman, E. D.; Lee, K.; Cutler, R. G.; Telljohann, R. S.; Egan, J. M.; Mattson, M. P. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus* **2008**, *18*, 1085–8.

101. Wu, H.-W.; Ren, L.-F.; Zhou, X.; Han, D.-W. A high-fructose diet induces hippocampal insulin resistance and exacerbates memory deficits in male Sprague-Dawley rats. *Nutr. Neurosci.* **2015**, *18*, 323–328.

102. Astrup, A.; Finer, N. Redefining type 2 diabetes: "diabesity" or "obesity dependent diabetes mellitus"? *Obes. Rev.* **2000**, *1*, 57–59.

103. Kahn, S. E.; Hull, R. L.; Utzschneider, K. M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* **2006**, *444*, 840–846.

104. Cserjsi, R.; Luminet, O.; Poncelet, A.-S.; Lenard, L. Altered executive function in obesity. Exploration of the role of affective states on cognitive abilities. *Appetite* **2009**, *52*, 535–539.

105. Jurdak, N.; Lichtenstein, A. H.; Kanarek, R. B. Diet-induced obesity and spatial cognition in young male rats. *Nutr. Neurosci.* **2008**, *11*, 48–54.

106. Jurdak, N.; Kanarek, R. B. Sucrose-induced obesity impairs novel object recognition learning in young rats. *Physiol. Behav.* **2009**, *96*, 1–5.

107. Johnson, P. M.; Kenny, P. J. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* **2010**, *13*, 635–41.

108. de Weijer, B. A.; van de Giessen, E.; van Amelsvoort, T. A.; Boot, E.; Braak, B.; Janssen, I. M.; van de Laar, A.; Fliers, E.; Serlie, M. J.; Booij, J. Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Res.* **2011**, *1*, 37.

109. Steele, K. E.; Prokopowicz, G. P.; Schweitzer, M. A.; Magunsuon, T. H.; Lidor, A. O.; Kuwabawa, H.; Kumar, A.; Brasic, J.; Wong, D. F. Alterations of central dopamine receptors before and after gastric bypass surgery. *Obes. Surg.* **2010**, *20*, 369–74.

110. De Souza, C. T.; Araujo, E. P.; Bordin, S.; Ashimine, R.; Zollner, R. L.; Boschero, A. C.; Saad, M. J. A.; Velloso, L. A. Consumption of a Fat-Rich Diet Activates a Proinflammatory Response and Induces Insulin Resistance in the Hypothalamus. *Endocrinology* **2005**, *146*, 4192–4199.

111. Moller, D. E.; Flier, J. S. Insulin Resistance — Mechanisms, Syndromes, and Implications. *N. Engl. J. Med.* **1991**, *325*, 938–948.

112. South, T.; Huang, X.-F. High-fat diet exposure increases dopamine D2 receptor and decreases dopamine transporter receptor binding density in the nucleus accumbens and caudate putamen of mice. *Neurochem. Res.* **2008**, *33*, 598–605.

113. Narayanaswami, V.; Thompson, A. C.; Cassis, L. A.; Bardo, M. T.; Dwoskin, L. P. Diet-induced obesity: dopamine transporter function, impulsivity and motivation. *Int. J. Obes.* **2013**, *37*, 1095–1103.

114. Le Foll, B.; Di Ciano, P.; Panlilio, L. V; Goldberg, S. R.; Ciccocioppo, R. Peroxisome proliferator-activated receptor (PPAR) agonists as promising new medications for drug addiction: preclinical evidence. *Curr. Drug Targets* **2013**, *14*, 768–76.

115. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates, 6th Edition*; Academic Press (Elsevier Inc): Waltham, MA, 2007.

Chapter 5

General Discussion

The global food supply is quickly shifting to a Westernized diet – cheap, processed foods are readily available in developed and developing countries more than ever before [reviewed in 1]. Heavily implicated in the global obesity epidemic, many of these contemporary "convenience" and pre-packaged foods are not only extremely cheap to procure, they exploit our innate preferences for sugars, salts, and fats [2] potentiating cravings and continued consumption in vulnerable individuals, long past the point of satiety [3]. Obesity is both a negative health outcome on its own, and a risk factor for other metabolic disorders [4] such as type 2 diabetes, whose comorbidity is highlighted by the term "diabesity" [5]. Disordered insulin signaling (such as that seen in type 2 diabetes) has been shown to impact the mesolimbic dopamine system [6,7], which is, in turn, implicated in maladaptive reward seeking [8].

The experiments described herein attempt to understand how poor quality diets can bias behavior towards maladaptive reward seeking. In Aim 1 (Chapters 2 and 3), we examined the behavioral consequences of a varied, palatable junk food diet on incentive motivation and decision-making. In Aim 2 (Chapter 4), we examined the behavioral and neurochemical consequences of diet-induced insulin resistance.

Junk Food Disrupts the Appropriate Use Of External Cues And Incentive Motivation

The experiments in Chapter 2 explore how junk food might disrupt the use of external cues to guide reward seeking in the absence of homeostatic need (i.e., in the sated state), akin to the compulsive and maladaptive overeating common in our contemporary food environment. In these experiments, we used the PIT paradigm and licking microstructure analysis to examine

how junk food might alter reward wanting and liking. We found that junk food-exposed rats showed altered reward seeking and liking, and that the specific effect depends on the pattern of feeding (i.e., ad libitum versus intermittent). Specifically, we found that ad libitum junk food exposure resulted in decreased responsiveness to reward-paired cues, while intermittent junk food exposure potentiated reward seeking in response to cues only loosely paired with reward. Interestingly, in Chapter 3, these two feeding protocols did not differ in their effects on decision-making tasks that required the use of external and interoceptive satiety cues to guide reward-seeking, where both junk food groups showed an inability to 1) adjust responding away from a devalued outcome, and 2) use environmental cues to guide reward seeking. This suggests that perhaps decision-making and incentive motivation are differentially affected by junk food-induced neuroadaptations.

Behavioral parallels between drug and food addiction

In these experiments, we see strong parallels to the drug addiction literature, as junk food is capable of increasing incentive motivation and disrupting decision-making, like many drugs of abuse [9–12]. Many argue that diet-induced obesity may be a product of "food addiction," which they contend should be considered a brain disorder like drug addiction, and included as a diagnostic category in the DSM [13,14]. Certainly, the pleasure obtained from eating palatable foods can be a powerful force driving overconsumption. When given a choice, rats will choose a calorie-free sweet solution over a cocaine reward [15], suggesting the drive for food can be comparable to, if not greater than, that for drugs. Further, sated rats will subject themselves to such aversive stimuli as extreme cold or footshock in order to obtain palatable food items (i.e., chocolate, candies, etc.) even when given the option of regular lab chow without aversive

stimulation [16,17]. Thus, even when not hungry, rats appear very driven and motivated to procure palatable foods, even subjecting themselves to discomfort and pain. Drug addicts show great motivation to procure their drug of choice, even in the face of negative consequences (e.g., threat of job or family loss, etc.). Compulsive overeaters (resulting in obesity or not) also continue to eat despite expressed desires to limit consumption and knowledge of negative consequences such as negative health problems and social criticism [18]. Many obese overeaters are so unsuccessful at reducing their consumption they undergo invasive bariatric surgery in an attempt to control their weight [19], yet are often unable to limit eating despite subsequent decreased subjective ratings of hunger [20]. This suggests that maladaptive and compulsive overeating shares with drug addiction a loss of inhibitory control over behavior, along with habitual and compulsive consumption despite knowledge of the negative health and social consequences.

Restricted access models drive addiction-like behaviors, while extended access models are associated with anhedonia

Importantly, maladaptive overeating as a behavioral disorder is complex in its etiology and clinical presentation, with clear distinctions between binge eating disorder and general overeating that can lead to obesity [13,21]. For some individuals, overeating is a steady, perhaps habitual action characterized by frequent snacking and large portion sizes of poor quality foods [22]. For others, it can be compulsive and driven, characterized by food binges and marked distress about overeating, as in the case of binge eating disorder [23]. Animal models of food addiction have sought to address these differences by using restricted versus extended access to palatable foods, such as in our experiments in Chapters 2 and 3.

Animal models of binge feeding report the development of addiction-like behaviors: escalation of intake [24], resistance to foot-shock as punishment [17], increased motivation to work for the reward [25,26] and withdrawal-induced anxiety [27]. Sucrose binging, in particular, appears to promote neuroadaptations that support cross-sensitization with other drugs of abuse. For instance, binge, but not extended, sucrose access results in increased psychostimulant-induced hyperactivity [28,29] (and vice versa [30]) and increased alcohol consumption [31]. Sucrose-binging rats also show naloxone-precipitated somatic signs of withdrawal such as teeth chattering, forepaw tremor, head shakes [32]. Abrupt cessation from regular, intermittent sucrose results in other signs of withdrawal such as decreased body temperature [33] and aggressive behavior [34].

Conversely, rats with extended access to palatable foods may be anhedonic, as demonstrated by decreased conditioned place preference for amphetamine [35], decreased ethanol consumption [36], decreased motivation to work for food on a progressive ratio task [37], and decreased incentive motivation and food seeking (Chapter 2). In contrast, *increased* reward seeking on some tasks may indicate an attempt to compensate for downregulated baseline hedonic tone, not unlike the mechanism proposed by the hedonic allostasis hypothesis [38]. Indeed, rats with a history of extended access to intravenous cocaine self-administration appear anhedonic and demonstrate increased ICSS (intracranial self-stimulation) thresholds (thought to reflect an *underactive* reward system), the intensity of which is proportional to the amount of cocaine they had consumed [39,40]. Further, rats with extended heroin access show similar decreases in sensitivity to rewards (reflected by increased ICSS thresholds), while rats with restricted access

show *increased* sensitivity to rewards (i.e., lowered ICSS thresholds) [41]. Likewise, using a similar ad libitum feeding protocol to that reported in Chapters 2 and 3, Johnson and Kenny [42] found reward hypofunction (as measured by increased ICSS thresholds) in rats chronically exposed to a junk food diet, but not in those fed intermittently. Blum and colleagues coined the term "reward deficiency syndrome" to describe a pattern of impulsive, compulsive and addiction-like reward seeking behaviors, suggesting that compulsive reward-seeking (for either drugs or food) may be due to a deficiency in baseline activation of mesolimbic reward circuits (reviewed in [43]). By this hypothesis, overeating and drug taking may be means by which the reward system is stimulated to reach a threshold that, for other people, is considered normal.

Taken together, these interpretations may initially appear contradictory, but likely represent differences in task, measured outcomes, and exposure timeline. Specifically, such results may reflect a complex, non-linear relationship between hedonia and reward seeking across time (**Fig. 1**): initially, exposure to experiences that stimulate the reward system (e.g., feeding, initial drug use) will quickly (but temporarily) downregulate reward circuitry, as reward satiety mechanisms

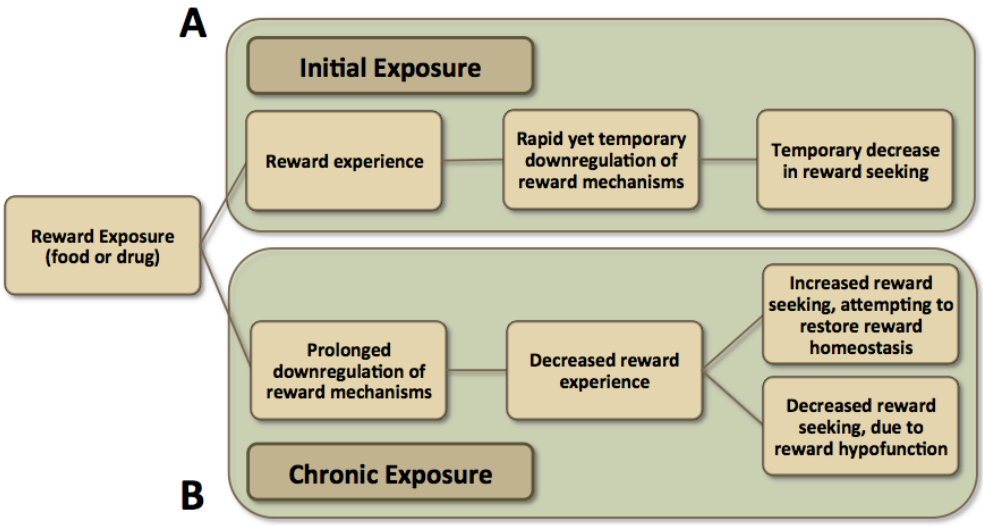

Figure 1. Effects of Chronic Reward Exposure. (A) With initial use, reward mechanisms are only temporarily downregulated. (B) With chronic reward exposure, reward mechanisms are persistently downregulated, resulting in decreased reward experience. As a result, behavioral compensations can manifest as either increased reward seeking in an attempt to normalize reward experience, or decreased reward seeking, as a result of reward hypofunction and anhedonia.

attempt to limit consumption (**Fig. 1A**). Such a mechanism makes intuitive sense: if you have just eaten, brain mechanisms supporting food seeking and craving should be downregulated in order to focus your attention elsewhere. Conversely, chronic, continued reward exposure (e.g., chronic overeating, potentially resulting in weight gain, or extended drug abuse; **Fig. 1B**) that persistently downregulates reward mechanisms may result in lasting neuroadaptations that reduce the hedonic response to rewards and the motivational impact of their predictors, in an effort to further limit consumption. This may, in turn, result in either increased reward seeking in an attempt to restore homeostasis to an altered, chronically downregulated reward system, or decreased reward seeking, due to anhedonia. It is possible that our ad libitum-fed rats from Chapter 2 fall somewhere along this continuum, experiencing prolonged downregulation of reward mechanisms, such as D2 receptor downregulation (discussed extensively below in *Potential mechanisms driving junk food-induced neuroadaptations*), and consequently reward hypofunction and anhedonia. Importantly, we found decreased reward liking suggestive of anhedonia only among ad libitum-fed rats that gained the most weight (Chapter 2, Figure 4), where they consistently had lower liking scores on both measures. This may also explain the decreased food (Chapter 2 supplement, Figure 5) and SCM (Chapter 3, Figure 2) consumption in ad libitum-exposed rats, where they consistently consumed less than other groups. Whether the conflicting reports of increased and decreased reward seeking after chronic reward exposure (**Fig. 1B**) occur sequentially along a timeline of exposure, or concurrently as a result of different measures across assays is unclear.

Interestingly, our data from Chapter 3 failed to demonstrate significant differences at test between the intermittent and ad libitum access feeding paradigms, results that may appear to

contradict those from Chapter 2. While these results remain unclear, this discrepancy is likely due to the different task under investigation, i.e., incentive motivation was evaluated in Chapter 2, versus decision-making in Chapter 3. These assays recruit from different brain regions: incentive motivation is overwhelmingly controlled by subcortical structures in mesolimbic regions [44], while decision-making is thought to be primarily regulated by cortical structures [45]. It is possible that pattern of access to junk food diets simply has differential, circuit-specific effects, and may not be as consequential on tasks recruiting from cortical structures, thus such potential differences warrant further inquiry.

Potential mechanisms driving junk food-induced neuroadaptations

While food and fluid intake is heavily regulated by survival needs and energy homeostasis, actual ingestive behavior is also influenced by both innate and learned food preferences and aversions. Animals have innate preferences for foods that signal efficient energy sources, such as high fat, high sugar, and high calorie foods. Similarly, they can *learn* to prefer foods, flavors, smells, and textures associated with these innate qualities, or those that become associated with positive postingestive effects, termed flavor-nutrient learning (discussed in detail in Chapter 3). The impact of junk foods on the brain may begin with sensory systems that project to regions mediating palatability and involved in learning about caloric and nutrient value. Food characteristics are processed by well-defined areas in the hind- and mid-brain, and synthesized with affective responses in subcortical and mesolimbic areas [46,47]. Affective food 'liking' responses as a result of sensory processing (i.e., the flavors, tastes, and textures) are heavily mediated by opioid signaling in subcortical mesolimbic areas [48,49], the intermittent or chronic

activation of which may result in hypersensitivity or apathy, respectively, to food-paired cues capable of driving reward-seeking.

Palatable foods activate the mesolimbic dopamine system

Activation of the rostral nucleus of the solitary tract as a result of a food's orosensory qualities potentiates glutamatergic afferents to the parabrachial nucleus (PBN) [50,51]. The PBN projects sensory information directly to the ventral tegmental area (VTA), engaging the nucleus accumbens (NAc) and subsequent areas involved in processing reward value and associated stimuli (e.g., mPFC, central amygdala (CeA)) [52,53]. Further, activation of the PBN, a known enkephalin 'hotspot' [54], might then activate a second circuit, driving enkephalin-mediated signaling on mu opioid receptors in the CeA and decreasing CeA activity [55,56]. As a result, GABAergic projections from the CeA to the VTA would be disinhibited, removing the 'brake' on VTA activity. Removing inhibitory control from the VTA activates dopaminergic projections to the NAc. By these two circuits, palatable foods can quickly and effectively engage mesolimbic dopamine systems.

Importantly, foods can induce NAc dopamine release independent of taste or flavor stimulation, relying instead on post-prandial and postingestive effects of different foods. Using knockout mice unable to taste sweetness, sucrose intake produced a significant increase in NAc dopamine, independent of taste [57]. Further, when tested with a non-caloric sucralose solution, wildtypes showed a larger dopamine increase vs. knockouts, but this difference disappeared when sucrose was used [57], i.e., caloric, sweet solutions evoked the same amount of dopamine release in wildtypes and knockout mice, but a non-caloric sweet solution evoked dopamine release only in

wildtypes that were able to taste the sweetness. This suggests that taste and palatability can induce mesolimbic dopamine activation, but are not required: dopamine signaling can occur simply in response to postingestive processes and nutrient availability, thereby signaling salient stimuli or reward (i.e., flavor-nutrient learning). Such postingestive signals might engage the mesolimbic dopamine system via insulin signaling [6], which is released in response to caloric availability [58].

Like drugs of abuse, intermittent access to a variety of palatable foods promotes mesolimbic dopamine signaling, and may, with repetition, sensitize brain areas and circuitry associated with taste, food and reward. If such dopamine signaling were repeatedly paired with specific contexts and cues, these neural systems could become sensitized, pathologically amplifying the excitatory and motivational effects of these cues, and driving food seeking, consistent with the incentive sensitization hypothesis of addiction [59]. Interestingly, like drug addicts, obese individuals that report craving food more do not report an increase in food 'liking' compared to healthy-weight controls [60], suggesting a similar dissociation between areas modulating 'wanting' versus 'liking.' Further, activation of the PBN's enkephalinergic projections via palatable foods could result in an opioid-induced downregulation of satiety systems, via the dysregulation of oxytocin and melanocortin signaling in the hypothalamus [61] thereby further stimulating feeding. Thus, continuous intermittent exposure to junk foods, like that described in our rats in Chapters 2 and 3, might sensitize mesolimbic circuitry and dopamine neuron firing, produce a vicious cycle of hypersensitivity to food-paired cues capable of driving compulsive food-seeking.

Indeed, intermittent sucrose access has been shown to repeatedly release dopamine in the NAc shell [62,63] and facilitate locomotor sensitization to a dopamine agonist [28,29,64], further suggesting that such dietary interventions may impact the dopaminergic systems involved in learning about and responding to reward-paired cues [65–68]. This ability to repeatedly potentiate mesolimbic dopamine release is a hallmark effect of all drugs of abuse, as foods typically invoke a large dopamine release event, which subsequently fades as the food loses its novelty [69]. Regular intermittent access, however, maintains this ability, stimulating dopamine release with each binge, an effect not seen after ad libitum access [62]. This repeated activation of the mesolimbic dopamine system may have played a role in the hypersensitivity to environmental cues seen in our intermittent-fed rats in Chapter 2. Interestingly, dopamine neurons will fire not just in response to reward-paired cues, but also in response to familiar stimuli non-predictive of reward, but to a lesser degree than firing in response to cues predicting reward [54,55], suggesting dopamine neurons may support stimulus generalization, consistent with the effects seen in our intermittent-exposed rats in Chapters 2 and 3.

Junk food and weight gain are associated with downregulated D2 receptors

While decreased D2 receptor availability has long been associated with prolonged and extensive drug abuse [70,71], a growing body of evidence has recently implicated junk foods, weight gain, and obesity in D2 receptor downregulation as well. Human brain imaging studies have reported blunted activation of the striatum associated with long-term weight gain, particularly in individuals with the Taq A1 allele, which is associated with decreased striatal D2 receptor expression [72]. Decreased striatal D2 receptors have also been shown in obese subjects [73], the extent of which is proportional to their BMI [74]. Not surprisingly, striatal D2 receptor binding is

also decreased in a genetic rat model of obesity [75], and after a junk food diet [76], especially in obesity-prone rats [77]. Further, D2 receptor availability is *upregulated* in individuals with anorexia nervosa (37), and after bariatric surgery-induced weight loss (38). In animal models, extended junk food exposure capable of inducing weight gain and associated with heightened cue-reactivity also downregulated D2 receptor mRNA [78]. In a particularly elegant experiment, Johnson and Kenny [42] demonstrated that artificial striatal D2 receptor knockdown almost instantaneously increased ICSS thresholds, accelerating the emergence of reward hypofunction in rats with extended junk food access, and that such reward hypofunction was positively correlated with junk food-induced weight gain. Importantly, diet-induced D2 receptor downregulation is also strongly associated with a poor quality diet, not just obesity: not all rats with D2 receptor downregulation fed palatable foods display weight gain. For instance, rats fed a glucose solution for 30 days did not gain more weight than their controls, but *did* show a decrease in D2 receptor availability [79]. While nearly all rats that *do* become obese (due to diet or genetic factors) show downregulated D2 receptor availability, not all rats that show D2 receptor availability are obese. These data offer strong support for junk food- and weight gain-induced changes in D2 receptor function as a likely mechanism for the anhedonia and apathy seen in our ad libitum-exposed rats in Chapter 2.

Notably, to our knowledge all studies showing downregulated D2 receptors in the absence of obesity have used a single-food feeding model, in which one food is fed continuously for the duration of the experiment (i.e., one high-fat or high-sucrose food) [79,80]. Such satiety on one food may quickly desensitize reward systems, resulting in a decreased capacity to evoke dopamine as the food loses its novelty [69]. Researchers commonly use the term ‘palatable diet’

when referring to a palatable food, but chronic access to the same food repeatedly is not likely to be palatable at all. This type of repeated, monotonous exposure is well know to quickly produce sensory specific satiety in humans and rats [81,82] which reflects a decrease in both 'liking' and 'wanting' [83], and is strongly associated with decreased dopamine signaling in the NAc [84,85]. Thus, in these models, it is unclear whether a palatable, varied diet alone can promote these neuroadaptations, or whether the results are strictly a product of weight gain or monodiet feeding. What remains to be tested is whether a *variety* of palatable foods, with the potential to continuously engage sensory and reward circuitry (e.g., modeling the 'thanksgiving effect' whereby overeating is easier and food is more rewarding when variety is available), will also produce these changes in the absence of weight gain and obesity.

Interestingly, when rats are given access to a high-fat diet and chow versus a high-fat diet only, only the rats with a *choice* showed decreased D2 receptor availability, which, again, was associated with significant weight gain [86]. This suggests that, while rats fed a poor quality diet with variety might develop downregulated D2 receptors, it is overwhelmingly more likely with the onset of obesity or if they were fed one type of food only (i.e., only high-fat, or only high-sugar pellets). Thus, perhaps decreased D2 receptor availability occurs via two mechanisms: 1) the development of an obese phenotype, and 2) chronic mono-feeding of an otherwise palatable food. While many studies report decreased D2 receptor availability after 'palatable' single-macronutrient feeding, perhaps this effect results from continuous palatable-food-*satiety*, not the food itself. Regardless, D2 receptor downregulation, whether due to obesity or chronic satiety on a single food may alter dopamine signaling sufficiently to further drive overeating, hastening insulin resistance and weight gain, resulting in reward hypofunction and further overeating. What

is lacking in the literature is evidence of D2 receptor downregulation after 'cafeteria diet' style junk food feeding *in the absence of obesity*. Indeed, while our extended access rats in Chapter 2 tended to gain more weight than their intermittent-fed or chow counterparts, many maintained comparable weight to other groups (Chapter 2, Figure 2), yet all appeared to display a behavioral phenotype consistent with D2 receptor downregulation.

Junk food-induced mesolimbic neuroadaptations may also disrupt impulse control

As discussed above, our intermittent and ad libitum junk food-fed rats from Chapter 3 displayed similar deficits on assays of decision-making in an outcome devaluation test or cue-guided action selection. Both groups displayed an initial insensitivity to outcome devaluation, and an inability to adaptively use cues to guide instrumental behavior, similar to the indiscriminate lever pressing seen in our intermittently-fed junk food rats from Chapter 2. While one hypothesis for these disruptions is altered flavor-nutrient satiety learning (discussed extensively in Chapter 3), an alternate explanation may be an increase in impulsive behavior, a key factor in poor decision making, where both groups engaged in indiscriminate lever pressing without regard for the implications. Indeed, increased impulsivity has been reported after high-fat, high-sugar, and palatable diets [87], and can even be passed on to offspring as a result of an "unfavorable intrauterine nutritional environment" [88]. A recent study found that junk food-fed rats with extended access were more sensitive to D2 receptor antagonism, resulting in increased impulsivity at lower doses, suggesting overall D2 receptors downregulation [76]. VTA dopamine neurons also project to regions of the prefrontal cortex involved in decision making [89], thus any food or weight gain-driven changes to mesolimbic dopamine neurons may also be consequential for the mesocortical dopamine system. For instance, reductions in striatal D2

receptors are also associated with decreased metabolism in cortical regions associated with impulse control and decision making [71,90–92], increased impulsivity [93], and decreased GABAergic receptor binding in the striatum [94] and the frontal cortex [93,95]. Finally, downregulated D2 receptor-associated impulsivity has long been implicated in an inability to abstain from reward seeking in drug abuse [96,97] and compulsive eating [73] disorders, though the degree to which impulsivity precedes addiction-like behavior or arises as a result of it remain unclear.

Because downregulated D2 receptor availability is most likely to occur after extended junk food access or weight gain, it's less likely that our intermittent-fed rats experienced this particular effect to any significant degree (although Bello et al. [98] reports decreased striatal dopamine D2 binding after intermittent sucrose access). However, binge eating disorder (modeled in our experiments by intermittent junk food access) is strongly associated with decreased response inhibition and increased impulsivity [99–101], the predisposition to make impulsive, risky decisions, and a decreased ability to use feedback appropriately [102]. While trait impulsivity is thought to play a *causative* role in these disorders [103,104], drug use has been shown to *exacerbate* impulsivity and disrupt response inhibition [105], creating a vicious cycle of impulsive drug seeking [106]. Given the behavioral and neurochemical similarities between drug addiction and binge eating disorder [107], it is possible that an impulsive phenotype may be both a product of intermittent palatable feeding, and a driving factor in humans with binge eating disorder. The neuromechanisms supporting increased impulsivity after intermittent junk food exposure remain unclear, though altered mesolimbic dopamine signaling remains a likely target.

A High-Fructose Diet Impairs Brain Insulin Signaling, Dopamine Reuptake, And Incentive Motivation

The experiments in Chapter 4 explore how diet-induced insulin resistance might exacerbate incentive motivation via disruptions within the dopamine system. Here, we propose a mechanistic hypothesis that diet-induced insulin resistance might downregulate dopamine transporter (DAT) membrane expression, allowing for an increase in dopamine sensitization in response to reward-paired cues, thereby facilitating incentive motivation. We found that rats exposed to an insulin-dysregulating diet did not experience increased incentive motivation for a reward-paired cue above and beyond that of controls, but instead more frequently for environmental stimuli only loosely paired with reward. This insulin-dysregulating diet was also associated with potentiated phasic dopamine signaling in response to these "neutral" cues, and decreased DAT-mediated reuptake.

Insulin resistance, dopamine signaling, and increased reward seeking: a vicious cycle

As already discussed in detail, the Westernization of global diets and excessive food consumption are strongly implicated in the development of type 2 diabetes [108]. Diets high in refined carbohydrates and sugars are well known to induce insulin resistance in the periphery, which, given correlated levels of insulin in the peripheral and central nervous systems [109], are likely to produce insulin resistance in the brain, as well. Importantly, insulin resistance exists on a continuum from insulin insensitivity, to insulin resistance, to pre-diabetes, to the onset of type 2 diabetes, such that a lack of a type 2 diabetes diagnosis does not rule out some degree of insulin signaling impairment. Alterations in insulin signaling have been repeatedly found to

impact the dopamine system, though the full significance of these effects remain poorly understood. If, consistent with out results from Chapter 4, insulin dysfunction potentiates associative learning between rewards and environmental stimuli, then such a consequence might further drive a susceptibility to the invigorating effects of reward-paired cues, and the consumption of highly palatable, yet poor quality foods, creating a vicious cycle.

While we report these changes using a model of diet-induced insulin resistance, it is possible that the early effects of insulin dysregulation prior to a clinical type 2 diabetes diagnosis might already be impacting behavior. Indeed, many studies report impacts to insulin signaling, and subsequently, mesolimbic dopamine, after manipulations as subtle as fasting and feeding [110,111,98]. Importantly, the administration of the insulin-sensitizing drug pioglitazone appeared to normalize our reported changes in dopamine reuptake and behavior, and might prove useful in the treatment of compulsive eating disorders, even in the absence of a clinical diabetes diagnosis. Indeed, PPARγ agonists such as pioglitazone have been shown to reduce reinstatement of alcohol-seeking [112] and decrease nicotine self-administration [113] in animal models. To our knowledge, such drugs have not been tested in the context of food addiction.

Drug users may be particularly vulnerable to altered insulin metabolism

Given the similarities between drug and food addiction (described in detail above), it is not unreasonable to speculate how diet-induced insulin resistance might also potentiate drug-seeking. Drug users have notoriously poor diets, diets that are strongly associated with metabolic syndrome and the development of insulin resistance. This includes insufficient calorie and nutrient consumption, and a preference for (and subsequent excessive consumption of) poor-

quality snacks and sugars [114–120]. Further, psychostimulant users regularly experience periods of anorexia due to appetite-suppressing drug effects [121], which has been shown to decrease circulating insulin [122] and downregulate the DAT [110,123]. For these reasons, drug users may be particularly vulnerable to the insulin-dopamine interactions reported in Chapter 4.

Further, a number of studies have shown that drug use (tobacco, alcohol, and illicit 'hard' drugs such as cocaine and methamphetamine) may negatively affect glucose and insulin metabolism. Emerging evidence suggests that regular illicit drug use is associated with decreased insulin sensitivity [124–127] and may hasten the onset of type 2 diabetes [128]. The pharmacokinetic effects of cocaine include increased corticotropin and cortisol concentrations [129,130], which increase blood glucose concentrations as well as inhibit pancreatic insulin secretion, activating glycogenolysis and gluconeogenesis. By these mechanisms, the net result is increased glucose production and decreased glucose clearance, which is a major characteristic of and risk factor for insulin resistance and type 2 diabetes [131]. Heroin, too, decreases insulin secretion, resulting in hyperglycemia [124], and heroin and methadone addicts appear to have reduced beta-cell response to glucose stimulation [124,132]. Limited evidence further incriminates tobacco smoking as increasing the risk of developing type 2 diabetes [128,133]. More generally, a history of illicit drug use accelerates the age of onset of type 2 diabetes by 6 years, and illicit drug use combined with heavy alcohol use was a remarkably strong predictor of early type 2 diabetes onset [128]. This effect was so strong the authors suggested perhaps screening patients for drug and alcohol misuse as potential risk factors for type 2 diabetes along with traditional factors such as family history and obesity [128].

Thus, drug use may confer a particular vulnerability to insulin dysregulation via promoting a lifestyle of poor diet choices, and via independent drug effects on insulin function. As a result, insulin signaling in the brains of many drug users may be sufficiently impaired to impact DAT function, despite the absence of a clinical type 2 diabetes diagnosis. If this is the case, our results in Chapter 4 may outline another dopamine-mediated mechanism by which drug abuse is maintained: by promoting a tendency to associate environmental stimuli with reward, insulin-impaired drug users may be more susceptible to the invigorating effects of reward-paired cues, promoting reward seeking and relapse.

Importantly, methodological complications suggest that research on the interactions of illicit drug use and insulin dysfunction in human populations may be incomplete. For instance, most research on this subject recruits from populations attending diabetes clinics and receiving diabetes monitoring and treatment. Drug users and addicts experience many of the same health problems that typify homeless and economically marginalized populations, and receive similarly poor access to health care [134–136]. Because illicit drug users are more likely to receive diabetes care sporadically from emergency healthcare settings (if at all), and not outpatient clinical settings where regular monitoring and maintenance may occur, the heaviest drug users (for whom insulin resistance and glycemic control may be the worst) are likely to be omitted from many studies [137]. Here, animal models may prove useful in filling these gaps; specifically, how and to what extent insulin dysfunction might enhance maladaptive drug seeking remains unclear.

While only a few studies have directly examined whether or how diabetes enhances a vulnerability to tobacco use via increasing dopamine signaling [138,139], to our knowledge none have been done for illicit drugs. Given our data from Chapter 4 and the epidemic increase in diet-induced insulin dysregulation in Westernized societies, how illicit drug users may be particularly affected seems rather important, especially considering the cyclical nature of drug abuse, withdrawal, and relapse. Insulin dysregulation, even at sub-diabetes thresholds, may impart a vulnerability to compulsive drug-taking via its effects on the dopamine system, making withdrawal and long term abstinence even more difficult. Overall, the degree to which chronic drug users regularly suffer from deficits in insulin signaling, the extent to which drug use and diabetes are comorbid, and how insulin dysfunction may promote addition-like behaviors remain understudied.

"Addiction transfer:" transference of compulsive reward seeking between drugs and foods may exacerbate insulin resistance, and drive compulsive reward seeking

A further consideration is the phenomenon by which drug addicts [140,141] and tobacco users [142–144] in recovery or withdrawal quickly gain weight. It is well known that tobacco users gain approximately 6-8 lbs upon quitting smoking [144,145], a weight difference which, over the next 10 years, becomes greatly exacerbated in former smokers versus those who never smoked at all [146]. This "rebound hyperphagia" is a likely mechanism by which users in recovery attempt to stimulate the mesolimbic dopamine reward system that, with abstinence, is no longer being stimulated by drug use [140,147], akin to the mechanism proposed by the reward deficiency syndrome [43,148]. Since weight gain, obesity and the consumption of poor diets are strongly associated with the development of diabetes, it is possible that addicts in recovery are further

increasing their risk of becoming insulin resistant, which, via its effects on the dopamine system, may exacerbate reward seeking behavior, making abstinence even more difficult.

Further, given the remarkable similarities in behavioral and neurochemical phenotypes for drug and food addiction, drug addicts in recovery may find themselves "transferring" their compulsive reward seeking to food. This may be a serious health concern given 1) the known comorbidity of drug addiction with other compulsive behaviors (such as pathological gambling [149] and hypersexual behavior [150]), 2) the behavioral and neurochemical similarities between drug and food addiction (discussed above), and 3) the weight gain experienced during recovery [140,141], which is strongly associated with insulin dysregulation [5]. Taken together, this suggests a predisposition for drug users to replace drug seeking with food seeking, which, when combined with increased insulin resistance, potentiates even further compulsive reward seeking and vulnerability to relapse or excessive food consumption. Further, a plethora of research demonstrates high comorbidity between drug use and eating disorders, particularly binge eating and bulimia [151–154], that can occur either concurrently or sequentially over the course of a lifetime [155]. While many researchers attribute this to a shared behavioral and genetic etiology (i.e., a high impulsivity phenotype or the Taq 1A allele genotype), the precise reason for the high comorbidity remains unclear. Thus, there is ample evidence to support a concern for 1) the transference of drug to food addiction, and 2) the development or exacerbation of insulin dysregulation during recovery, which may impair efforts at abstinence (discussed in [156]).

Interestingly, addiction transfer may be a two-way street. In a follow-up on otherwise successful bariatric surgery patients, researchers have found that some patients developed new compulsive

disorders, such as alcoholism, gambling or compulsive shopping (reviewed in [43]). This occurs even though alcoholism and drug addiction are contraindications for bariatric surgery, strongly suggesting that the development of these compulsive behaviors occurred *after* the surgery, not concurrently with overeating [157]. The case of "trading" one addiction for another supports the hypothesis that a predisposition to compulsive reward-seeking is not drug specific, and that junk food, which is legal and readily available in all Westernized nations, may be just as powerful and rewarding. If genetic or trait predispositions (i.e., high trait impulsivity or the Taq 1A allele genotype) already support a bias for addictive and compulsive behaviors, diet-induced insulin resistance and its downstream effects may further hinder efforts at moderation.

Conclusion

The classification of compulsive overeating as an addiction disorder remains hotly debated, but undoubtedly food and drug addiction share remarkable similarities. Specifically, the overconsumption of food or drugs of abuse produce similar deficits in mesolimbic dopamine transmission and striatal D2 receptor expression, and the specific patterns of reward exposure (i.e., restricted versus extended) may differentially impact these effects. Behaviorally, junk food and drug overconsumption can result in compulsive reward seeking, a hypersensitivity to reward-paired cues, reward hyposensitivity and anhedonia, and impulsive and poor decision making. Obesity is frequently treated as a symptom of food addiction, and is subject to behavioral treatment (e.g., therapy) or medical interventions (e.g., bariatric surgeries) when attempting "recovery," paralleling many treatment options used in drug addiction.

Importantly, the mechanisms by which diet might contribute to maladaptive drug or food seeking remain poorly understood. Growing evidence suggests that insulin resistance, obesity, and chronic junk food exposure might exacerbate compulsive reward seeking via several mechanisms (e.g., downregulated inhibitory control circuitry, sensitized dopamine and sensory systems, increased cue-induced dopamine transmission, downregulated DAT and D2 receptors). If these mechanisms can be identified and understood, novel behavioral or pharmacological targets for treating food or drug addiction might be developed.

References

1. Swinburn, B. A.; Sacks, G.; Hall, K. D.; McPherson, K.; Finegood, D. T.; Moodie, M. L.; Gortmaker, S. L. The global obesity pandemic: shaped by global drivers and local environments. *Lancet (London, England)* **2011**, *378*, 804–14.

2. Krebs, J. R. The gourmet ape: evolution and human food preferences. *Am. J. Clin. Nutr.* **2009**, *90*, 707S–711S.

3. Johnson, F.; Wardle, J. Variety, palatability, and obesity. *Adv. Nutr.* **2014**, *5*, 851–9.

4. Kopelman, P. Health risks associated with overweight and obesity. *Obes. Rev.* **2007**, *8 Suppl 1*, 13–7.

5. Astrup, A.; Finer, N. Redefining type 2 diabetes: “diabesity” or “obesity dependent diabetes mellitus”? *Obes. Rev.* **2000**, *1*, 57–59.

6. Stouffer, M. A.; Woods, C. A.; Patel, J. C.; Lee, C. R.; Witkovsky, P.; Bao, L.; Machold, R. P.; Jones, K. T.; de Vaca, S. C.; Reith, M. E. A.; Carr, K. D.; Rice, M. E. Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. *Nat. Commun.* **2015**, *6*, 8543.

7. Figlewicz, D. P.; Szot, P.; Chavez, M.; Woods, S. C.; Veith, R. C. Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res.* **1994**, *644*, 331–334.

8. Berridge, K. C.; Robinson, T. E. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* **1998**, *28*, 309–369.

9. Ostlund, S. B.; Leblanc, K. H.; Kosheleff, A. R.; Wassum, K. M.; Maidment, N. T. Phasic mesolimbic dopamine signaling encodes the facilitation of incentive motivation produced by repeated cocaine exposure. *Neuropsychopharmacology* **2014**, *39*, 1–9.

10. Kosheleff, A. R.; Grimes, M.; O’Dell, S. J.; Marshall, J. F.; Izquierdo, A. Work aversion and associated changes in dopamine and serotonin transporter after methamphetamine exposure in rats. *Psychopharmacology (Berl).* **2011**, *219*, 411–420.

11. Paulus, M. P.; Hozack, N.; Frank, L.; Brown, G. G.; Schuckit, M. a Decision making by methamphetamine-dependent subjects is associated with error-rate-independent decrease in prefrontal and parietal activation. *Biol. Psychiatry* **2003**, *53*, 65–74.

12. LeBlanc, K. H.; Maidment, N. T.; Ostlund, S. B. Repeated cocaine exposure facilitates the expression of incentive motivation and induces habitual control in rats. *PLoS One* **2013**, *8*, e61355.

13. Davis, C.; Carter, J. C. Compulsive overeating as an addiction disorder. A review of theory and evidence. *Appetite* **2009**, *53*, 1–8.

14. Gearhardt, A. N.; Corbin, W. R.; Brownell, K. D. Food addiction: an examination of the

diagnostic criteria for dependence. *J. Addict. Med.* **2009**, *3*, 1–7.

15. Lenoir, M.; Serre, F.; Cantin, L.; Ahmed, S. H. Intense sweetness surpasses cocaine reward. *PLoS One* **2007**, *2*, e698.

16. Cabanac, M.; Johnson, K. G. Analysis of a conflict between Palatability and cold exposure in rats. *Physiol. Behav.* **1983**, *31*, 249–253.

17. Oswald, K. D.; Murdaugh, D. L.; King, V. L.; Boggiano, M. M. Motivation for palatable food despite consequences in an animal model of binge eating. *Int. J. Eat. Disord.* **2011**, *44*, 203–11.

18. Puhl, R. M.; Moss-Racusin, C. A.; Schwartz, M. B.; Brownell, K. D. Weight stigmatization and bias reduction: perspectives of overweight and obese adults. *Health Educ. Res.* **2008**, *23*, 347–58.

19. Yurcisin, B. M.; Gaddor, M. M.; DeMaria, E. J. Obesity and bariatric surgery. *Clin. Chest Med.* **2009**, *30*, 539–53, ix.

20. Saunders, R. Compulsive eating and gastric bypass surgery: what does hunger have to do with it? *Obes. Surg.* **2001**, *11*, 757–61.

21. Fichter, M. M.; Quadflieg, N.; Brandl, B. Recurrent overeating: An empirical comparison of binge eating disorder, bulimia nervosa, and obesity. *Int. J. Eat. Disord.* **1993**, *14*, 1–16.

22. Drewnowski, A.; Darmon, N. The economics of obesity: dietary energy density and energy cost. *Am. J. Clin. Nutr.* **2005**, *82*, 265S–273S.

23. Bean, M. K.; Stewart, K.; Olbrisch, M. E. Obesity in America: Implications for Clinical and Health Psychologists. *J. Clin. Psychol. Med. Settings* **2008**, *15*, 214–224.

24. Avena, N. M.; Rada, P.; Hoebel, B. G. Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci. Biobehav. Rev.* **2008**, *32*, 20–39.

25. Avena, N. M.; Long, K. A.; Hoebel, B. G. Sugar-dependent rats show enhanced responding for sugar after abstinence: evidence of a sugar deprivation effect. *Physiol. Behav.* **2005**, *84*, 359–62.

26. Wojnicki, F. H. E.; Roberts, D. C. S.; Corwin, R. L. W. Effects of baclofen on operant performance for food pellets and vegetable shortening after a history of binge-type behavior in non-food deprived rats. *Pharmacol. Biochem. Behav.* **2006**, *84*, 197–206.

27. Avena, N. M.; Bocarsly, M. E.; Rada, P.; Kim, A.; Hoebel, B. G. After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance. *Physiol. Behav.* **2008**, *94*, 309–315.

28. Avena, N. M.; Hoebel, B. G. A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience* **2003**, *122*, 17–20.

29. Gosnell, B. A. Sucrose intake enhances behavioral sensitization produced by cocaine. *Brain*

Res. **2005**, *1031*, 194–201.

30. Avena, N. M.; Hoebel, B. G. Amphetamine-sensitized rats show sugar-induced hyperactivity (cross-sensitization) and sugar hyperphagia. *Pharmacol. Biochem. Behav.* **2003**, *74*, 635–9.

31. Avena, N. M.; Carrillo, C. A.; Needham, L.; Leibowitz, S. F.; Hoebel, B. G. Sugar-dependent rats show enhanced intake of unsweetened ethanol. *Alcohol* **2004**, *34*, 203–209.

32. Colantuoni, C.; Rada, P.; McCarthy, J.; Patten, C.; Avena, N. M.; Chadeayne, A.; Hoebel, B. G. Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obes. Res.* **2002**, *10*, 478–88.

33. Wideman, C. H.; Nadzam, G. R.; Murphy, H. M. Implications of an animal model of sugar addiction, withdrawal and relapse for human health. *Nutr. Neurosci.* **2005**, *8*, 269–76.

34. Galic, M. A.; Persinger, M. A. Voluminous Sucrose Consumption in Female Rats: Increased "Nippiness" during Periods of Sucrose Removal and Possible Oestrus Periodicity. *Psychol. Rep.* **2002**, *90*, 58–60.

35. Davis, J. F.; Tracy, A. L.; Schurdak, J. D.; Tschöp, M. H.; Lipton, J. W.; Clegg, D. J.; Benoit, S. C. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. *Behav. Neurosci.* **2008**, *122*, 1257–63.

36. Vendruscolo, L. F.; Gueye, A. B.; Vendruscolo, J. C. M.; Clemens, K. J.; Mormède, P.; Darnaudéry, M.; Cador, M. Reduced alcohol drinking in adult rats exposed to sucrose during adolescence. *Neuropharmacology* **2010**, *59*, 388–394.

37. Blaisdell, A. P.; Lau, Y. L. M.; Telminova, E.; Lim, H. C.; Fan, B.; Fast, C. D.; Garlick, D.; Pendergrass, D. C. Food quality and motivation: a refined low-fat diet induces obesity and impairs performance on a progressive ratio schedule of instrumental lever pressing in rats. *Physiol. Behav.* **2014**, *128*, 220–5.

38. Koob, G. F.; Le Moal, M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* **2001**, *24*, 97–129.

39. Ahmed, S. H.; Kenny, P. J.; Koob, G. F.; Markou, A. Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. *Nat. Neurosci.* **2002**, *5*, 625–6.

40. Markou, A.; Koob, G. F. Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology* **1991**, *4*, 17–26.

41. Kenny, P. J.; Chen, S. A.; Kitamura, O.; Markou, A.; Koob, G. F. Conditioned Withdrawal Drives Heroin Consumption and Decreases Reward Sensitivity. *J. Neurosci.* **2006**, *26*, 5894–5900.

42. Johnson, P. M.; Kenny, P. J. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* **2010**, *13*, 635–41.

43. Blum, K.; Bailey, J.; Gonzalez, A. M.; Oscar-Berman, M.; Liu, Y.; Giordano, J.; Braverman, E.; Gold, M. Neuro-Genetics of Reward Deficiency Syndrome (RDS) as the Root Cause of

"Addiction Transfer": A New Phenomenon Common after Bariatric Surgery. *J. Genet. Syndr. gene Ther.* **2011**, *2012*.

44. Robinson, T.; Berridge, K. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.* **1993**, *18*, 247–291.

45. Ernst, M.; Paulus, M. P. Neurobiology of decision making: a selective review from a neurocognitive and clinical perspective. *Biol. Psychiatry* **2005**, *58*, 597–604.

46. Yamamoto, T.; Ueji, K. Brain mechanisms of flavor learning. *Front. Syst. Neurosci.* **2011**, *5*, 76.

47. Small, D. M. Flavor is in the brain. *Physiol. Behav.* **2012**, *107*, 540–52.

48. Peciña, S.; Smith, K. S. Hedonic and motivational roles of opioids in food reward: implications for overeating disorders. *Pharmacol. Biochem. Behav.* **2010**, *97*, 34–46.

49. Berridge, K. C. Food reward: Brain substrates of wanting and liking. *Neurosci. Biobehav. Rev.* **1996**, *20*, 1–25.

50. Sclafani, A.; Azzara, A. V; Touzani, K.; Grigson, P. S.; Norgren, R. Parabrachial nucleus lesions block taste and attenuate flavor preference and aversion conditioning in rats. *Behav. Neurosci.* **2001**, *115*, 920–33.

51. Norgren, R.; Leonard, C. M. Taste Pathways in Rat Brainstem. *Science (80-.).* **1971**, *173*, 1136–1139.

52. Karádi, Z.; Lukáts, B.; Papp, S.; Szalay, C.; Egyed, R.; Lénárd, L.; Takács, G. Involvement of forebrain glucose-monitoring neurons in taste information processing: electrophysiological and behavioral studies. *Chem. Senses* **2005**, *30 Suppl 1*, i168-9.

53. Sakai, N.; Yamamoto, T. Effects of excitotoxic brain lesions on taste-mediated odor learning in the rat. *Neurobiol. Learn. Mem.* **2001**, *75*, 128–39.

54. Poulin, J.-F.; Chevalier, B.; Laforest, S.; Drolet, G. Enkephalinergic afferents of the centromedial amygdala in the rat. *J. Comp. Neurol.* **2006**, *496*, 859–76.

55. Zhu, W.; Pan, Z. Z. Mu-opioid-mediated inhibition of glutamate synaptic transmission in rat central amygdala neurons. *Neuroscience* **2005**, *133*, 97–103.

56. Gosnell, B. A. Involvement of μ opioid receptors in the amygdala in the control of feeding. *Neuropharmacology* **1988**, *27*, 319–326.

57. de Araujo, I. E.; Oliveira-Maia, A. J.; Sotnikova, T. D.; Gainetdinov, R. R.; Caron, M. G.; Nicolelis, M. A. L.; Simon, S. A. Food reward in the absence of taste receptor signaling. *Neuron* **2008**, *57*, 930–41.

58. Koch, L.; Wunderlich, F. T.; Seibler, J.; Könner, A. C.; Hampel, B.; Irlenbusch, S.; Brabant, G.; Kahn, C. R.; Schwenk, F.; Brüning, J. C. Central insulin action regulates peripheral glucose and fat metabolism in mice. *J. Clin. Invest.* **2008**, *118*, 2132–47.

59. Robinson, T. E.; Berridge, K. C. Incentive-sensitization and addiction. *Addiction* **2001**, *96*, 103–14.

60. Cox, D. N.; van Galen, M.; Hedderley, D.; Perry, L.; Moore, P. B.; Mela, D. J. Sensory and hedonic judgments of common foods by lean consumers and consumers with obesity. *Obes. Res.* **1998**, *6*, 438–47.

61. Olszewski, P. K.; Levine, A. S. Central opioids and consumption of sweet tastants: when reward outweighs homeostasis. *Physiol. Behav.* **2007**, *91*, 506–12.

62. Rada, P.; Avena, N. M.; Hoebel, B. G. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience* **2005**, *134*, 737–744.

63. Avena, N. M.; Rada, P.; Moise, N.; Hoebel, B. G. Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience* **2006**, *139*, 813–820.

64. Foley, K. K. A.; Fudge, M. A. M.; Kavaliers, M.; Ossenkopp, K.-P. K. Quinpirole-induced behavioral sensitization is enhanced by prior scheduled exposure to sucrose: A multi-variable examination of locomotor activity. *Behav. Brain Res.* **2006**, *167*, 49–56.

65. Corwin, R. L.; Avena, N. M.; Boggiano, M. M. Feeding and reward: Perspectives from three rat models of binge eating. *Physiol. Behav.* **2011**, *104*, 87–97.

66. Wise, R. A. Dopamine, learning and motivation. *Nat. Rev. Neurosci.* **2004**, *5*, 483–94.

67. Schultz, W.; Hollerman, J. R. Dopamine neurons report an error in the temporal prediction of rewardduring learning. *Nat. Neurosci.* **1998**, *1*, 304–309.

68. Flagel, S. B.; Clark, J. J.; Robinson, T. E.; Mayo, L.; Czuj, A.; Willuhn, I.; Akers, C. A.; Clinton, S. M.; Phillips, P. E. M.; Akil, H. A selective role for dopamine in stimulus-reward learning. *Nature* **2011**, *469*, 53–7.

69. Bassareo, V.; Di Chiara, G. Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. *J. Neurosci.* **1997**, *17*, 851–861.

70. Wang, G. J.; Smith, L.; Volkow, N. D.; Telang, F.; Logan, J.; Tomasi, D.; Wong, C. T.; Hoffman, W.; Jayne, M.; Alia-Klein, N.; Thanos, P.; Fowler, J. S. Decreased dopamine activity predicts relapse in methamphetamine abusers. *Mol. Psychiatry* **2012**, *17*, 918–25.

71. Volkow, N. D.; Chang, L.; Wang, G. J.; Fowler, J. S.; Ding, Y. S.; Sedler, M.; Logan, J.; Franceschi, D.; Gatley, J.; Hitzemann, R.; Gifford, a; Wong, C.; Pappas, N. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *Am. J. Psychiatry* **2001**, *158*, 2015–21.

72. Stice, E.; Spoor, S.; Bohon, C.; Small, D. M. Relation Between Obesity and Blunted Striatal Response to Food Is Moderated by TaqIA A1 Allele. *Science (80-.).* **2008**, *322*.

73. Volkow, N. D.; Wang, G.-J.; Telang, F.; Fowler, J. S.; Thanos, P. K.; Logan, J.; Alexoff, D.;

Ding, Y.-S.; Wong, C.; Ma, Y.; Pradhan, K. Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. *Neuroimage* **2008**, *42*, 1537–43.

74. Wang, G.-J.; Volkow, N. D.; Logan, J.; Pappas, N. R.; Wong, C. T.; Zhu, W.; Netusll, N.; Fowler, J. S. Brain dopamine and obesity. *Lancet* **2001**, *357*, 354–357.

75. Hajnal, A.; Margas, W. M.; Covasa, M. Altered dopamine D2 receptor function and binding in obese OLETF rat. *Brain Res. Bull.* **2008**, *75*, 70–6.

76. Robertson, S. H.; Rasmussen, E. B. Effects of a cafeteria diet on delay discounting in adolescent and adult rats: Alterations on dopaminergic sensitivity. *J. Psychopharmacol.* **2017**, 26988111773575.

77. Alsiö, J.; Olszewski, P. K.; Norbäck, A. H.; Gunnarsson, Z. E. A.; Levine, A. S.; Pickering, C.; Schiöth, H. B. Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. *Neuroscience* **2010**, *171*, 779–87.

78. Robinson, M. J.; Burghardt, P. R.; Patterson, C. M.; Nobile, C. W.; Akil, H.; Watson, S. J.; Berridge, K. C.; Ferrario, C. R. Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology* **2015**.

79. Colantuoni, C.; Schwenker, J.; McCarthy, J.; Rada, P.; Ladenheim, B.; Cadet, J. L.; Schwartz, G. J.; Moran, T. H.; Hoebel, B. G. Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport* **2001**, *12*, 3549–52.

80. Spangler, R.; Wittkowski, K. M.; Goddard, N. L.; Avena, N. M.; Hoebel, B. G.; Leibowitz, S. F. Opiate-like effects of sugar on gene expression in reward areas of the rat brain. *Brain Res. Mol. Brain Res.* **2004**, *124*, 134–42.

81. Berridge, K. C. Modulation of taste affect by hunger, caloric satiety, and sensory-specific satiety in the rat. *Appetite* **1991**, *16*, 103–120.

82. Rolls, B. J.; Rolls, E. T.; Rowe, E. A.; Sweeney, K. Sensory specific satiety in man. *Physiol. Behav.* **1981**, *27*, 137–142.

83. Havermans, R. C.; Janssen, T.; Giesen, J. C. A. H.; Roefs, A.; Jansen, A. Food liking, food wanting, and sensory-specific satiety. *Appetite* **2009**, *52*, 222–5.

84. Ahn, S.; Phillips, A. G. Dopaminergic correlates of sensory-specific satiety in the medial prefrontal cortex and nucleus accumbens of the rat. *J. Neurosci.* **1999**, *19*, RC29.

85. Ahn, S.; Phillips, A. G. Modulation by Central and Basolateral Amygdalar Nuclei of Dopaminergic Correlates of Feeding to Satiety in the Rat Nucleus Accumbens and Medial Prefrontal Cortex. *J. Neurosci.* **2002**, *22*, 10958–10965.

86. van de Giessen, E.; la Fleur, S. E.; de Bruin, K.; van den Brink, W.; Booij, J. Free-choice and no-choice high-fat diets affect striatal dopamine D2/3 receptor availability, caloric intake, and adiposity. *Obesity (Silver Spring).* **2012**, *20*, 1738–40.

87. Marwitz, S. E.; Woodie, L. N.; Blythe, S. N. Western-style diet induces insulin insensitivity and hyperactivity in adolescent male rats. *Physiol. Behav.* **2015**, *151*, 147–54.

88. Wu, T.; Deng, S.; Li, W.-G.; Yu, Y.; Li, F.; Mao, M. Maternal obesity caused by overnutrition exposure leads to reversal learning deficits and striatal disturbance in rats. *PLoS One* **2013**, *8*, e78876.

89. Carr, D. B.; Sesack, S. R. GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. *Synapse* **2000**, *38*, 114–123.

90. Volkow, N. D.; Fowler, J. S.; Wang, G.-J.; Hitzemann, R.; Logan, J.; Schlyer, D. J.; Dewey, S. L.; Wolf, A. P. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse* **1993**, *14*, 169–177.

91. Volkow, N. D.; Wang, G.-J.; Telang, F.; Fowler, J. S.; Logan, J.; Jayne, M.; Ma, Y.; Pradhan, K.; Wong, C. Profound Decreases in Dopamine Release in Striatum in Detoxified Alcoholics: Possible Orbitofrontal Involvement. *J. Neurosci.* **2007**, *27*, 12700–12706.

92. Volkow, N. D.; Fowler, J. S. Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cereb. Cortex* **2000**, *10*, 318–25.

93. Jupp, B.; Caprioli, D.; Saigal, N.; Reverte, I.; Shrestha, S.; Cumming, P.; Everitt, B. J.; Robbins, T. W.; Dalley, J. W. Dopaminergic and GABA-ergic markers of impulsivity in rats: evidence for anatomical localisation in ventral striatum and prefrontal cortex. *Eur. J. Neurosci.* **2013**, *37*, 1519–1528.

94. Caprioli, D.; Sawiak, S. J.; Merlo, E.; Theobald, D. E. H.; Spoelder, M.; Jupp, B.; Voon, V.; Carpenter, T. A.; Everitt, B. J.; Robbins, T. W.; Dalley, J. W. Gamma aminobutyric acidergic and neuronal structural markers in the nucleus accumbens core underlie trait-like impulsive behavior. *Biol. Psychiatry* **2014**, *75*, 115–23.

95. Sandoval-Salazar, C.; Ramírez-Emiliano, J.; Trejo-Bahena, A.; Oviedo-Solís, C. I.; Solís-Ortiz, M. S. A high-fat diet decreases GABA concentration in the frontal cortex and hippocampus of rats. *Biol. Res.* **2016**, *49*, 15.

96. Lee, B.; London, E. D.; Poldrack, R. a; Farahi, J.; Nacca, A.; Monterosso, J. R.; Mumford, J. A.; Bokarius, A. V; Dahlbom, M.; Mukherjee, J.; Bilder, R. M.; Brody, A. L.; Mandelkern, M. a Striatal dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. *J. Neurosci.* **2009**, *29*, 14734–40.

97. Everitt, B. J.; Belin, D.; Economidou, D.; Pelloux, Y.; Dalley, J. W.; Robbins, T. W. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos. Trans. R. Soc. London* **2008**, *363*, 3125–35.

98. Bello, N. T.; Lucas, L. R.; Hajnal, A. Repeated sucrose access influences dopamine D2 receptor density in the striatum. *Neuroreport* **2002**, *13*, 1575–8.

99. Schag, K.; Schönleber, J.; Teufel, M.; Zipfel, S.; Giel, K. E. Food-related impulsivity in obesity and binge eating disorder--a systematic review. *Obes. Rev.* **2013**, *14*, 477–95.

100. Rosval, L.; Steiger, H.; Bruce, K.; Israël, M.; Richardson, J.; Aubut, M. Impulsivity in women with eating disorders: problem of response inhibition, planning, or attention? *Int. J. Eat. Disord.* **2006**, *39*, 590–3.

101. Nasser, J. A.; Gluck, M. E.; Geliebter, A. Impulsivity and test meal intake in obese binge eating women. *Appetite* **2004**, *43*, 303–7.

102. Svaldi, J.; Brand, M.; Tuschen-Caffier, B. Decision-making impairments in women with binge eating disorder. *Appetite* **2010**, *54*, 84–92.

103. Velázquez-Sánchez, C.; Ferragud, A.; Moore, C. F.; Everitt, B. J.; Sabino, V.; Cottone, P. High trait impulsivity predicts food addiction-like behavior in the rat. *Neuropsychopharmacology* **2014**, *39*, 2463–72.

104. Dawe, S.; Loxton, N. J. The role of impulsivity in the development of substance use and eating disorders. *Neurosci. Biobehav. Rev.* **2004**, *28*, 343–351.

105. Jentsch, J. D.; Taylor, J. R. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl).* **1999**, *146*, 373–90.

106. de Wit, H. Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addict. Biol.* **2009**, *14*, 22–31.

107. Wonderlich, S. A.; Connolly, K. M.; Stice, E. Impulsivity as a risk factor for eating disorder behavior: Assessment implications with adolescents. *Int. J. Eat. Disord.* **2004**, *36*, 172–182.

108. Hu, F. B. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* **2011**, *34*, 1249–57.

109. Schwartz, M. W.; Sipols, A.; Kahn, S. E.; Lattemann, D. F.; Taborsky, G. J.; Bergman, R. N.; Woods, S. C.; Porte, D. Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid. *Am. J. Physiol.* **1990**, *259*, E378-83.

110. Patterson, T. A.; Brot, M. D.; Zavosh, A.; Schenk, J. O.; Szot, P.; Figlewicz, D. P. Food deprivation decreases mRNA and activity of the rat dopamine transporter. *Neuroendocrinology* **1998**, *68*, 11–20.

111. Bello, N. T.; Sweigart, K. L.; Lakoski, J. M.; Norgren, R.; Hajnal, A. Restricted feeding with scheduled sucrose access results in an upregulation of the rat dopamine transporter. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *284*, R1260-8.

112. Stopponi, S.; Somaini, L.; Cippitelli, A.; Cannella, N.; Braconi, S.; Kallupi, M.; Ruggeri, B.; Heilig, M.; Demopulos, G.; Gaitanaris, G.; Massi, M.; Ciccocioppo, R. Activation of Nuclear PPARγ Receptors by the Antidiabetic Agent Pioglitazone Suppresses Alcohol Drinking and Relapse to Alcohol Seeking. *Biol. Psychiatry* **2011**, *69*, 642–649.

113. Panlilio, L. V; Justinova, Z.; Mascia, P.; Pistis, M.; Luchicchi, A.; Lecca, S.; Barnes, C.; Redhi, G. H.; Adair, J.; Heishman, S. J.; Yasar, S.; Aliczki, M.; Haller, J.; Goldberg, S. R. Novel Use of a Lipid-Lowering Fibrate Medication to Prevent Nicotine Reward and Relapse:

Preclinical Findings. *Neuropsychopharmacology* **2012**, *37*, 1838–1847.

114. Gambera, S. E.; Clarke, J. A. Comments on dietary intake of drug-dependent persons. *J. Am. Diet. Assoc.* **1976**, *68*, 155–7.

115. Morabia, A.; Fabre, J.; Ghee, E.; Zeger, S.; Orsat, E.; Robert, A. Diet and Opiate Addiction: a quantitative assessment of the diet of non-institutionalized opiate addicts. *Addiction* **1989**, *84*, 173–180.

116. el-Nakah, A.; Frank, O.; Louria, D. B.; Quinones, M. A.; Baker, H. A vitamin profile of heroin addiction. *Am. J. Public Health* **1979**, *69*, 1058–60.

117. Santolaria-Fernández, F. J.; Gómez-Sirvent, J. L.; González-Reimers, C. E.; Batista-López, J. N.; Jorge-Hernández, J. A.; Rodríguez-Moreno, F.; Martínez-Riera, A.; Hernández-García, M. T. Nutritional assessment of drug addicts. *Drug Alcohol Depend.* **1995**, *38*, 11–8.

118. Zador, D.; Wall, P. M. L.; Webster, I. High sugar intake in a group of women on methadone maintenance in South Western Sydney, Australia. *Addiction* **1996**, *91*, 1053–1062.

119. Isganaitis, E.; Lustig, R. H. Fast food, central nervous system insulin resistance, and obesity. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 2451–62.

120. Pereira, M. A.; Kartashov, A. I.; Ebbeling, C. B.; Van Horn, L.; Slattery, M. L.; Jacobs, D. R.; Ludwig, D. S. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. *Lancet (London, England)* **2005**, *365*, 36–42.

121. Demellweek, C.; Goudie, A. J. Behavioural tolerance to amphetamine and other psychostimulants: The case for considering behavioural mechanisms. *Psychopharmacology (Berl).* **1983**, *80*, 287–307.

122. Olefsky, J. M. Effects of fasting on insulin binding, glucose transport, and glucose oxidation in isolated rat adipocytes: relationships between insulin receptors and insulin action. *J. Clin. Invest.* **1976**, *58*, 1450–60.

123. Owens, W. A.; Sevak, R. J.; Galici, R.; Chang, X.; Javors, M. A.; Galli, A.; France, C. P.; Daws, L. C. Deficits in dopamine clearance and locomotion in hypoinsulinemic rats unmask novel modulation of dopamine transporters by amphetamine. *J. Neurochem.* **2005**, *94*, 1402–1410.

124. Giugliano, D. Morphine, opioid peptides, and pancreatic islet function. *Diabetes Care* **1984**, *7*, 92–8.

125. Passariello, N.; Giugliano, D.; Quatraro, A.; Consoli, G.; Sgambato, S.; Torella, R.; D'Onofrio, F. Glucose tolerance and hormonal responses in heroin addicts. A possible role for endogenous opiates in the pathogenesis of non-insulin-dependent diabetes. *Metabolism* **1983**, *32*, 1163–1165.

126. Hwang, S. W.; Bugeja, A. L. Barriers to appropriate diabetes management among homeless people in Toronto. *CMAJ* **2000**, *163*, 161–5.

127. Ceriello, A.; Giugliano, D.; Passariello, N.; Quatraro, A.; Dello Russo, P.; Torella, R.; D'Onofrio, F. Impaired glucose metabolism in heroin and methadone users. *Horm. Metab. Res.* **1987**, *19*, 430–3.

128. Johnson, K. H.; Bazargan, M.; Cherpitel, C. J. Alcohol, tobacco, and drug use and the onset of type 2 diabetes among inner-city minority patients. *J. Am. Board Fam. Pract.* **2001**, *14*, 430–6.

129. Umpierrez, G. E.; Kelly, J. P.; Navarrete, J. E.; Casals, M. M.; Kitabchi, A. E. Hyperglycemic crises in urban blacks. *Arch. Intern. Med.* **1997**, *157*, 669–75.

130. Warner, E. A.; Greene, G. S.; Buchsbaum, M. S.; Cooper, D. S.; Robinson, B. E. Diabetic ketoacidosis associated with cocaine use. *Arch. Intern. Med.* **1998**, *158*, 1799–802.

131. Haffner, S. M.; Miettinen, H. Insulin Resistance Implications for Type II Diabetes Mellitus and Coronary Heart Disease. *Am. J. Med.* **1997**, *103*, 152–162.

132. Saunders, S. A.; Democratis, J.; Martin, J.; Macfarlane, I. A. Intravenous drug abuse and Type 1 diabetes: financial and healthcare implications. *Diabet. Med.* **2004**, *21*, 1269–73.

133. Falck, R. S.; Wang, J.; Siegal, H. A.; Carlson, R. G. Current physical health problems and their predictors among a community sample of crack-cocaine smokers in Ohio. *J. Psychoactive Drugs* **2003**, *35*, 471–8.

134. Butters, J.; Erickson, P. G. Meeting the health care needs of female crack users: a Canadian example. *Women Health* **2003**, *37*, 1–17.

135. Weiss, L.; McCoy, K.; Kluger, M.; Finkelstein, R. Access to and use of health care: perceptions and experiences among people who use heroin and cocaine. *Addict. Res. Theory* **2009**.

136. Andersen, M. Health Needs of Drug Dependent Clients. *Women Health* **2008**, *5*, 23–34.

137. Sheldon, B.; Quin, J. Diabetes and illicit drug use. *Pract. Diabetes Int.* **2005**, *22*, 222–224.

138. Richardson, J. R.; Pipkin, J. A.; O'Dell, L. E.; Nazarian, A. Insulin resistant rats display enhanced rewarding effects of nicotine. *Drug Alcohol Depend.* **2014**, *140*, 205–7.

139. O'Dell, L. E.; Nazarian, A. Enhanced vulnerability to tobacco use in persons with diabetes: A behavioral and neurobiological framework. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2015**.

140. Edge, P. J.; Gold, M. S. Drug withdrawal and hyperphagia: lessons from tobacco and other drugs. *Curr. Pharm. Des.* **2011**, *17*, 1173–9.

141. Hodgkins, C. C.; Cahill, K. S.; Seraphine, A. E.; Frost-Pineda, K.; Gold, M. S. Adolescent drug addiction treatment and weight gain. *J. Addict. Dis.* **2004**, *23*, 55–65.

142. Hall, S. M.; Ginsberg, D.; Jones, R. T. Smoking cessation and weight gain. *J. Consult. Clin. Psychol.* **1986**, *54*, 342–346.

143. Hall, S. M.; Tunstall, C. D.; Vila, K. L.; Duffy, J. Weight gain prevention and smoking cessation: cautionary findings. *Am. J. Public Health* **1992**, *82*, 799–803.

144. Filozof, C.; Fernández Pinilla, M. C.; Fernández-Cruz, A. Smoking cessation and weight gain. *Obes. Rev.* **2004**, *5*, 95–103.

145. Williamson, D. F.; Madans, J.; Anda, R. F.; Kleinman, J. C.; Giovino, G. A.; Byers, T. Smoking cessation and severity of weight gain in a national cohort. *N. Engl. J. Med.* **1991**, *324*, 739–45.

146. Flegal, K. M.; Troiano, R. P.; Pamuk, E. R.; Kuczmarski, R. J.; Campbell, S. M. The influence of smoking cessation on the prevalence of overweight in the United States. *N. Engl. J. Med.* **1995**, *333*, 1165–70.

147. Orsini, C. A.; Ginton, G.; Shimp, K. G.; Avena, N. M.; Gold, M. S.; Setlow, B. Food consumption and weight gain after cessation of chronic amphetamine administration. *Appetite* **2014**.

148. Blum, K.; Braverman, E. R.; Holder, J. M.; Lubar, J. F.; Monastra, V. J.; Miller, D.; Lubar, J. O.; Chen, T. J.; Comings, D. E. Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. *J. Psychoactive Drugs* **2000**, *32 Suppl*, i–iv, 1-112.

149. Dannon, P. N.; Lowengrub, K.; Shalgi, B.; Sasson, M.; Tuson, L.; Saphir, Y.; Kotler, M. Dual psychiatric diagnosis and substance abuse in pathological gamblers: a preliminary gender comparison study. *J. Addict. Dis.* **2006**, *25*, 49–54.

150. Black, D. W. The epidemiology and phenomenology of compulsive sexual behavior. *CNS Spectr.* **2000**, *5*, 26–72.

151. Ricca, V.; Castellini, G.; Mannucci, E.; Monami, M.; Ravaldi, C.; Gorini Amedei, S.; Lo Sauro, C.; Rotella, C. M.; Faravelli, C. Amphetamine derivatives and obesity. *Appetite* **2009**, *52*, 405–9.

152. Wolfe, W. L.; Maisto, S. A. The relationship between eating disorders and substance use. *Clin. Psychol. Rev.* **2000**, *20*, 617–631.

153. Holderness, C. C.; Brooks-Gunn, J.; Warren, M. P. Co-morbidity of eating disorders and substance abuse review of the literature. *Int. J. Eat. Disord.* **1994**, *16*, 1–34.

154. Bulik, C. M.; Sullivan, P. F.; Carter, F. A.; Joyce, P. R. Lifetime comorbidity of alcohol dependence in women with bulimia nervosa. *Addict. Behav.* **1997**, *22*, 437–446.

155. Davis, C.; Claridge, G. The eating disorders as addiction. *Addict. Behav.* **1998**, *23*, 463–475.

156. Merlo, L. J.; Gold, M. S. Addiction Research and Treatment. *Psychiatr. Times* **2008**, *25*, 1–11.

157. Buchwald, H.; Rudser, K. D.; Williams, S. E.; Michalek, V. N.; Vagasky, J.; Connett, J. E.

Overall mortality, incremental life expectancy, and cause of death at 25 years in the program on the surgical control of the hyperlipidemias. *Ann. Surg.* **2010**, *251*, 1034–40.