IMPACT OF DIET ON LEARNING, MEMORY AND COGNITION

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IMPACT OF DIET ON LEARNING, MEMORY AND COGNITION

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Changes in food composition and availability have contributed to the dramatic increase in obesity over the past 30-40 years in developed and, increasingly, in developing countries. The modern diet now contains many foods that are rich in saturated fat and refined sugar. People who eat excessive amounts of this diet are not only likely to become overweight, even obese, develop metabolic and cardiovascular diseases, some forms of cancer, but also undergo a more rapid rate of normal age-related cognitive decline and more rapid progression of neurological diseases such as dementia. A central problem is why people persist in consuming this diet in spite of its adverse health effects and when alternative food choices are available. As high fat / high sugar foods are inherently rewarding, eating for pleasure, like taking psychoactive drugs, can modulate reward neurocircuitry, causing changes in responsiveness to reward-predicting stimuli and incentive motivation. Indeed, the excessive ingestion in modern societies and the resulting obesity epidemic may be viewed as a form of food addiction. Thus, a diet high in palatable foods is proposed to impact upon reward systems in the brain, modulating appetitive learning and altering reward thresholds.

Impairments in other forms of cognition have been associated with obesity, and these have a rapid onset. The hippocampus appears to be particularly vulnerable to the detrimental effects of high fat and high sugar diets. Recent research has shown that as little as one week of exposure to a high fat, high sugar diet leads to impairments in place but not object recognition memory in the rat. Excess sugar alone had similar effects, and the detrimental effects of diet consumption was linked to increased inflammatory markers in the hippocampus, a critical region involved in memory. Furthermore, obesity-related inflammatory changes have also been described in the human brain that may lead to memory impairments. These memory deficits may contribute to pathological eating behaviour through changes in the amount consumed and timing of eating.

The aim of this eBook is to present up-to-date information about the impact of diet and diet-induced obesity on reward driven learning, memory and cognition, encompassing both animal and human literature, and also potential therapeutic targets to attenuate such deficits.

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EDITORIAL
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Editorial: Impact of Diet on Learning, Memory and Cognition

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Keywords: diet, obesity, cognition, memory, reward, adolescent, fMRI neuroimaging, behavior

Editorial on the Research Topic

Impact of Diet on Learning, Memory and Cognition

The so-called western diet is rich in saturated fat and refined carbohydrates. Excessive consumption of this diet is associated not only with the development of obesity but also with reduced global cognitive function, cognitive decline, and dementia (Morris et al., 2015). This Research Topic explores the effects of diet and diet-induced obesity on learning, memory, and cognition in both experimental and epidemiological settings.

High fat and high sugar foods are highly rewarding and excessive consumption leads to enduring alterations in brain regions involved in learning, memory, and reward. These changes are proposed to drive overconsumption by promoting food seeking behaviors. Moreover, alterations in brain regions essential for learning, memory, and behavioral control induced by this diet appear to be especially profound in the immature brain (Boitard et al.; Gainey et al.; Reichelt).

The neuroplasticity mechanisms that underpin cognitive and behavioral alterations are reviewed by Morin et al., with particular reference to neuronal alterations in the hippocampus and prefrontal cortex (PFC), brain regions essential for encoding memories and controlling behavior, as well as in the amygdala and nucleus accumbens, regions involved in processing and seeking rewards. The abundance of palatable foods in modern environments contributes to their overconsumption, increases in body weight and progression to obesity. Kendig et al. examined whether food-seeking behaviors in rodents differ in an environment associated with junk foods vs. one that contained regular chow. The important result was that food seeking behavior in the environment associated with junk food became relatively inflexible and habit based, whereas food seeking in the chow associated environment was flexible and goal-directed. These and other findings (e.g., Furlong et al., 2014) may provide new insights into environmental determinants of over-consumption. Stressful experiences are also involved in triggering overconsumption in binge-eating disorder (BED). Lyu and Jackson utilized functional imaging (fMRI) following exposure to an acute stressor (cold pressor test) and observed reduced inhibitory hippocampal responsiveness to food cues in BED-symptomatic women.

Consumption of a western style diet in rats altered levels of the neurotransmitter dopamine and associated metabolites in the striatum and hippocampus, suggesting a mechanistic basis by which such diets may alter food related learning and memory processes (Nguyen et al.). Recent evidence has begun to link the gut microbiome with dietary- and metabolic-associated hippocampal impairment. High fat and/or high sugar diets alter gut bacteria (microbiotal) colonies and in turn increase intestinal permeability and reduce blood brain barrier integrity (Noble et al.). This creates a vulnerability to the influx of toxins from the circulation to the brain, potentially underpinning diet-induced cognitive dysfunction.

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This Research Topic contains epidemiological and experimental evidence of sensitive or critical time points at which diet can alter brain and cognitive development with enduring consequences. For example, childhood obesity is increasingly prevalent and is associated with diminished cognition. Reichelt reviews preclinical evidence that exposure to high fat and high sugar diets during adolescence produces more profound cognitive deficits than such exposure during adulthood. Such diets may be especially injurious to cognition when consumed across adolescence because this is a period of heightened neuroplasticity due to age-specific maturational processes, including pruning of dopamine receptors in the prefrontal cortex.

Studies exploring the reversibility of diet-induced cognitive deficits in young animals through both dietary and pharmacological methods are presented in this Research Topic. Gainey et al. demonstrated that administration of glyburide, a second-generation drug for the treatment of type-2-diabetes that stimulates insulin release, attenuated high fat diet evoked deficits in anxiety and memory in young mice. Acute glyburide administration reversed high fat diet evoked memory deficits in a novel object recognition memory task and alleviated anxiety-like behaviors in mice fed a high fat diet across adolescence. Drugs that stimulate insulin secretion may thus have potential for the treatment of obesity-associated cognitive dysfunction. Furthermore, Boitard et al. showed that switching rodents to a standard chow diet for 12 weeks following adolescent consumption of a high fat diet for 12 weeks was sufficient to restore aspects of diet induced changes in cognitive and emotional processing. Rats returned to the chow diet exhibited greater hippocampal neurogenesis measured by doublecortin immunoreactivity, and reduced HPA axis reactivity measured by blood corticosterone, and amygdala activity by c-Fos, as well as better conditioned odor avoidance memory.

Poor diet is a potential risk factor for the development of cognitive impairment; conversely, dietary nutrients are protective against such impairments. Lu et al. reported a cross-sectional study which examined the impact of dietary nutrients on the development of mild cognitive impairment (MCI). Dietary intake of nutrients was compared between MCI patients and cognitively normal subjects. Carotenoids, vitamin C, and vitamin B6 were identified as the dietary nutrients with the highest protective capacity against MCI, potentially due to their antioxidant properties. Moreover, adequate dietary intake of monounsaturated fatty acids and cholesterol were significantly associated with decreased risk of MCI. Wang et al. examined the association between widespread scarcity of food at various childhood developmental stages (fetal exposure—late childhood exposure) on subsequent cognitive performance in an adult Chinese cohort (age range 51–65). These investigators found that famine during the fetal period was associated with subsequent global cognitive decline and increased risk of MCI, and that famine during mid- and late-childhood was associated with deficits in executive function in adulthood. Critically, this study highlights the importance of nutrient availability during early life on adult cognitive function.

What is apparent from the studies presented in this Research Topic is the pervasive influence of diet and food-associated environments on cognition, motivation, and behavioral control. The papers collected in this Research Topic offer new and valuable insights into the psychological processes and neural mechanisms underpinning this pervasive influence of diet and food-associated environments, be it through excessive consumption of fat and sugar, or malnutrition across the lifespan. The findings reported will form the basis for novel theoretical ideas and applications to an increasingly severe public health issue.

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AR, RW, and MM wrote the editorial and served as editors for the Research Topic.

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REVIEW
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Palatable Hyper-Caloric Foods Impact on Neuronal Plasticity

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Neural plasticity is an intrinsic and essential characteristic of the nervous system that allows animals "self-tuning" to adapt to their environment over their lifetime. Activity-dependent synaptic plasticity in the central nervous system is a form of neural plasticity that underlies learning and memory formation, as well as long-lasting, environmentally-induced maladaptive behaviors, such as drug addiction and overeating of palatable hyper-caloric (PHc) food. In western societies, the abundance of PHc foods has caused a dramatic increase in the incidence of overweight/obesity and related disorders. To this regard, it has been suggested that increased adiposity may be caused at least in part by behavioral changes in the affected individuals that are induced by the chronic consumption of PHc foods; some authors have even drawn attention to the similarity that exists between over-indulgent eating and drug addiction. Long-term misuse of certain dietary components has also been linked to chronic neuroimmune maladaptation that may predispose individuals to neurodegenerative conditions such as Alzheimer's disease. In this review article, we discuss recent evidence that shows how consumption of PHc food can cause maladaptive neural plasticity that converts short-term ingestive drives into compulsive behaviors. We also discuss the neural mechanisms of how chronic consumption of PHc foods may alter brain function and lead to cognitive impairments, focusing on prenatal, childhood and adolescence as vulnerable neurodevelopmental stages to dietary environmental insults. Finally, we outline a societal agenda for harnessing permissive obesogenic environments.

Keywords: obesity, overweight, adiposity, food addiction, indulgent eating, hedonics, neuroinflammation, neural plasticity

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INTRODUCTION

Given the abundance and omnipresence of palatable hyper-caloric (PHc) foods, overweight and obesity have become a pandemic phenotype in a large portion of the world's population (WHO, 2016a). Thus, an increased understanding of the underlying causes of obesity is warranted in order to better prevent and treat this growing and global health problem.

Short-term homeostatic control of food intake is essential for animal survival. In addition to this, top-down modulation of homeostatic circuits including palatability and post-prandial rewarding effects modulate food ingestion and seeking behavior (Tulloch et al., 2015). Those drives can support and motivate long-term foraging strategies and planning. In the modern calorie-permissive societies, in which lower energy investments are required to obtain PHc food, those hard-wired capacities, which once evolved to cope with uncertain caloric availability in the wilderness and were evolutionary acquired as adaptive characters, now clearly became maladaptive and do not promote health. Evidence reviewed here suggest that PHc food consumption is self-reinforcing and may further lead to health problems, including cognitive impairments and possibly neurodegenerative diseases that produce a decrease in general wellbeing and productivity. But how eating densely caloric foods can modify brain and behavior in such drastic ways?

In this review article we will explore the brain plasticity mechanism that contribute to persistent overeating and thus causing overweight/obesity, focusing on the overlap of learning and memory, addictive behaviors and indulgent eating. As well we pinpoint critical neurodevelopmental periods for dietary environmental insults. Graphical summaries are depicted on **Figures 1**, **2** and key terms definitions can be found as glossary on **Table 1**.

Neural Plasticity and Addictive Behaviors

One of the most outstanding properties of the nervous system is its ability to modify its structure and function in response to experience, thus allowing individual ontogenic "self-tuning" to particular environmental drivers. The phenomenon of neural plasticity is known to underlie the learning, consolidation and refinement of both adaptive and maladaptive behaviors (Abbott and Nelson, 2000; Citri and Malenka, 2008; Sehgal et al., 2013). At the synaptic level, activity-dependent modifications of the strength or efficacy of synaptic transmission shape the response properties of neural circuits. The versatility and complexity of neural computations is made possible by a huge diversity of cellular plasticity mechanisms (Nelson and Turrigiano, 2008). Those include Hebbian-type plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), as well as homeostatic synaptic scaling and metaplasticity (Pérez-Otaño and Ehlers, 2005).

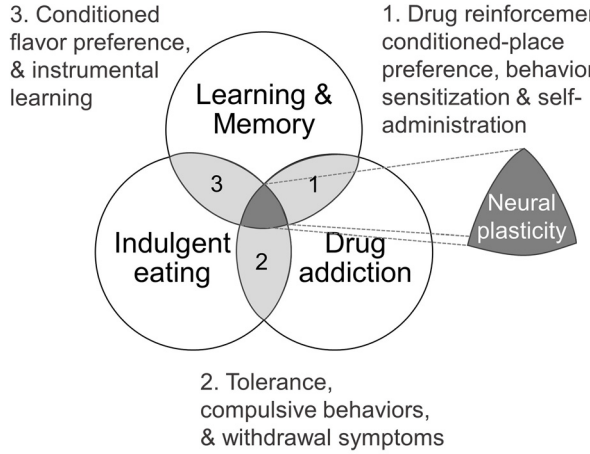

FIGURE 1 | Theoretical framework as Venn diagram showing intersections of learning and memory, drug addiction and indulgent eating (see text for details).

Some studies have suggested that the development of addictive behaviors share common features with traditional learning models (**Figure 1**; Jones and Bonci, 2005). For example, N-methyl-D-aspartate (NMDA) receptors blockade, which effectively blocks LTP and LTD in many brain regions (Malenka and Bear, 2004), also prevents many behavioral adaptations normally associated with drug reinforcement, such as conditioned-place preference, behavioral sensitization and self-administration (Mameli and Lüscher, 2011). Furthermore, relapse caused by exposure to cues associated with the drug experience is a major clinical problem that contributes to the persistence of addiction, and its underlying mechanisms are thought to depend at least in part on the phenomenon of pattern completion in the hippocampal CA3 region, which is a hallmark of contextual memory retrieval (Kauer and Malenka, 2007; Kesner et al., 2016). On the other hand, synaptic scaling of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-receptors surface expression in the nucleus accumbens (NAcc) neurons has been observed with the appearance of addictions (Sun and Wolf, 2009; Tang and Dani, 2009; Reimers et al., 2014). In addition, a single cocaine administration induces metaplasticity in the ventral tegmental area (VTA) through increased synaptic non-GluA2 containing AMPA receptors as well as NR2B containing NMDA receptors, contributing to sensitization upon further exposure, as well as possibly lowering the threshold for further plasticity events in the VTA—NAcc pathway (Creed and Lüscher, 2013). More controversial, however, is the idea that humans can develop "food-dependence" through learning and habit-formation, and that obesity may be seen, at least in some cases, as a clinical manifestation of "food addiction" (Volkow and Wise, 2005; Blumenthal and Gold, 2010; Volkow et al., 2013a; García-García et al., 2014; Carlier et al., 2015). Even though food, as opposed to drugs of abuse, is needed for an organism's survival, dependence on PHc foods in humans and animal models shares characteristics with drug addiction (**Figure 1**). These include activation of the mesolimbic dopaminergic system (Blackburn et al., 1986; Hernandez and Hoebel, 1988), the activation of similar brain structures (Robinson et al., 2016), as well as an overlapping symptomatology such as the appearance of tolerance, compulsive behaviors (Johnson and Kenny, 2010; Rossetti et al., 2014) and withdrawal symptoms in relation to PHc food that has been consistently observed in obese individuals (Iemolo et al., 2012; García-García et al., 2014). In this regard, there are many similarities between the eating behavior of some obese individuals and the diagnostic criteria for substances dependence on the *Diagnostic and Statistical Manual of Mental Disorders* (DSM -IV, -5). For instance, both patterns of behavior show

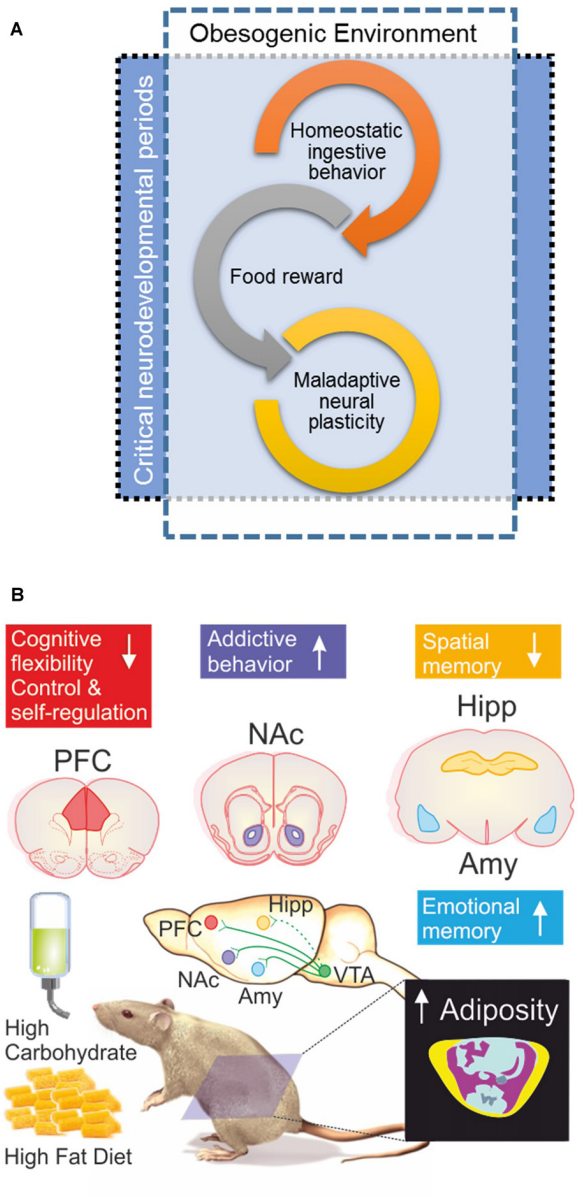

FIGURE 2 | (A) When the obesogenic environment overlaps critical neurodevelopmental periods, enhanced maladaptive neural plasticity may be expected; which could eventually lead to uncontrolled ingestive behavior (food addiction). Interplay of food reward and homeostatic ingestive behavior may evolve in wilderness to promote biological fitness under extremely different evolutionary pressures; e.g., scarcity and unpredictable access to food, low dense caloric food, large caloric investments in foraging/hunting.
(B) Obesogenic environment driven by palatable hyper-caloric (PHc) food can be experimentally modeled in rodents by exposure to high carbohydrate/high fat diet (HFD) resulting in increased adiposity evidenced by body composition analysis by micro-computed tomography; yellow = sub cutaneous fat/pink = visceral fat, blue = lean mass, ultimately causing diet induced obesity (DIO). Experimental evidence documents that exposure to a high carbohydrate/HFD negatively impact on cognitive functions, with increased sensitivity during prenatal, childhood and adolescence neurodevelopmental stages. In particular, hippocampal (Hipp) and pre-frontal cortex (PFC) dependent tasks are negatively impaired; whereas amygdala (Amy) dependent
(*Continued*)

FIGURE 2 | Continued
function seems to be enhanced. Cognitive impairments are accompanied (or preceded) by ingestive addictive behaviors driven by the dopaminergic reward system that initiates its projections on the ventral tegmental area (VTA) directly innervating the Amy, PFC, as well as the nucleus accumbens (NAcc; Lisman and Grace, 2005; Russo and Nestler, 2013), the brain structure assessing the hedonic and saliency stimuli properties. It should be remarked that direct projections from VTA to Hipp are on current debate (Takeuchi et al., 2016), thus are depicted with a dash-line. The "reward deficiency syndrome" propose that addiction vulnerability results on from hyporesponsiveness of the midbrain dopaminergic system, leading individuals to seek out and engage in addictive behaviors in order to compensate for underarousal (George et al., 2012), which is in line to the theory of food addiction (Volkow and Wise, 2005; Davis et al., 2011) in particular for PHc food (Ifland et al., 2009; Schulte et al., 2015).

signs of: tolerance; withdrawal; substances taken in larger amounts or for longer time than intended; unsuccessful efforts to control usage; a large amount of time spent obtaining, using, or recovering from use of the substance; a neglect of social, occupational, or recreational activities; and continued use despite a recurrent physical or psychological problem caused or exacerbated by the substance (Davis et al., 2011). Following this rationale and aiming to develop a reliable tool for diagnosing food addiction, the DSM-IV criteria for substance dependence have been adapted to create the *Yale Food Addiction Scale* (*YFAS*, Gearhardt et al., 2009, 2016).

Additionally, it is important to recognize that purified and concentrated ingredients used to produce PHc food do resemble the production of addictive drugs that refine cocaine from coca leaf or heroin from poppies (Ifland et al., 2009). There is still scientific debate and no consensus has been reached on the etiological magnitude of food addiction on explaining obesity (Carter et al., 2016), however it is clear by now that in particular PHc foods, like addictive drugs, may produce powerful changes in the brain reward circuitry that we did not evolve for, leading to overconsumption and weight gain. Supporting this view, recent evidence indicates that the addictive effect of food, as for drugs, may be dependent on the rate of its absorption and metabolism; foods reported to be more addictive are rapidly digested and absorbed (Schulte et al., 2015; Criscitelli and Avena, 2016) and are also highly rewarding as we will comment on the next section.

Reward-Modulated Nutrient Intake

In addition to the homeostatic circuitry that underlie eating (reviewed in Morton et al., 2014), food intake is strongly regulated by hedonic or reward-based signals, which can often override the homeostatic pathways during periods of relative energy abundance by increasing the desire to consume palatable foods (Lutter and Nestler, 2009). Presentation of palatable foods induces potent release of dopamine into the NAcc, originating in the VTA projection, contributing to the motivational and rewarding value of food (**Figure 2B**). Crucially, the activation of this pathway during meals is related to a loss of control over food intake in some individuals (Stoeckel et al., 2008).

The hedonic component of food intake can be further divided in palatability and post-prandial reward. The palatability

TABLE 1 | Glossary.

Diet induced obesity (DIO)	Procedure to expose experimental subjects to a hypercaloric diet intervention (e.g., HFD, Western diet).
High fat diet (HFD)	Diet used on pre-clinical experiments usually with at least 45 kcal% from fat (predominately lard). In contrast a control diet contains 10 kcal% from fat.
Homeostatic synaptic scaling	Homeostatic synaptic scaling or simply synaptic scaling is a post-synaptic synaptic plasticity mechanism that changes the global level of postsynaptic AMPA receptors according to a neuron's activity history.
Long term depression (LTD)	Sustained, use-dependent decrease of the efficiency of a connection between two or more neurons
Long term potentiation (LTP)	Sustained, use-dependent increase of the efficiency of a connection between two or more neurons
Indulgent eating	Indulgent behavior caused by loss of self-control is characterized by time-inconsistent preferences, or a tendency to overweigh short-term rewards relative to more distant ones, and a tendency in the short term to ignore the costs of one's actions. Thus indulgent eating in some case might be the first step of overeating and other eating behavior disorders.
Metaplasticity	Phenomenon by which the activity history of a given synapse determines its susceptibility to further activity-dependent modification as well as the nature of such modification.
Outcome devaluation	Outcome devaluation occurs when a food reward used during training is devalued by allowing free access to it or by pairing it with an aversive consequence such as gastric malaise.
Overeating/hyperphagia	Is the excess food ingestion in relation to the energy that an organism expends, resulting in overweight/obesity phenotype. It might be related to hypothalamic hyperphagia disorders.
Palatability	Is the hedonic reward provided by foods which often varies relative to the homeostatic satisfaction of nutritional, water, or energy needs.
Pattern completion	Ability to recall an entire memory when presented with a partial sensory cue.
Roux-En-Y gastric bypass surgery	Surgical procedure in which the proximal part of the stomach is cut from the rest. The small intestine is then cut and its distal part is attached to the newly formed pouch below the esophagus, while the proximal part (connected to the larger remaining portion of the stomach) is attached further down. This procedure has been successfully employed in humans to treat morbid obesity.
Synaptic pruning	Widespread process of synapse elimination that occurs during childhood and adolescence, in an experience-dependent fashion.
Synaptic stripping	Removal of dysfunctional synapses by activated microglia.
Western diet (also known as cafeteria diet)	Diet used on pre-clinical experiments where the animal self-selects from palatable, readily available foods including cookies, candy, cheese and processed meats. These foods contain a substantial amount of salt, sugar and fat, which are meant to simulate the human Western diet.

subcomponent can be inferred since mammals have innate preference for sweet-flavored solutions over bitter ones independently of their caloric content, and rats learn to prefer a saccharin-sweetened solution over water once it is recognized as safe (Bermúdez-Rattoni, 2004; Yarmolinsky et al., 2009; Drewnowski et al., 2012). Consumption of sucralose, a non-caloric artificial sweetener, induces increases in NAcc dopamine release at levels comparable to sucrose (de Araujo et al., 2008). However, taste palatability alone, independent of its nutritive properties fails to elicit the full rewarding effect of a "good meal", which integration is dependent upon the summation of relatively independent multisensory "layers of reward", that include not only taste pleasantness and post-prandial reward, but also visual and olfactory anticipatory cues (de Araujo, 2011).

Post-prandial reward perception is thought to play a central role in the modulation of eating habits (Antoni et al., 2016). In fact, recent evidence has shown that rodents can learn to identify food as rewarding based solely on its caloric content, independently of their taste. For example, ageusic trpm5$^{-/-}$ mice, though initially failing to distinguish between water and a sucrose solution, later develop a preference for sucrose that is indistinguishable from that of wild-types (de Araujo et al., 2008; Simon et al., 2008; Domingos et al., 2011). Pre- and post-absorptive signals from the gut that could alter dopaminergic activity and hence account for the taste-independent rewarding value of sugar are thought to be involved (de Araujo et al., 2012). Indeed, recent evidence has shown that the hormone leptin interfered with the ability of sucrose to produce taste-independent dopaminergic neurons firing. Conversely, other evidences suggest that in addition to its well-established orexygenic effects, the gut peptide ghrelin may have a role in post-prandial reward processing (Müller et al., 2015; Reichelt et al., 2015).

PHc Food Consumption and Neural Plasticity

Post-prandial reward processing in food consumption involves dopamine efflux in the dorsal striatum (de Araujo et al., 2012). In rodents, this region contains distinct neural circuits that are involved in goal-directed behavior, in the case of the dorsomedial striatum, whereas in habit-based behavior, in the case of the dorsolateral striatum (**Figure 2B**). Imbalance in these action-control systems is thought to underlie a wide range of neuropsychiatric disorders (Balleine and O'Doherty, 2010). Indeed, there is an extensive overlap between the neural circuits activated by PHc food and drugs of abuse (Kenny, 2011a). In recent years, efforts have been deployed to unveil whether obesity and drug addiction share some common mechanisms, for instance in the long-term modification of reward-seeking behavior (Benton and Young, 2016). In this regard, one crucial question is to ask whether exposure to PHc foods can produce long-term plastic changes in the neural circuitry underlying goal-directed and habit-based behavior? If PHc foods cause some kind of addiction, a shift towards habit-based behavior is expected. This issue was recently addressed by a group of researchers who exposed rats to restricted access to sweetened condensed milk (i.e., PHc food) during

5 weeks and then measured their sensitivity to outcome-devaluation (Furlong et al., 2014). In this case, the task of outcome-devaluation makes use of an instrumental learning paradigm in which animals learn to lever-press for a food reward; once the task is well learned the outcome—the food pellet—is subsequently devalued by allowing free access to it or by pairing it with an aversive consequence such as gastric malaise; so lever-pressing is expected to diminish in animals using a goal-directed strategy. When the task was accomplished via a habit-based strategy instead, the outcome devaluation will not affect the operant response such as pressing a lever. Interestingly, they observed that animals with previous exposure to PHc food, showed greater persistence in lever pressing compared to controls, suggesting that those animals had acquired a habit-based strategy. Also they showed enhanced activation of the dorsolateral striatum, a region involved in habitual behavior. Accordingly, AMPA or dopamine (D)1-receptors antagonism in the dorsolateral striatum rescued behavior to the level of controls. Therefore these results show that a history of consumption of PHc foods may facilitate a shift towards habitual-type control of behavior (Furlong et al., 2014). Importantly, it has recently been shown that behavioral sensitivity to outcome-devaluation is also compromised in obese young men (Horstmann et al., 2015). A study modeling PHc food in rats showed that high fat diet (HFD) exposure from weaning to adulthood reduced instrumental performance and decreased sensitivity to outcome devaluation, suggesting impaired motivation, increased habitual behavior, or both (Tantot et al., 2017). Importantly, these behavioral impairments could be abolished by training adults with a task that reinforces goal-directed behavior (Tantot et al., 2017).

Chronic consumption of PHc food, as it is the case for drugs of abuse, can lead to long-term modifications in the brain circuits involved in reward-seeking behavior (Kenny, 2011b; Volkow et al., 2013b). But food ingestion, as we mentioned, is a complex behavior involving many multisensory reward "layers". So what characteristic of PHc food is more likely to cause changes in the brain's circuitry, and ultimately in behavior? To address this issue, a recent study evaluated whether neuronal modifications observed after sustained consumption of PHc correlated with the hedonic value of food, or with its caloric contents (Guegan et al., 2013). For this, they trained mice to lever-press for food rewards that were either normal chow, hypercaloric or palatable isocaloric food and analyzed dendritic spine morphology. In addition, they compared the persistence of food seeking behavior in the three groups of mice once food restriction was relieved. Interestingly, mice trained to obtain isocaloric palatable food showed higher persistence of lever pressing than the two other groups, while having access to food *ad libitum*. Furthermore, non-rewarded lever-press was also higher in mice presented with palatable isocaloric food, suggesting this diet also promoted impulsive-like behavior. Importantly, this behavioral change was not observed in the KO mice for the cannabinoid receptor type 1 ($CB1^{-/-}$), suggesting a role for this endocannabinoid receptors in impulsive food-seeking. When examining dendritic morphology in the three groups, the authors observed that dendritic spine density was increased in the medial prefrontal cortex (PFC) and NAcc shell, regions associated with addictive behavior, in the palatable isocaloric food group, compared to mice that ate hypercaloric food or normal chow. Consistently, this phenomenon was also shown to be dependent on CB1 receptors (Guegan et al., 2013). However, the degree to which neural plasticity mechanisms driven by post-prandial reward interact with those related to learned pleasantness of taste perception remains to be established. It is interesting to note meanwhile, that surgical treatments that have been shown to effectively treat obesity in humans (e.g., bypass surgery) may effectively dampen sweet appetite by interfering with post-prandial striatal dopamine release, as evidenced in a rodent study (Han et al., 2016). In addition, *Roux-En-Y* gastric bypass surgery in rats was shown to alter neural activity in brain regions related to taste perception and reward (Thanos et al., 2015).

As we have reviewed, certain environmental factors and behavior patterns may lead to "food addiction" and ultimately to obesity. Moreover certain lines of evidence suggest that some gene clusters may predispose individuals to both diet induced obesity (DIO) as well as brain inflammation (Heber and Carpenter, 2011). Certain people may therefore be genetically predisposed to absorb fat more efficiently. In addition, DIO by HFD exposure was recently shown to depend on neurotensin, a neuropeptide with significant dopaminergic interactions, and longitudinal studies in humans have shown that pro-neurotensin plasma level is a reliable predictor for the eventual development of obesity (Li et al., 2016). Even though such hereditary view of obesity may slightly downplay the role of behavior and dietary control on obesity, it clearly highlights the fact that a sedentary lifestyle and western diet are at odds with our evolutionary capacity to optimally absorb fats (Bellisari, 2008). In addition, it pinpoints clear pharmacological strategies that may be used in addition to changes in lifestyle and dieting.

Cognitive Consequences of PHc Food Exposure and Increased Adiposity

It has been reported that PHc foods that lead to obesity are related to a reduced ability to express synaptic plasticity in certain brain areas related to cognition (Dingess et al., 2016; Klein et al., 2016; Tran et al., 2016). For instance, chronic HFD consumption disrupts intracellular cascades involved in synaptic plasticity and insulin signaling/glucose homeostasis (Dutheil et al., 2016) and affects neuronal plasticity-related protein levels (Cai et al., 2016). Nutritional imbalance triggered by this diet eventually impacts glutamate neural pathways, up regulating glial glutamate transporters (GLT-1 and GLAST), down regulating glutamate-degrading enzymes, diminishing basal synaptic transmission and hindering NMDA-induced LTD (Valladolid-Acebes et al., 2012).

Consistently, obesogenic dietary factors, such as simple carbohydrate and saturated fatty acids, have been linked to memory impairments and hippocampal dysfunction (Kanoski,

2012; Sobesky et al., 2014) and evidence suggests that the brain may be particularly vulnerable to obesogenic diets during sensitive neurodevelopmental periods such as pre-natal, infancy and adolescence stages (**Figure 2A**; Valladolid-Acebes et al., 2013; Noble and Kanoski, 2016; Reichelt, 2016). In rodents, evidence shows that HFD exposure impairs memory of a variety of behavioral test, such as Morris' water maze, Barnes' maze, radial arm maze, Y- and T-maze, and novel object recognition (Cordner and Tamashiro, 2015). Interestingly, whereas abundant evidence shows that HFD impairs long-term memory and cognitive flexibility in spatial learning tasks (mainly dependent on hippocampus integrity), some learning processes, such as those that include an anxiogenic or aversive component (amygdala-dependent) may actually be enhanced by such diets (**Figure 2B**). For instance a recent study found increased emotional memory and amygdala plasticity in rats exposed to HFD from weaning to adulthood, through a mechanism that is dependent on glucocorticoid receptors in the amygdala (Boitard et al., 2015).

Studies in humans have shown that HFD consumption, obesity and metabolic syndrome are associated with poor cognitive performance in children (Bauer et al., 2015; Martin et al., 2016) and adults (Singh-Manoux et al., 2012; Papachristou et al., 2015; Lehtisalo et al., 2016; Yao et al., 2016), and increases risk for development of dementia (Francis and Stevenson, 2013; Freeman et al., 2014). Intake of a HFD that includes mostly omega-6 and saturated fatty acids is associated with worse performance on a cognitive tasks (Kalmijn et al., 1997) and with increased risk for Alzheimer's disease (Kalmijn et al., 1997; Luchsinger et al., 2002) hypertension and diabetes (Fowler, 2016). In this regards, caloric restriction has been shown to partially revert the HFD effects (Murphy et al., 2014). Individuals adhering to anti-hypertensive diet combined with caloric restriction and exercise show significant improvements in both executive-function memory learning and psychomotor speed when evaluated at 4 months following intervention (Smith et al., 2010). Interestingly, there is strong evidence suggesting that dietary restriction in adult non-human primates has beneficial effects on the preservation of cognitive performance during the course of aging (Colman et al., 2009; Mattison et al., 2012). In addition, a recent meta-analysis suggested that bariatric surgery is generally followed by improved cognitive functions in human patients (Handley et al., 2016), although it should also be warned that under certain circumstances, neuropsychiatric complications, such as increased suicide risk may also occur after this surgical treatment (Peterhänsel et al., 2013; Yen et al., 2014).

New research with animal models has begun to shed light on the neuroinflammatory mechanisms that may underlie the cognitive impairments observed in obese individuals (Castanon et al., 2015). For example, recent evidence in rats showed that fat transplantation produced microglial activation in the hippocampus while lipectomy had opposite effects. The authors went on to show that the cytokine interleukin (IL)-1 positively correlated with adiposity levels as well as cognitive impairments, and IL-1 receptor antagonism rescued the cognitive deficits observed in these animals (Erion et al., 2014; Sobesky et al., 2014). Furthermore, HFD exposure was recently shown to provoke a decrease in hippocampal dendritic spine density as well as synaptic plasticity deficits due to synaptic stripping by microglia, which could be reversed by diet suspension (Hao et al., 2016).

Prevention and Sensitive Periods to Nutritional Environmental Insults

As is the case for many other diseases, there seems to be critical periods for the development of obesity. Early studies established that gestation, the period between 5 and 7 years of age, and adolescence are critical for the risk of developing long-term obesity (Dietz, 1994), although a more recent longitudinal study suggested that childhood obesity is itself highly dependent on the mother's diet during pregnancy (Glavin et al., 2014). Studies in rats showed that offspring of dams fed with HFD had higher leptin concentration and glucose intolerance along with increased adiposity (Tamashiro et al., 2009). Similarly in mice, offspring of HFD fed dams show strikingly increased preference for sucrose as well as non-caloric sweetener solution when tested as adults. Interestingly, these mice also show increased sensitivity to cocaine and amphetamine, as well as reduced basal dopamine levels in the striatum and the VTA, which is consistent with higher motivation to obtain food reward (Peleg-Raibstein et al., 2016).

At the neurodevelopmental level, adolescence is characterized by extensive experience-dependent synaptic pruning (Petanjek et al., 2011), as well as changes in gliogenesis and myelination (Fields, 2005; Barbarich-Marsteller et al., 2013; Estes and McAllister, 2016). Moreover, it was recently suggested that blood-brain barrier permeability may be increase by HFD exposure (Kanoski et al., 2010; Hsu and Kanoski, 2014) and is differentially modulated during adolescence (Brenhouse and Schwarz, 2016). Some regions, such as the PFC, which matures up until early adulthood, undergo extensive remodeling and functional plasticity during this period (Reichelt, 2016). In recent years, adolescence has also been established as a critical period for the development of obesity and obesity-related cognitive impairments as some of the underlying neural mechanism are starting to be elucidated (Labouesse et al., 2016; Reichelt, 2016). In a series of experiments, mice were fed HFD during adolescence and later tested in novel location recognition memory, a task that is highly dependent on proper hippocampus function and that is particularly sensitive to manipulations in dorsal CA1 (Assini et al., 2009; Vogel-Ciernia and Wood, 2014). When tested as adults, these mice were less efficient than their control counterparts in this task and this difference was observable even after being switched to food restriction during a 5-week period. In contrast, the same HFD treatment had no effect when administered during adulthood. Intriguingly, this impairment in spatial memory was accompanied by increased neural cell adhesion molecule (NCAM, also known as CD56) accumulation and dendritic spine density increase

in the hippocampal CA1 region (Valladolid-Acebes et al., 2013). More recently, adolescent HFD exposure was also shown to alter the levels of the extracellular matrix glycoprotein reelin and impair LTD at PFC synapses (Labouesse et al., 2016). Also it has been observed a diminished neurogenesis and behavioral flexibility in hippocampus-dependent tasks in mice exposed to HFD during adolescence (Boitard et al., 2012). Supporting the notion that PHc food lead to cognitive impairments in particular during vulnerable periods, it has been reported that a HFD supplemented with 10% sucrose was also shown to produce learning and memory impairments in juvenile mice (Xu et al., 2015). More recently, a study demonstrated that rats fed with so-called Western diet (i.e., PHc food) during adolescence had post-traumatic stress responsivity as adults. The study also showed a significant decrease in hippocampal volumes as well as enlarged lateral ventricles in these animals (Kalyan-Masih et al., 2016). Importantly, a promising study showed that by suppressing HFD exposure during adulthood, neurocognitive deterioration seems to be restored in rats even when they were chronically exposed to this diet during adolescence (Boitard et al., 2016).

Outlook, Living in and Harnessing Permissive Obesogenic Environments

Together, these data provide rationale for particular beneficial effects of early educational/psychosocial interventions, as well as a more aggressive campaigning of warning the effects of PHc food consumption targeting sensitive neurodevelopmental periods; i.e., pregnancy, childhood and adolescence. For instance, it was recently demonstrated that when healthy nutrition is presented as choices that are coherent with adolescent values (such as independence from parents or other figures of authority and freedom from the influence of mass advertising by junk food giants companies), USA eight graders were more likely to stick to a healthy dietary choices (Bryan et al., 2016). Additionally, direct negative monetary incentives were also shown to modulate consumer choice by taxation. For example, in an audacious move trying to control the extreme high prevalence of overweight/obesity, and considering that caloric beverages were major sources of energy among children and adults (Stern et al., 2014), the Mexican government announced the implementation a 10% tax on sugar-sweetened beverages as well as on non-essential food with high caloric density, starting on January 2014. Indeed, a recent analysis confirmed that by December 2014, sales had already dropped by 12% and the data suggested that Mexican consumers were indeed switching to cheaper and healthier alternatives (Colchero et al., 2016).

To increase sales, industrialized food enhances rewarding properties by manipulating salt, sugar, fat, flavors and other food additives to make such foods more like addictive commodities (Cocores and Gold, 2009; Gearhardt et al., 2011; Carter et al., 2016). In the other hand, minimal regulation from governmental health agencies limits food industry and so far there is no public warning about the potential addiction and health problems of PHc food consumption. In this regard, as for other addictive substances like nicotine or alcohol, additional societal support might encourage policy-making bodies to: (a) to start warning about the potential addiction towards PHc food; (b) to regulate PHc food consumption for children, as the first step in modulating adult access to addictive food (Carter et al., 2016); (c) to foster additional research aiming to define the addictive properties of different refined food ingredients/additives as well as its mixture; and (d) to empower consumers by providing clear and straightforward health information in food labels as well as on advertising campaigns.

In summary, recent but indubitable experimental and clinical evidence have documented the deleterious health effects of the permissive obesogenic environment that most western countries are facing, as we have reviewed here, now evidently extending to mental health due to dysregulation in neuronal plasticity (**Figure 2A**). It is clear that our human physiology did not evolved to face constant and ubiquitous challenges imposed by obesogenic environments, resulting in an overweight/obesity pandemic (WHO, 2016a) that is challenging health systems by imposing unprecedented economic loads (OECD, 2014). Thus it is urgent and necessary to develop comprehensive, long lasting and multidimensional societal agendas to control and revert obesogenic environments by: (a) empowering citizens to take knowledge-based decision and become responsible consumers; (b) protecting consumers in vulnerable stages (i.e., pregnant women, children and adolescents) either by taxation, regulation or bans (WHO, 2016b); and last but not least (c) promoting economic growth based in innovation-driven healthy food alternatives.

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All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication. In particular: J-PM, LFR-D, GP-L, SD-C, GF, CP-C and KG-R performed literature review. J-PM, GP-L, SD-C and CP-C wrote the manuscript. GP-L designed the figures.

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frontiers in Behavioral Neuroscience

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Contexts Paired with Junk Food Impair Goal-Directed Behavior in Rats: Implications for Decision Making in Obesogenic Environments

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The high prevalence of obesity and related metabolic diseases calls for greater understanding of the factors that drive excess energy intake. Calorie-dense palatable foods are readily available and often are paired with highly salient environmental cues. These cues can trigger food-seeking and consumption in the absence of hunger. Here we examined the effects of palatable food-paired environmental cues on control of instrumental food-seeking behavior. In Experiment 1, adult male rats received exposures to one context containing three "junk" foods (JFs context) and another containing chow (Chow context). Next, rats were food-deprived and trained to perform instrumental responses (lever-press) for two novel food rewards in a third, distinct context. Contextual influences on flexible control of food-seeking behavior were then assessed by outcome devaluation tests held in the JF, chow and training contexts. Devaluation was achieved using specific satiety and test order was counterbalanced. Rats exhibited goal-directed control over behavior when tested in the training and chow-paired contexts. Notably, performance was habitual (insensitive to devaluation) when tested in the JF context. In Experiment 2 we tested whether the impairment found in the JF context could be ameliorated by the presentation of a discrete auditory cue paired with the chow context, relative to a second cue paired with the JF context. Consistent with the results of Experiment 1, the devaluation effect was not significant when rats were tested in the JF context with the JF cue. However, presenting the chow cue increased the impact of the devaluation treatment leading to a robust devaluation effect. Further tests confirmed that performance in the chow context was goal-directed and that sensory-specific satiety in the JF context was intact. These results show that environments paired with palatable foods can impair goal-directed control over food-seeking behavior, but that this deficit was improved by a cue paired with chow. This has promising implications for assisting individuals in controlling their eating behavior in environments designed to dysregulate it.

Keywords: instrumental conditioning, Pavlovian conditioning, stimulus, habit, junk food, context, rat

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INTRODUCTION

Obesity is now widespread across the developed and developing world, with the number of obese individuals recently estimated to exceed that of underweight people worldwide (World Health Organisation, 2016). A key driver of excess energy intake and long-term weight gain is the abundance of highly palatable and energy-dense foods. These products are typically

advertised with highly salient cues that are ubiquitous in day-to-day life and which are explicitly designed to influence consumption. For example, one study found that children ate significantly more after viewing advertisements for food than for non-food products, regardless of body weight, and that the amount eaten was positively correlated with how many adverts were recognized (Halford et al., 2004).

A substantial proportion of eating now occurs outside of the home, and these meals are associated with greater energy intake and lower micronutrient content (Stroebele and De Castro, 2004; Lachat et al., 2012). These external environments are riddled with stimuli designed to promote food purchase and consumption. While attending to food cues was highly adaptive in earlier periods of human history, relying too heavily on external cues may undermine body weight regulation in modern environments (Berthoud, 2007, 2012). Indeed, there is ample evidence for stimulatory effects of food cues on consumption in the short term. Animal models of *cue-potentiated feeding* show that cues paired with the delivery of food to hungry rats elicit consumption of this food when rats are no longer food-deprived (Weingarten, 1983; Petrovich, 2013), with similar effects found in people (e.g., Cornell et al., 1989). However, long-term effects of food-cue exposure on weight gain have not been established, in part due to the difficulty of testing this hypothesis. For example, in animal models the effects of food cues are sometimes tested within-subjects (Boggiano et al., 2009) and animals are commonly food deprived to encourage learning of the cue-food association, constraining body weight change (but see Reppucci and Petrovich, 2012).

Of course, food cues may affect eating behavior in ways other than prompting immediate consumption. Food is not always readily available in the presence of food cues; for example, when driving past a fast-food sign or walking through a shopping center food court. In these instances, cues may influence consumption via a series of cognitive processes involving where, what and how much food to procure. How food cues affect the decision-making processes that precede actual consumption is relatively less studied and was the focus of the present experiments. To explore this, we applied a framework based on principles of instrumental learning that distinguishes between behavior that is volitional (i.e., goal-directed) and that which is habitual (Dickinson, 1985). Performance of a goal-directed behavior, such as pressing a lever for food, relies on the contingency between the lever press (action) and food reward (outcome) and the fact that the food reward is currently valued. Therefore, manipulating the value of the reward should produce corresponding changes in performance of the action if the behavior is goal-directed, and no change or a reduced change if the behavior is under habitual control (Dickinson and Balleine, 1994). The outcome devaluation paradigm is a behavioral assay used to determine whether an action is under goal-directed or habitual control. The value of a reward is manipulated either by specific satiety or by inducing sickness (via lithium chloride) and performance of the action that earns the devalued outcome is compared either with conditions where the same outcome is valued or with a second action earning a different outcome for which value is intact (Adams and Dickinson, 1981; Balleine and Dickinson, 1998). Goal-directed behaviors are sensitive to changes in outcome value and, therefore, manifest as a selective reduction of the action earning the devalued outcome. By contrast, behaviors under habitual control are insensitive to changes in outcome value and are evident in responding that is not selectively sensitive to manipulation of the outcome of responding.

Recent studies have shown that habitual control over behavior can be accelerated by chronic access to diets high in sugar and/or fat in rats (Kendig et al., 2013; Furlong et al., 2014) and that higher BMI was associated with reduced sensitivity to devaluation in people (Horstmann et al., 2015). Here we focused not on lasting changes produced by long-term diet but on whether contexts paired with highly palatable foods could alter sensitivity to devaluation. The general experimental procedure was modeled on that used in two studies demonstrating that contexts paired with drugs of abuse promoted habitual control over behavior. In the first, rats were injected with ethanol and placed in one distinct context and injected with saline then placed in another context, prior to instrumental training conducted in a third environment. Devaluation tests revealed that responding was insensitive to devaluation when rats were tested in the alcohol-paired context but goal-directed in the saline context (Ostlund et al., 2010). The second study used a similar procedure to demonstrate habitual control over behavior produced by contexts paired with methamphetamine (Furlong et al., 2015). Importantly, instrumental performance was reinforced with food rather than drug rewards and the animals were drug-free at test, indicating that the contexts, rather than acute intoxication, influenced the decision-making processes that promoted habitual responding. We adopted a similar experimental procedure to Ostlund et al. (2010) and Furlong et al. (2015) to assess whether junk food (JF)-paired contexts would disrupt sensitivity to outcome devaluation.

The two experiments reported here each began with Pavlovian context conditioning in which non-deprived rats received repeated exposures to one context paired with standard lab chow and another paired with highly palatable JFs. Rats were then food-deprived for instrumental training in a third context where two lever-press responses for two novel food rewards were trained. Sensitivity to outcome devaluation was then examined in the JF, chow and training contexts. Experiment 1 found that the JF context promoted habitual control over behavior. Experiment 2 attempted to reverse this effect by exploring whether the presentation of a discrete cue paired with chow and satiety would restore goal-directed control over behavior in the JF context.

EXPERIMENT 1

Materials and Methods

Subjects

All experimental procedures were carried out in accordance with the recommendations of the Australian code for the care and use of animals for scientific purposes 8th edition (2013),

and were approved by the Animal Ethics Committee at the University of Sydney. Twenty-eight adult male hooded Wistar rats were used. These animals were tested in two replications (n = 16 and n = 12) that underwent identical experimental procedures. Rats were sourced from the University of Adelaide, were experimentally naïve, and were group-housed (n = 4/cage) in temperature- and humidity-controlled ventilated cages in a colony room maintained on a 12:12 light:dark cycle (lights on 7 am–7 pm). Testing was conducted between 2–5 pm each day. Chow and water were available *ad libitum* during context conditioning, but food access was restricted during instrumental training (see below). Rats were handled regularly prior to the beginning of the experiment.

Apparatus

All behavioral procedures were conducted in operant chambers (Med-Associates, St. Alban, VT, USA) contained within light- and sound-attenuating shells. The top and side walls of these chambers were Plexiglas and the floor consisted of steel bars. A recessed magazine was centered on one wall of the chamber between two retractable levers. Illumination was provided by a houselight centered at the top of the wall opposite the levers. For context conditioning, visual, tactile and olfactory cues were used to form two distinct contexts that were paired with JFs and chow in a counterbalanced fashion. Thus, one context contained a smooth plastic floor insert, was scented with vanilla essence (10% v/v in water; Queen, Queensland) and had top and side walls decorated with black and white stripes. The second context was scented with peppermint odor (10% v/v in water; Queen, Queensland), had black spots on a white background surrounding the top and side walls, and contained a floor insert covered with rough sandpaper. Odors were pipetted onto folded paper towels that were inserted into the front edge of the bedding tray. Wall decorations were laminated sheets of paper fitted around the exterior of the chamber. Instrumental training was conducted in the same operant chambers with all cues removed to form a "training" context. The houselight was on during all context conditioning and instrumental training sessions. The rewards used in instrumental training were 45 mg pellets (grain-based formula, BioServ, USA) and 20% w/v sucrose solution (∼0.1 ml per reward), which are both highly palatable to rats and greatly preferred to chow. Devaluation pre-feeding was conducted in individual acrylic cages with metal bar tops located in a separate room to operant chambers.

Procedure

Context conditioning

Context conditioning lasted for 14 days and consisted of seven, 1 h exposures each to the Chow and JF contexts in an alternating sequence (chow, JF, chow, JF, etc.). Laboratory chow (Specialty Feeds®; 14.23 kJ/g) was provided in the chow context. In the JF context three palatable foods were provided: Oreos (Nabisco, East Hanover, NJ, USA; 20.33 kJ/g), Pringles (Pringles, Battle Creek, MI, USA; 22 kJ/g), and Jelly Snakes (Nestlé, Australia, 14.2 kJ/g). The total weight of food available in JF and Chow sessions was approximately 15 g. Foods were presented in white ceramic dishes centered against the side wall of the chamber. Food was weighed before and after the session to determine intake, which was converted from grams to kJ for analyses and summed for the three foods in the JF context.

Instrumental training

Immediately after day 14 of context conditioning, home-cage chow was removed and a restricted feeding schedule introduced wherein rats were fed 14–15 g of chow per rat each day. Instrumental training began 2 days after the last day of context conditioning with a magazine training session where 20 pellets and 20 sucrose rewards were delivered to the magazine on independent random-time 60 s schedules. The left and right levers were then assigned to earn these rewards in a counterbalanced fashion. For the first 6 days of instrumental training, left and right levers were trained in separate sessions that ended either after 30 rewards were earned or 45 min elapsed. The sessions were separated by a minimum of an hour and whether the pellet or sucrose outcome was trained first was alternated each day. For days 1 and 2 of training each lever press was rewarded (i.e., continuous reinforcement). Thereafter, the reinforcement schedule was increased to random-ratio (RR) 5 on days 3 and 4 and RR10 on days 5–7. On day 7 the two levers were trained in the same session. In this session the left lever was inserted until five rewards were earned and then retracted. After 10 s, the right lever was inserted until five rewards of the other outcome were earned. This sequence repeated until 30 rewards of each outcome were earned, or until 60 min had elapsed. This two-outcome procedure was used for all subsequent re-training days between tests. This procedure is similar to that used by Ostlund et al. (2010) but with a shorter delay between levers.

Devaluation tests

Devaluation tests were held in the JF, Chow and Training contexts. The order of these three tests was counterbalanced and test days were separated by a single day of re-training using the two-outcome procedure described for training day 7 above. Devaluation was achieved by specific satiety: rats were placed in individual feeding cages and allowed to consume pellets or sucrose solution *ad libitum* for 1 h. Approximately 15 g pellets or 30 g sucrose solution were provided during pre-feeding; rats never consumed more than these amounts. Rats were familiarized to pre-feeding cages on two occasions for 20-min during instrumental training (after daily sessions). The devalued outcome was held constant across tests and counterbalanced, such that pellets were devalued for half of the rats and sucrose solution was devalued for the other half. Immediately after devaluation treatment rats were transferred to the context (JF, Chow or Training) for a 15-min test. Levers were not inserted for the first 10 min of this test to promote attention toward the contexts. After 10 min, both levers were inserted simultaneously for a 5-min test. Presses were recorded but not reinforced.

Data analysis

Consumption of chow and JF in context conditioning sessions (kJ/rat) was analyzed using a (2) × (7) within-subjects ANOVA. The dependent measure during instrumental training was the response rate (lever presses/minute) averaged across pellet

and sucrose levers. Response rates across days were analyzed in a within-subjects ANOVA. Responding on devalued and non-devalued levers in the three context tests was compared using a within-subjects (2) × (3) ANOVA. Preliminary analyses included devalued outcome (sucrose or pellets) as an additional between-subjects factor but, as it did not interact with the context (Experiment 1) or cue (Experiment 2) effects of interest, we collapsed across this variable for subsequent analyses. Significant interaction effects were followed by tests of simple effects, results for which $p < 0.05$ were considered statistically significant.

Results

Context Conditioning

Consumption during training is shown in **Figure 1**. Rats rapidly increased their consumption of JF in the JF context but ate minimal chow in the Chow context. This was supported statistically by a significant effect of session (linear trend: $F_{(1,27)} = 84.92$, $p < 0.001$) and a significant context × session interaction ($F_{(1,27)} = 79.88$, $p < 0.001$) in a (2) × (7) ANOVA. Averaged over sessions, rats ate significantly more in the JF than Chow context (context main effect: $F_{(1,27)} = 149.30$, $p < 0.001$). Despite being non-deprived during this phase, by the end of context training, rats were consuming around eight times more energy in the JF context than in the Chow context.

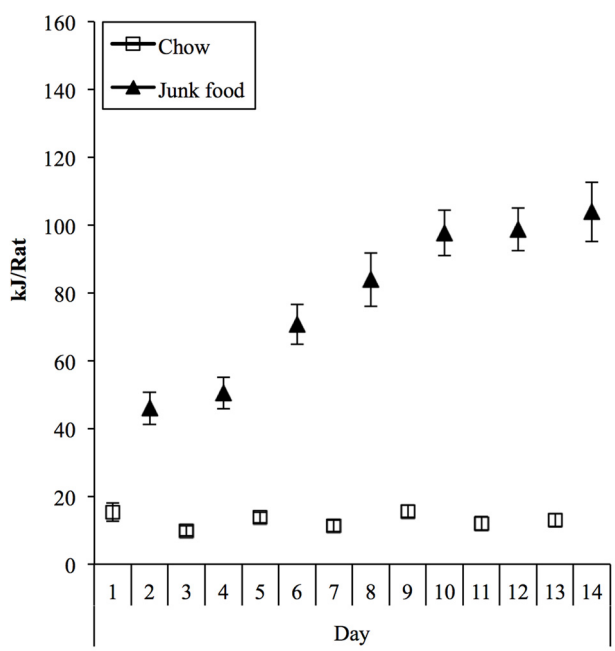

FIGURE 1 | Experiment 1 context conditioning. In 1-h daily sessions, rats were exposed to a context containing three "junk" foods (JFs) or to another context containing chow (Chow). Consumption in the JF context was substantially greater from the first session onwards and increased steadily during training, while consumption in the chow context remained low.

Instrumental Training

All rats learned both instrumental responses. Response rates are shown in **Figure 2** and significantly increased during training (linear trend: $F_{(1,27)} = 301.56$, $p < 0.001$). Two rats showed an extreme response bias by responding four times more on the pellet lever than the sucrose lever across training. Since this bias would likely obscure the devaluation effect, these rats were not included in test analyses.

Devaluation Tests

Pre-feeding

Consumption during pre-feeding did not change significantly over the three test days ($F_{(2,48)} = 1.53$, $p = 0.227$). On average, rats pre-fed with pellets consumed 8.58 ± 0.29 g, while rats pre-fed with sucrose consumed 16.18 ± 0.54 g. However, when expressed as reward equivalents (1 pellet reward = 45 mg and 1 sucrose reward = 0.1 g), consumption was greater in pellet-fed rats (190.6 ± 0.6) than sucrose-fed rats (161.9 ± 5.4). In both cases, consumption far exceeded what rats earned in instrumental training sessions (30 rewards) and rats had stopped eating by the end of the 1-h period, indicating they were satiated.

Test

Compiled devaluation test data are displayed in **Figure 3** and were analyzed in a (2) × (3) ANOVA (devaluation × context). This analysis found a significant devaluation effect ($F_{(1,25)} = 5.47$, $p = 0.028$) that, critically, interacted with the context in which rats were tested ($F_{(2,50)} = 3.65$, $p = 0.033$). There were no differences in overall responding between contexts ($F < 1$).

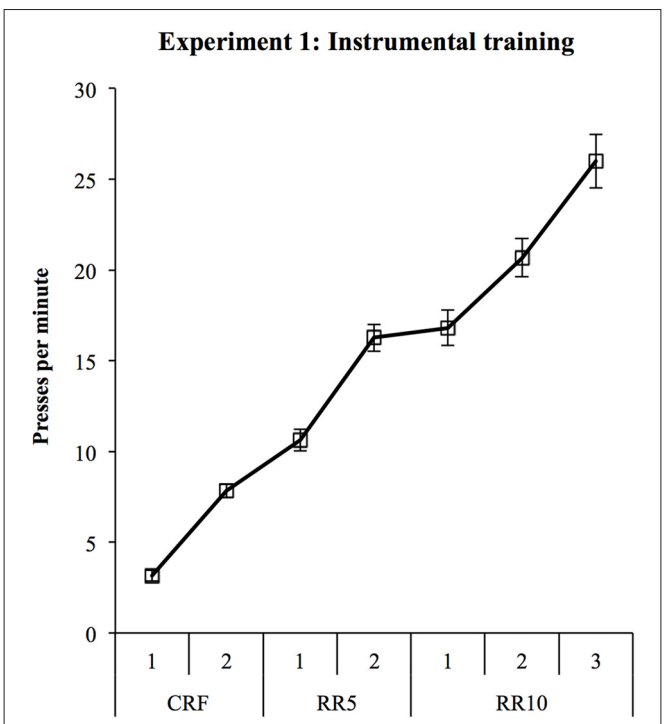

FIGURE 2 | Experiment 1 instrumental training. Rats were trained to make two lever presses for pellets and 20% sucrose solution.

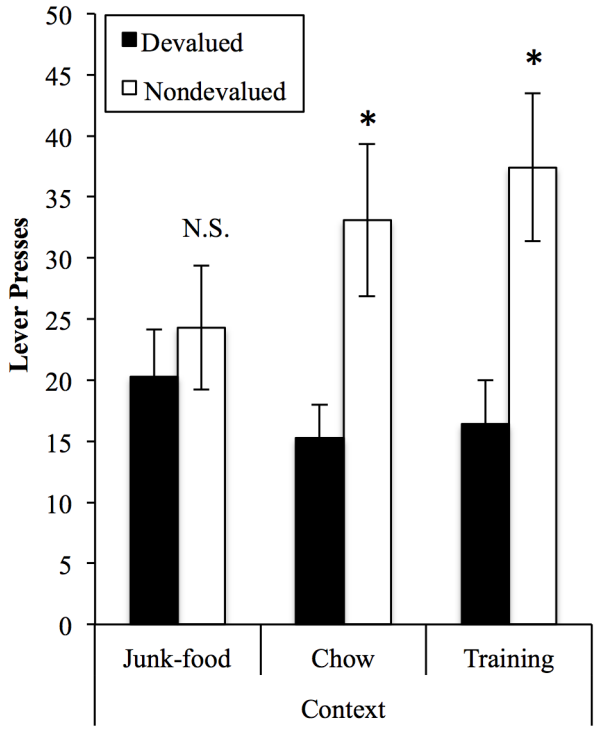

FIGURE 3 | A JF context impairs sensitivity to outcome devaluation. Sensitivity to devaluation was tested in the three contexts, within-subjects and in a counterbalanced order. After devaluation of one outcome by specific satiety, rats selectively reduced responding on the lever that had earned that outcome in training, but only in the chow and training contexts. When tested in the JF context, performance was insensitive to devaluation, with no overall difference in responding between contexts. *Indicates $p < 0.05$, N.S., non-significant.

Sensitivity to devaluation in each context was then assessed using tests of simple effects. These found that rats showed significant devaluation effects when tested in the Training ($F_{(1,25)} = 7.85$, $p = 0.01$) and Chow contexts ($F_{(1,25)} = 6.27$, $p = 0.019$) but that responding on the devalued and non-devalued levers did not differ in the JF context ($F_{(1,25)} = 0.32$, $p = 0.576$).

Discussion

Experiment 1 trained non-deprived rats to associate one context with highly palatable JFs and another with bland chow. After repeated, alternating exposures to these environments, rats were food-deprived and trained to perform two instrumental responses for distinct food rewards in a third environment. At test we assessed whether the ability to direct food-seeking behavior according to the current value of those foods would be affected by the context in which rats were tested. Rats showed sensitivity to devaluation when tested in a context previously paired with chow or in the environment in which instrumental training occurred. The key finding from this experiment is that these same rats were insensitive to devaluation when tested in the context previously paired with palatable food. Importantly, overall responding did not differ between the three contexts, suggesting that this impairment was not driven by some non-specific effect on overall responding. Rather, rats pressed at a similar rate in this environment but were unable to adjust behavior in accordance with the current value of the outcomes. Therefore, contexts paired with highly palatable JFs undermined goal-directed control over food-seeking behavior.

EXPERIMENT 2

Loss of goal-directed control over food-seeking behaviors could be an obstacle to changing one's eating behavior. In Experiment 2 we explored whether additional conditioning manipulations could ameliorate this impairment. We modeled our approach on a body of literature studying the effects of discrete stimuli paired with the extinction of previously learned associations, often termed "e-cues", which are thought to serve as reminders of extinction training and have been shown to promote expression of extinction (Brooks and Bouton, 1993). Under most conditions, extinction of an instrumental response does not erase original learning but rather produces new learning that the response no longer leads to reward. Because responding recovers under a variety of circumstances (Bouton et al., 2012), interventions that protect or strengthen extinction learning are important for reducing these recovery phenomena, particularly in the context of food-related behavior (Bouton, 2011). To this end, Brooks and Bouton (1993) found that a visual cue presented during extinction of a tone-food association (e-cue) attenuated the spontaneous recovery of conditioned responding to the tone when rats were tested 6 days later. Using a similar experimental procedure, Brooks and Bouton (1994) found that presentation of an e-cue prevented ABA renewal, a phenomenon where a response learned in one context ("A") and extinguished in a second context ("B") recovers with a return to the first ("A"). A recent study found similar effects of an e-cue on ABA renewal in rats trained to nose-poke for alcoholic beer (Willcocks and McNally, 2014). The typical interpretation of these results is that the presentation of the e-cue facilitates the retrieval of the extinction memory to buffer against returned expression of the original learning (Brooks and Bouton, 1993).

Related to these findings, Ostlund et al. (2010) found that the contextual promotion of habitual responding was reversed by providing response-contingent feedback in the form of outcome delivery. Together, these results suggest that where two conflicting systems compete for behavioral control (original learning vs. extinction, or goal-directed vs. habit systems), stimuli that "remind" the rat of extinction or the devalued state of the outcome can influence behavior to favor the cued learning. Thus, in Experiment 2 rather than attempting to extinguish the JF context, we examined whether a reminder of a relatively unpalatable food; chow, could override the effects of the context previously paired with the palatable JF and promote sensitivity to devaluation. To this end, we presented discrete auditory stimuli in the JF and Chow contexts so that consumption of JF and Chow were paired with a "JF-cue" and a "Chow-cue" in

addition to the contexts. We then assessed whether presentation of the Chow-cue would improve sensitivity to devaluation in the JF context relative to when the JF cue was presented in this environment. An additional aim of Experiment 2 was to measure sensitivity to devaluation in terms of consumption as well as instrumental responding. We hypothesized that, just as the e-cue reminds rats of conditions of non-reinforcement (e.g., Willcocks and McNally, 2014), or as outcome delivery reminds animals of changes in outcome value following devaluation (Ostlund et al., 2010), presenting a cue previously paired with chow would remind rats of reduced palatability, and/or satiety, to enhance sensitivity to devaluation in the JF context.

Materials and Methods

Subjects

Twenty adult male Long-Evans rats were used. Animals were bred in-house at the Brain and Mind Centre at the University of Sydney, Australia, and were housed 2–4 per cage in ventilated cages contained in a temperature- and humidity-controlled room. The colony room was maintained on a 12:12 reverse dark:light cycle (lights off 9 am–9 pm). Behavioral testing occurred between 2–6 pm each day. During context conditioning rats had free access to chow and water in home cages. During instrumental training rats were fed approximately 12 g chow daily. Rats were handled regularly in the week prior to the start of the experiment.

Design

Context conditioning in Experiment 2 was identical to Experiment 1 except that a discrete auditory cue was also paired with each context. These cues were a white noise and pure tone and were paired with Chow and JF contexts in a counterbalanced fashion. Ten 2-min presentations of these stimuli occurred in every 1-h training session and were separated by a variable ITI (range: 1–4 min). To prevent hearing an inappropriate stimulus from adjacent boxes, rats were run in two groups of 10 rats according to stimulus type. Home-cage chow intake was monitored each day during context conditioning. On the day after the last context conditioning session, rats were pre-exposed in home cages for 2 h to pellets (Bioserv; grain-based formula) and 20% sucrose solution, the outcomes to be used for instrumental training. Food was then removed overnight, and from the following day the restricted feeding scheduled was introduced. Instrumental training was conducted as described for Experiment 1 except that two sessions of the two-outcome procedure were held prior to tests (rather than one).

The first two devaluation tests were conducted in the JF context and compared the effects of the JF and Chow cues (order counterbalanced). Devaluation was achieved by specific satiety as in Experiment 1. For the first 10 min of each test no levers were available and no stimuli were presented. After 10 min both levers were inserted for a 5-min choice extinction test. When levers were inserted, either the Chow- or JF-cue was turned on and played constantly for the remainder of the test. Lever presses were recorded in 1-min bins. On the following day rats received a single session of instrumental re-training using the two-outcome procedure described above. The second devaluation test was identical to the first except that rats tested with the chow cue in Test 1 now received the JF cue, and vice versa. Rats were then given 3 days of re-training prior to a second set of devaluation tests held in the chow context in order to confirm goal-directed responding in this context and test whether the presence of the JF cue was sufficient to impair sensitivity to devaluation. Rats pre-fed with pellets for Tests 1 and 2 were pre-fed with sucrose solution for these tests, and vice versa. The order in which JF- and Chow-paired cues were tested was counterbalanced, and for each rat was the reverse of the order used in tests 1 and 2.

We were also interested to examine whether the habitual performance in the JF-paired context could be explained by impaired sensitivity to sensory specific satiety in this context. Therefore, we examined whether rats would selectively reduce *consumption* of the pre-fed outcome in the JF-paired context. For this test, rats were pre-fed either with pellets ($n = 10$) or sucrose solution ($n = 9$) for 1-h in devaluation pre-feeding cages before a 10-min test of pellet consumption in the JF context with the JF-cue played continuously. Pellets were the test food for all tests due to the logistical difficulty of fixing a bottle of sucrose within operant chambers.

Results

Context Conditioning

Consumption in Chow and JF sessions during training is shown in **Figure 4**. As in Experiment 1, rats ate substantial amounts

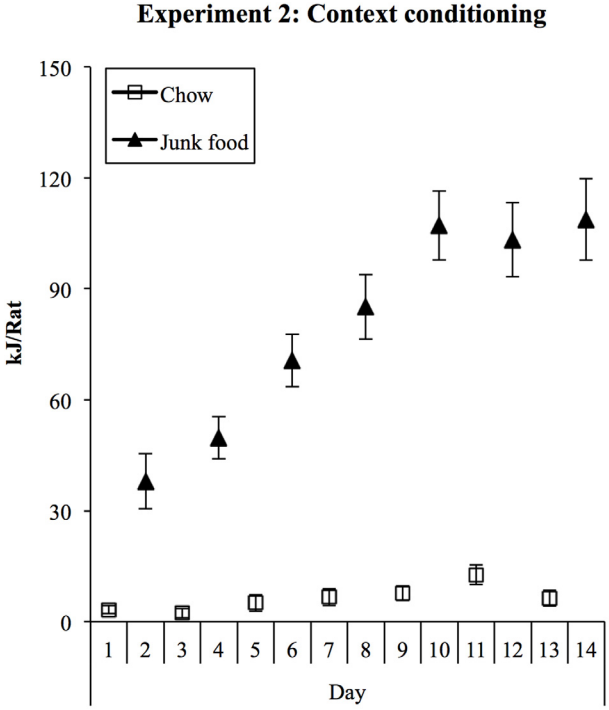

FIGURE 4 | Experiment 2 context conditioning. As in Experiment 1, rats rapidly increased their intake of palatable foods in the JF context and ate minimal amounts of chow in the chow context.

of the palatable foods in the JF context but little chow in the Chow context. Consumption of all foods was converted to kilojoules, summed across the three foods in the JF context, and analyzed in a (2) × (7) repeated-measures ANOVA. This analysis showed a significant increase in consumption across sessions ($F_{(1,19)} = 40.28$, $p < 0.001$) and a significant interaction between context and session ($F_{(1,19)} = 31.633$, $p < 0.001$), indicating a greater increase in consumption in the JF- than Chow-paired context. Averaged over sessions, consumption was greater in the JF context ($F_{(1,19)} = 150.71$, $p < 001$).

Each day, home-cage chow intake was measured when rats were in context conditioning sessions. Total energy intake was then calculated on a per-cage basis by adding home-cage chow intake to the total consumption in the context session by the rats in each cage. Consumption in each day's training session (kJ/rat) was added to home-cage consumption (kJ/rat) in the following 24-h. This resulted in a measure of 24-h energy intake for each of the six cages on each day of training. Subsequently, we compared total energy intake between chow and JF-training days to assess the extent to which rats compensated for the kJ consumed in JF sessions. Total daily energy intakes were analyzed in a within-subjects (2) × (7) ANOVA, with day type (JF- or Chow-paired day) and "session" as factors. This analysis found a main effect of "day type" ($F_{(1,5)} = 38.86$, $p = 0.002$) indicating that energy intake was higher on days beginning with a JF-session. The difference in average total energy intake indicated by this result is shown in **Figure 5**. There was no significant linear change in energy intake over days ($F_{(1,5)} = 6.17$, $p = 0.056$).

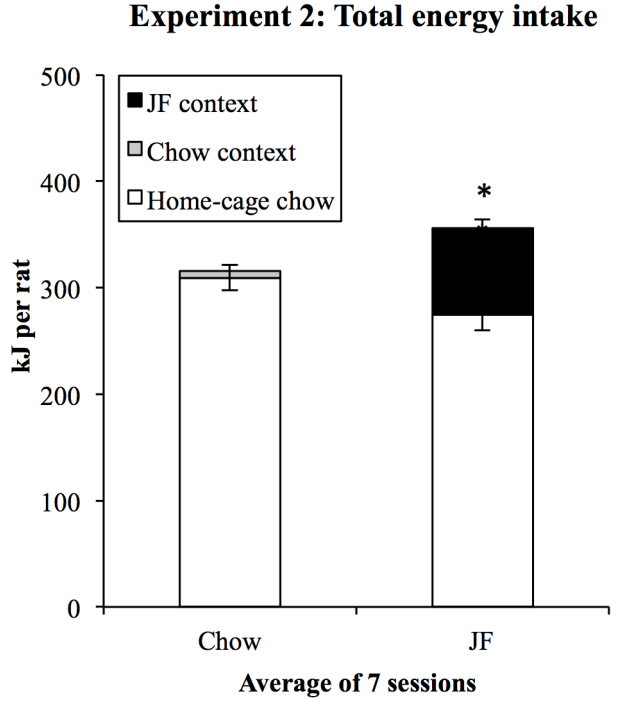

FIGURE 5 | Total energy intake during context conditioning in Experiment 2. On average, rats ate more in 24-h periods beginning with a JF context session *$p = 0.002$. Since rats were group-housed, consumption in daily context sessions was summed for each cage.

Instrumental Training

Nineteen rats learned both instrumental responses; the 20th failed to respond for sucrose solution and therefore could not be tested. Average daily responding is displayed in **Figure 6**. Responding in the first block of training prior to the first test was analyzed in a within-subjects ANOVA. This analysis found a significant linear increase in response rates over sessions ($F_{(1,18)} = 154.19$, $p < 0.001$). These response rates were maintained throughout subsequent re-training sessions.

Tests

Pre-feeding

Familiarization to the pre-feeding cages was as described for Experiment 1. Rats were pre-fed either with sucrose solution or pellets for both JF context tests, and the other reward (pellets or sucrose) for both Chow context tests. On average, rats consumed 14.13 ± 0.87 g sucrose and 7.81 ± 0.45 g pellets; this was equivalent to 179.32 ± 8.43 pellet rewards and 144.53 ± 8.54 sucrose reward.

Effects of the JF- and Chow-cues on sensitivity to devaluation in the JF context

Presses on the devalued and non-devalued levers in the chow-cue and JF-cue test are shown in **Figure 7A** and were analyzed in a (2) × (2) within-subjects ANOVA. This analysis found a significant effect of devaluation ($F_{(1,18)} = 11.14$, $p = 0.004$) and no main effect of cue ($F < 1$). Importantly,

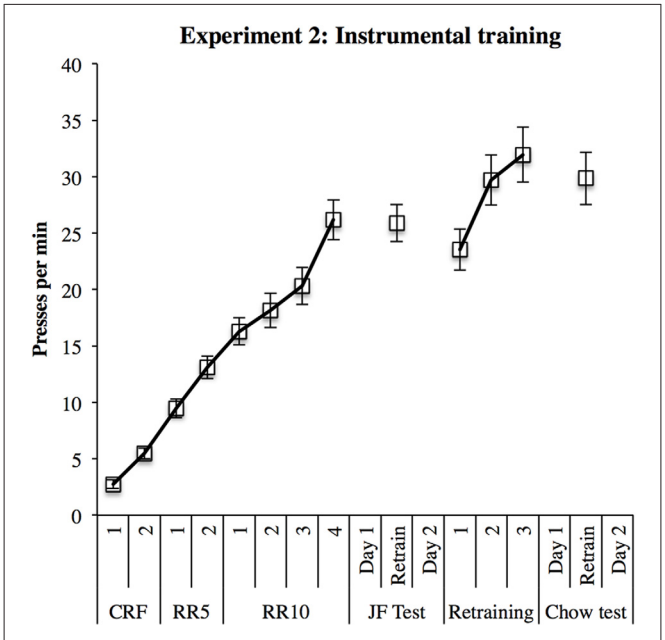

FIGURE 6 | Instrumental training in Experiment 2. Responding increased steadily throughout training and remained high during re-training sessions between outcome devaluation tests.

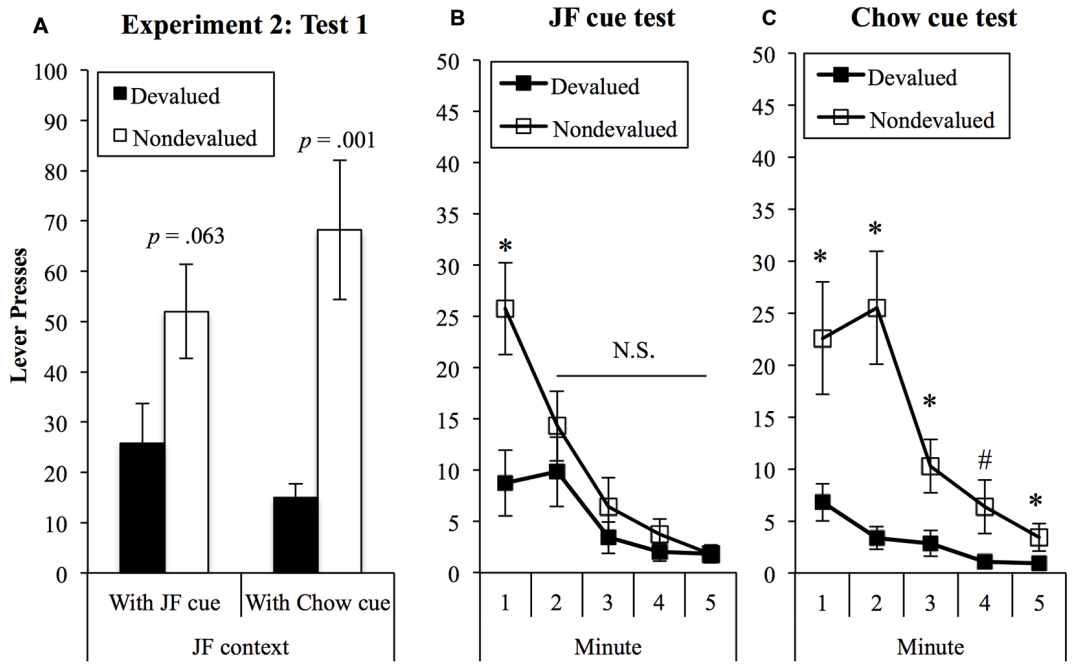

FIGURE 7 | Devaluation tests in the JF contexts. (A) Sensitivity to devaluation in the JF context was significantly improved when the chow cue was presented (interaction $p = 0.038$). *p*-values show tests of simple effects in each cue test. Analysis of bin data showed that sensitivity was lost rapidly in the presence of the JF cue **(B)** but remained statistically significant throughout the chow-cue test **(C)**. $^{*}p < 0.05$; $^{\#}p = 0.057$.

there was a significant interaction between devaluation and cue ($F_{(1,18)} = 4.99$, $p = 0.038$), indicating that sensitivity to devaluation treatment varied according to whether the JF- or Chow-paired cue was present during the test. Simple effects analyses were then conducted to explore the nature of the interaction. These analyses found a significant devaluation effect when the Chow-cue was presented in the JF context ($F_{(1,18)} = 15.54$, $p = 0.001$) but not when the JF-cue was presented ($F_{(1,18)} = 3.93$, $p = 0.063$).

To explore the devaluation × cue interaction in greater detail, we examined 1-min bin data for JF-cue and chow-cue tests, shown in **Figures 7B,C**, respectively. Examining these data suggested that initial sensitivity to devaluation in both tests was rapidly lost in the presence of the JF-cue, but sustained by the chow-cue. To examine this, we added "bin" as a third factor with five levels to a 3-way within-subjects (5) × (2) × (2) ANOVA. This analysis found a significant 3-way interaction between cue, lever and bin ($F_{(4,72)} = 3.09$, $p = 0.021$), indicating that the difference between responding on devalued and non-devalued levers over the five bins varied between JF- and Chow-cue tests. In the JF-cue test, the devaluation effect was significant in the first minute ($F_{(1,18)} = 6.64$, $p = 0.019$) but not in minutes 2, 3, 4, or 5 (largest $F_{(1,18)} = 1.09$). By contrast, during the Chow-cue test the devaluation effect was significant during all five 1-min bins, save for a marginally significant result in minute 4 (minute 1: $F_{(1,18)} = 6.95$, $p = 0.017$; minute 2: $F_{(1,18)} = 16.02$, $p = 0.001$; minute 3: $F_{(1,18)} = 6.18$, $p = 0.023$; minute 4: $F_{(1,18)} = 4.13$, $p = 0.057$; minute 4: $F_{(1,18)} = 5.95$, $p = 0.025$).

Sensitivity to devaluation in the Chow context

Responding on the devalued and non-devalued levers in the chow context tests is shown in **Figure 8**. The effects of the JF- and Chow-paired cues on performance were assessed using a (2) × (2) within-subjects ANOVA, with cue (JF and Chow) and lever (devalued vs. non-devalued). This analysis found a significant devaluation effect ($F_{(1,18)} = 7.17$, $p = 0.015$) but no effect of cue ($F < 1$) and no interaction between cue and lever ($F < 1$).

Sensitivity to devaluation measured with consumption in the JF context

For the consumption test in the JF context, 10 rats were pre-fed with pellets and nine were pre-fed with sucrose solution for 20 min prior to a 10-min test of pellet consumption in the JF context. Consumption during test is shown in **Figure 9**. Pellet consumption in the JF context was significantly reduced in rats pre-fed with pellets relative to those pre-fed with sucrose solution ($F_{(1,17)} = 57.29$, $p < 0.001$); thus, specific satiety itself was intact even when rats were tested in the JF context.

Discussion

In Experiment 2, we tested whether the effects of the JF context on sensitivity to devaluation would be affected by the presentation of discrete cues paired with JF and Chow. Results indicated that presenting the Chow cue in the JF context improved sensitivity to the devaluation treatment and promoted goal-directed performance across the 5-min of the test. By

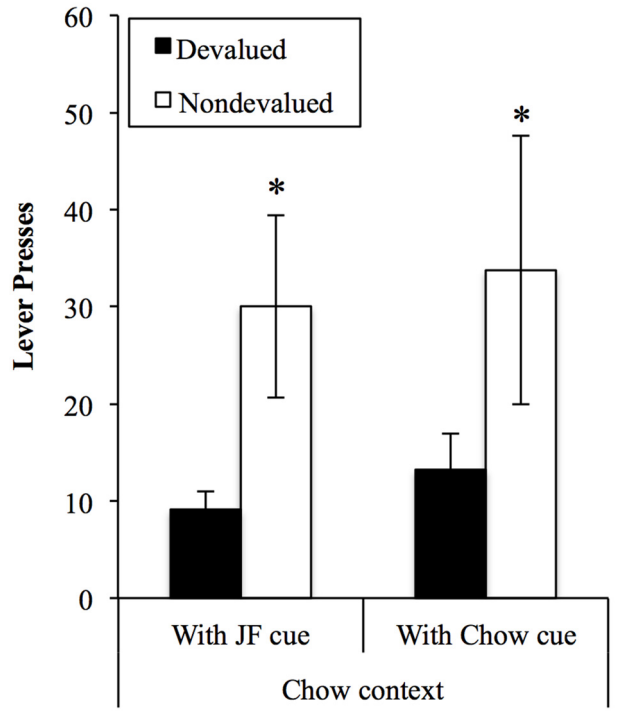

FIGURE 8 | Devaluation tests in the Chow context. Performance was sensitive to outcome devaluation and unaffected by which cue was presented when rats were tested in the chow context, despite an overall reduction in responding from the first set of tests. *Indicates $p = 0.015$ for devaluation effect.

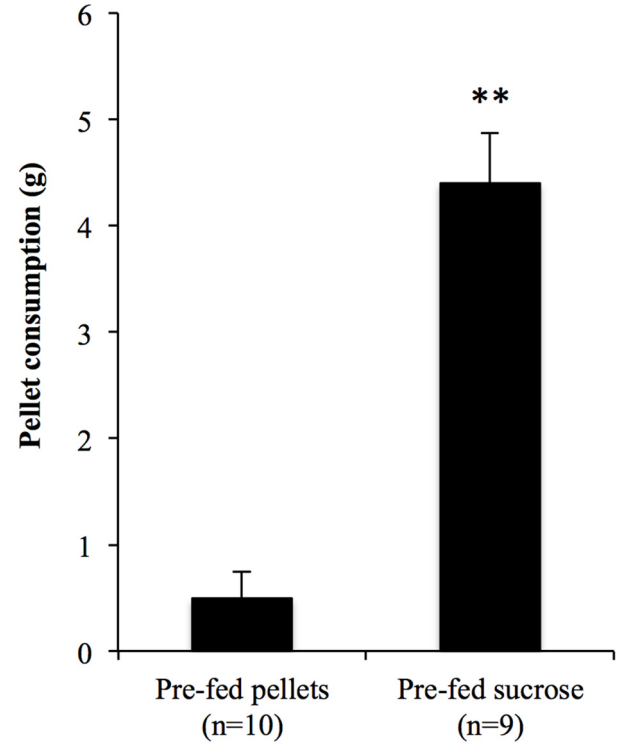

FIGURE 9 | Sensitivity to outcome devaluation measured by consumption. To test whether sensory specific satiety was intact in the JF context, pellet consumption in this context was compared between rats pre-fed with pellets and sucrose solution. Those pre-fed with pellets ate significantly less than those pre-fed with sucrose solution, **indicates $p < 0.001$.

contrast, when the rats were tested in the JF context with the cue that was present in this context during training, the devaluation effect was not statistically significant. Because numerically the impact of the JF context did not appear as complete as in Experiment 1, analysis of bin data characterized this effect further, showing that the devaluation effect was significant in the first minute of the test but not in minutes 2–5. The initial sensitivity to devaluation may be because the JF context was somewhat degraded at the beginning of the test due to the absence of the auditory cue that was present during training and which was likely to have been a salient element of the context. Thus, in the first 10 min of the test, the absence of the JF-cue rendered the context incomplete. As the onset of the cue "completed" the context, goal-directed behavior was then undermined, but this effect was not apparent until the second minute of the test. Future studies could examine the effects of the auditory cues alone (or other individual elements of the context) to examine their contribution to the observed effect.

Next, we confirmed that sensitivity to devaluation was intact in the Chow context and unaffected by the presentation of the JF- or Chow-cue. These tests were conducted separately and after a period of re-training, because our primary aim was to test a means for restoring goal-directed control in the JF context after observing impaired performance in this environment. A consequence of this approach is that the order of the four devaluation tests was not fully counterbalanced. Not surprisingly, overall responding was lower in the chow context tests (compare **Figures 7A**, **8**) likely due to cumulative extinction of responding resulting from the multiple tests. Importantly, significant devaluation effects were still found in both tests.

Consumption of JFs in the JF context steadily increased over context conditioning sessions such that, by the seventh exposure to this context, rats consumed approximately 30% of their daily calories in a single hour. It is possible, then, that rats associated the JF context not only with palatable tastes, but also with satiety signals and—perhaps—resistance to this satiety. Indeed, studies of cue-potentiated feeding find that contextual food cues can promote consumption even in non-deprived rats that have been pre-fed with the test food (Petrovich et al., 2007). Therefore, we explored whether insensitivity to devaluation in the JF context could be explained by poorer sensory-specific satiety in this environment and to rule out whether altered expression of satiety specifically within the JF context undermined the effectiveness of

the devaluation treatment. Results of the consumption test in the JF context showed that this was not the case: rats pre-fed with pellets ate significantly less of that same food than did rats pre-fed with sucrose, indicating that consumption was sensitive to the current value of pellets. The impact of this treatment, however, did not translate into changes in instrumental performance.

GENERAL DISCUSSION

The present experiments sought to further understand how food cues alter food-seeking behavior in ways distinct from consumption. Rats learned to associate one context with the consumption of highly palatable JFs and another with chow, prior to instrumental training conducted in a third environment. We then compared whether these contexts would modulate sensitivity to outcome devaluation. Experiments 1 and 2 found that rats failed to show sensitivity to devaluation in an environment previously paired with consumption of palatable foods. By contrast, rats' performance was goal-directed when tested in the context previously paired with chow and in the training context. Presentation of a discrete cue previously associated with chow restored sensitivity to devaluation when rats were tested in the JF context. Importantly, these effects of context (Experiment 1) and of the chow-cue (Experiment 2) were not attributable to floor or ceiling effects in responding. Rather, it was the distribution of responding between devalued and non-devalued levers that was impaired by the JF context in Experiment 1. Likewise, presentation of the chow-cue in Experiment 2 significantly improved the ability to direct responding toward the non-devalued outcome.

The current findings are consistent with past studies showing similar impairments in sensitivity to devaluation in contexts paired with ethanol (Ostlund et al., 2010) and methamphetamine (Furlong et al., 2015). While rats ate more JF than chow, and thus may have associated eating freely available food with the JF context which may, in some way, have interfered with having to earn food, as noted above, rats continued to respond in the JF context, they just did so indiscriminately. Furthermore, given the similarity between the current results and those seen in drug-paired contexts, it seems unlikely that the results can be explained by previous consumption. The novel result of the present study is that presentation of a chow-cue significantly improved performance in the JF context.

Although caution should be taken when comparing across experiments, it is worth noting that the reduction in goal-directed control within the JF context appeared more complete in Experiment 1. Responding on devalued and non-devalued levers in the JF context was all but equivalent in Experiment 1, but in the comparable test in Experiment 2 (JF context with JF cue) the devaluation effect approached statistical significance (p = 0.063, see **Figure 7A**). We are confident, however, that this does not reflect inadequate statistical power: all testing was within-subjects, and the above result was generated from the data of 19 animals, which is highly powered to detect devaluation effects. Moreover, the most important result in Experiment 2 was that performance in the JF-context was significantly improved by the presentation of the Chow-cue, as supported by a significant interaction between cue and devaluation. Here it may be useful to consider that, while goal-directed and habit-based control are conceptualized as distinct systems competing for control over behavior (Corbit, 2016), variability within them is still meaningful. Thus, the transition from goal-directed to habitual control over behavior does not occur instantaneously, but instead shifts gradually with extended training (Dickinson et al., 1995) and can be accelerated by exposure to drugs of abuse (e.g., Nelson and Killcross, 2006) or to high-sugar/high-fat diets (Kendig et al., 2013; Furlong et al., 2014). A relevant parallel to consider is that extinction-paired "E-cues" reduce, rather than completely block, the relapse from extinction produced by various manipulations (Brooks and Bouton, 1993, 1994; Willcocks and McNally, 2014). In the present studies, goal-directed behavior was significantly poorer in a context associated with highly palatable food and, in turn, was improved by a discrete cue paired with chow. These incremental changes in sensitivity to devaluation are relevant to food-seeking because the regulation of energy intake is as much a question of what and how much to eat as it is whether to eat or not (Wansink, 2004). Both of the present experiments demonstrated poorer sensitivity to devaluation in the JF context, despite differences in the extent of this impairment, while Experiment 2 demonstrated that the presentation of the chow-cue significantly improved performance.

The use of a "chow cue" in Experiment 2 drew from literature exploring how discrete cues paired with extinction protect against the recovery of the original response that occurs following various manipulations (e.g., renewal, reinstatement etc.; Bouton, 2002). However, an important difference in our approach was that the cue we used to "rescue" performance was not associated with extinction of the JF context but rather had been paired with another distinct environment paired with chow. It is worth noting that consumption of chow during context conditioning was minimal. Therefore, it is difficult to determine the extent to which rats associated the chow cue with chow consumption and the relative value of chow in a non-deprived state, or with an environment in which JFs were unavailable. However, it seems likely that any association formed with chow itself would be with its taste and relative palatability upon sampling, given consumption was appreciable, but low. Therefore, presenting this cue in the JF context may have primed memory of the less-palatable chow or, possibly, of the other elements of the chow context. Importantly, the chow cue was not simply a distraction in the JF context, since overall response rates were unaffected. Instead, instrumental responding was better distributed toward the currently-valued outcome in the presence of the chow cue, indicating some restoration of evaluative processes guiding instrumental performance. By contrast, the high levels of JF consumption in the JF context provided opportunity for the JF cue to become associated with the palatable taste and hedonic properties of the JFs and, potentially, with short-term satiety occurring toward the end of the 1-h conditioning session. Regardless, when this cue was

presented in the chow-context (in Test 2) responding was still goal-directed. In summary, our data indicate that the chow cue was effective in disrupting the influence of the JF context to promote goal-directed performance like that seen in the Chow-context, perhaps by retrieving some aspect of that that context, or the chow within it, to improve the efficacy of the devaluation treatment.

The current experiments demonstrated contextual influences on sensitivity to devaluation using a within-subjects design. This is an interesting complement to past research showing that chronic exposure to diets high in sugar, or sugar and fat, promotes habitual performance as assessed by outcome devaluation (Kendig et al., 2013; Furlong et al., 2014). Taken together, these results show that highly palatable foods can impair sensitivity to devaluation both transiently (i.e., the current results) and over the longer term. It is interesting to speculate that in people, repeated exposures to palatable food-paired environments might come to disrupt decision-making processes that alter what and how much individuals eat in these environments. In turn, this increases consumption of high-fat, high-sugar foods contained in these environments, predisposing individuals toward a more lasting expression of habitual behavior toward foods. This tentative suggestion bears some resemblance to the "vicious cycle" model of obesity posited by Davidson et al. (2005) which centers on environmental factors that produce and perpetuate hippocampal insult (see also Hargrave et al., 2016). Hippocampal effects would not appear to contribute to the present results, since sensitivity to devaluation is unaffected by lesions of the hippocampus (Corbit and Balleine, 2000) and the shift between goal-directed and habitual performance instead relies on functional changes to corticostriatal circuits (Corbit, 2016).

In summary, the key message from the present experiments is that decision-making processes can be altered by diet and environments associated with consumption of highly palatable foods. Entering an environment where a certain food type is routinely consumed may bias decision-making processes that mediate future food choices. In places where there has been a history of eating so-called JFs—for example, food courts—this conditioning history may predispose people toward poorer food choices and perpetuate consumption of JFs. This might manifest as a decision to buy food despite a recent meal; selecting a less healthy option; or continuing to eat when no longer hungry. Our data also suggest that relatively simple interventions, such as reminders of reduced food value or interrupting the automatic processing of JF cues, might assist individuals in restoring control in environments where control over eating behavior is compromised. Smartphone apps designed to encourage healthy food choices and prevent "binge" episodes are one example, though their efficacy is still unclear, at least in clinical populations (e.g., Fairburn and Rothwell, 2015). Other manipulations of the external environment may also be effective. For example, one study found that college students selected healthier food options when signs throughout a food court highlighted healthy rather than unhealthy foods (e.g., salads vs. burgers; Mollen et al., 2013). A specific hypothesis prompted by the present results is to test whether a chow-paired cue produces similarly beneficial effects in animals showing habit-based performance following chronic diet exposure of the kind described above.

AUTHOR CONTRIBUTIONS

MDK conducted experiments with assistance from AMKC and JSR. MDK and LHC drafted the manuscript with assistance from AMKC and JSR. Statistical analyses were performed by MDK. LHC conceived and directed the project. All authors approved the final submission of the manuscript.

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frontiers in Behavioral Neuroscience

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Acute Stressors Reduce Neural Inhibition to Food Cues and Increase Eating Among Binge Eating Disorder Symptomatic Women

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Stressors can trigger binge-eating but researchers have yet to consider their effects on both neural responses to food cues and food consumption among those at risk. In this experiment, we examined the impact of acute stressors on neural activation to food images and subsequent food consumption within binge-eating disorder (BED) and non-eating disordered control groups. Eighteen women meeting DSM-IV BED criteria and 26 women serving as non-eating disordered controls were randomly assigned to unpleasant stressor (painful cold pressor test (CPT) followed by negative performance feedback) or less unpleasant stressor (non-painful sensory discrimination task followed by positive performance feedback) conditions. Subsequently, they were scanned with functional magnetic resonance imaging (fMRI) while viewing food and neutral images. After the scans, participants completed a self-report battery in an environment conducive to snacking. During exposure to food images, BED-symptomatic women in the unpleasant stressor condition reported more liking of high calorie food images and showed less activation in one inhibitory area, the hippocampus, compared to controls in this condition. BED-symptomatic women exposed to unpleasant stressors also consumed more chocolate than any other group during the post-scan questionnaire completion. Crucially, reduced hippocampal activation to high calorie food images predicted more chocolate consumption following fMRI scans within the entire sample. This experiment provides initial evidence suggesting unpleasant acute stressors contribute to reduced inhibitory region responsiveness in relation to external food cues and later food consumption among BED-symptomatic women.

Keywords: binge eating, fMRI, acute stress, external food cues, food consumption, cognitive control

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INTRODUCTION

Theory and research have linked unpleasant stressors to binge-eating episodes within binge-eating disorder (BED) symptomatic groups yet associated neural responses are not well understood. One plausible hypothesis is that stressors enhance reward region responsiveness to external food cues, precipitating increases in consumptive behavior. Alternately, unpleasant stressors might decrease activation in regions associated with inhibition or cognitive control in the presence of such cues, ultimately fostering increased food consumption. Towards clarifying this issue, we assessed

the impact of unpleasant acute stressors on neural responses to external food images and food consumption in average weight BED-symptomatic women and non-eating disordered controls.

BED is characterized by episodes of consuming unusually large amounts of food accompanied by a perceived loss of control. Affected persons experience marked distress related to bingeing but do not engage in compensatory behaviors such as purging, fasting, or excessive exercise following binges (American Psychiatric Association, 2013). Lifetime prevalence in the USA was estimated at about 3.5% for women and 2.0% for men (Hudson et al., 2007). However, binge-eating disturbances have also become increasingly common in highly populated non-Western nations such as China (Chen and Jackson, 2008; Jackson and Chen, 2010; Tong et al., 2014). For example, Tong et al. (2014) estimated 3.53% of young Chinese women met criteria for BED in a two-stage epidemiological study of eating disorder prevalence in Wuhan China. BED is distinct from other eating disorders and obesity, but psychiatric and medical comorbidity is common (Grilo et al., 2009).

The affect regulation model offers one explanation of links between stressors and BED symptoms (Hawkins and Clement, 1984; Haedt-Matt and Keel, 2011; Leehr et al., 2015). From this perspective, elevations in negative affect trigger binge eating episodes because food provides comfort and distraction from distress. Furthermore, given that binge eating is effective in reducing negative affect, at least in the short-run, future risk for binge-eating is increased (Haedt-Matt and Keel, 2011). As such, negative affect is especially likely to induce binge-eating among people who have binged previously compared to non-binge eaters. Support for the model hinges, in part, upon data demonstrating relatively high or rising levels of unpleasant affect prior to binge episodes among binge-eaters.

Presumably, unpleasant stressors are a common source of negative affect among binge-eaters. Related research has implicated acute stressors as influences on increased food consumption, particularly of high-fat and high-sugar foods (Epel et al., 2001; Dallman et al., 2003, 2005); for example, elevations in perceived stress, reported hassles and stressful life events predict more frequent binge eating episodes and increases in unhealthy eating (Wolff et al., 2000; Crowther et al., 2001; Pendleton et al., 2001; O'Connor et al., 2008; Klatzkin et al., 2015; Mason and Lewis, 2015; Zhu et al., 2016). Stress has also been implicated both in increasing vulnerability to BED maintenance (Striegel-Moore et al., 2002).

Illustrating possible causal effects of stress, Gluck et al. (2004) found that an obese BED group reported more hunger and a stronger desire to binge eat than obese non-BED controls did following a cold pressor test (CPT). Laessle and Schulz (2009) assessed effects of a threatening social stressor, the Trier Social Stress Test vs. a neutral task (reading a newspaper for the same duration) on the subsequent rate and duration of pudding consumption in BED patients and non-BED controls. The BED group ate more rapidly and consumed more pudding though, notably, this difference was isolated to a more stressful condition (Laessle and Schulz, 2009). Later, Schulz and Laessle (2012) reported a BED-symptomatic group showed more motivation to eat and less deceleration of eating in a stressful compared to a less stressful condition while non-BED controls had a complementary pattern. Together, these studies suggest that BED-symptomatic groups are especially prone to over-eating following exposure to unpleasant stressors.

Regardless, neural responses underlying stressor-consumption relations are not well understood. One plausible hypothesis is that stressors differentially affect reward circuitry responsiveness to external food cues among BED-symptomatic groups compared to non-disordered controls. Reviews of neuroimaging literatures on obesity and eating disturbances have implicated the medial orbitofrontal cortex (OFC), caudate, putamen, nucleus accumbens (NAc), ventral striatum and insula as key reward areas with increased activation interpreted as evidence for enhanced reward region responsiveness to external food cues and/or food anticipation (Rolls, 2004; Schäfer et al., 2010; Carnell et al., 2012; Alonso-Alonso et al., 2015). Results can vary on the basis of reward region operationalizations, hunger state and task (e.g., passive viewing vs. imagined eating; Martin et al., 2010; Dimitropoulos et al., 2012; Frankort et al., 2012) but these contentions have drawn support from functional magnetic resonance imaging (fMRI) research wherein an overweight BED group reported more reward sensitivity and showed stronger medial OFC responses while viewing food images compared to a bulimia nervosa (BN) group, obese controls, or average weight controls (Schienle et al., 2009). Subsequently, using positron emission tomography, Wang et al. (2011) found obese BED patients had more dopaminergic activity than did controls in the caudate/putamen during exposure to food stimulation.

Alternately, acute stressors and increases in unpleasant affect might result in reduced responsiveness of brain regions implicated in cognitive control or behavior inhibition among BED-symptomatic persons exposed to external food cues. General reviews of response inhibition studies (Gray and McNaughton, 2000; Buchsbaum et al., 2005; Simmonds et al., 2008) and those specific to obesity and eating disturbances (Benoit et al., 2010; Carnell et al., 2012) have identified the dorsolateral prefrontal cortex (DLPFC), superior frontal gyrus (SFG), middle frontal gyrus (MFG), inferior frontal gyrus (IFG), ventrolateral prefrontal cortex (vlPFC), hippocampus, and/or anterior cingulate cortex (ACC) as potentially important inhibitory control regions.

Representative research from Yokum and Stice (2013) found cognitive reappraisal strategies aimed at suppressing appetitive responses to palatable food images increased inhibitory region activity in the SFG, DLPFC and vlPFC. Marsh et al. (2011) concluded that diminished IFG and ACC activity contribute to reduced control over eating among BN patients. Finally, aside from its hypothesized involvement in general behavior inhibition (Gray and McNaughton, 2000), some researchers have linked impaired hippocampus functioning to heightened food intake, increased appetitive behavior and problems inhibiting responses to external food cues (e.g., Tracy et al., 2001; DelParigi et al., 2004; Davidson et al., 2009).

Despite evidence implicating food reward and cognitive control regions in binge-eating responses, it is not known whether acute stressors contributing to unpleasant affect increase reward region activation and/or reduce activity in cognitive control areas in the presence of external food cues nor is it clear which activation differences between BED-symptomatic groups and controls are salient to subsequent food consumption levels. Moreover, despite evidence suggesting that substantial percentages of people with BED are not obese (Kessler et al., 2013), very little is known about behavioral and neural responses within non-obese BED-symptomatic groups due to the near exclusive reliance upon obese BED-symptomatic groups within neuroimaging research.

Highlighting links between obesity and BED symptoms, select USA-based questionnaire research has estimated nearly 70% of those who report binge eating also endorse a body mass index (BMI) in the obese range (Grucza et al., 2007), yet rigorous, large-scale multinational research based on structured interviews has reported substantially lower obesity rates within subgroups fulfilling a DSM-IV BED diagnosis. Specifically, Kessler et al. (2013) assessed more than 24,000 participants from 14 mostly upper middle and high income Western countries including the USA They found 63.7% and 58.3% of those who met all criteria for BED during their lifetimes and past 12 months, respectively, were not obese. A substantial minority (26.5%) diagnosed with BED within the past 12 months even had a BMI in the average range. While the point prevalence information on BMI at time of diagnosis was not reported, these data suggest BED is not inevitably related to being obese and substantial percentages with the diagnosis are not obese.

On a related note, cross-national obesity data underscores how USA obesity prevalence estimates do not reflect obesity rates in highly populated non-Western nations including China. Flegal et al. (2012) reported an age-adjusted mean BMI of 28.7 for USA men and women aged 20 years and older in 2009–2010; more than one third of those sampled (35.7%) were obese. In contrast, the 2011 China Health and Nutrition Survey (Mi et al., 2015) reported an age-adjusted mean BMI of 23.8 for mainland Chinese adult men and women in the same age range and a far lower age-adjusted obesity rate (11.3%). Other recent China-based epidemiological research concluded that eating disorder rates among young Chinese women are similar to those found in Western nations (Tong et al., 2014) while mean BMIs of adolescent and young adult Chinese subgroups with binge-eating pathology have more typically fallen at the low end of the average range not the obese range (e.g., Chen and Jackson, 2008; Jackson and Chen, 2010, 2015). Based on these data, the continued neglect of non-obese BED-symptomatic samples within neuroimaging research seems unfounded, particularly when considering large Asian nations where much of the planet's population is concentrated.

Based on the preceding review, we examined the impact of acute stressors on: (1) neural activation responses to visual food cues as well as; (2) post-fMRI food consumption levels of average weight BED-symptomatic groups and non-eating disordered controls. Drawing upon assumptions of the affect regulation model and previous research (Laessle and Schulz, 2009; Schulz and Laessle, 2012), we hypothesized that BED-symptomatic participants exposed to unpleasant stressors would eat more chocolate subsequently than BED-symptomatic participants exposed to neutral stressors or non-eating disordered controls in either of these conditions. Second, within the unpleasant stressor condition, we expected BED-symptomatic participants would show more activation than controls in a priori-selected reward/motivation regions of interest (ROIs; i.e., OFC, putamen, caudate, vmPFC, nucleas accumbens and/or insula) and/or less activity in ROIs reflecting response inhibition (i.e., DLPFC, SFG, MFG, IFG, hippocampus, ACC) during exposure to visual food images. Conversely, in the neutral stressor condition, fewer salient ROI activation differences were expected between the BED-symptomatic group and controls. Finally, we hypothesized that ROIs differentiating BED-symptomatic groups from controls during fMRI scans would predict later chocolate consumption levels within the entire sample.

MATERIALS AND METHODS

Participants

The final sample included 18 women who endorsed all DSM-IV BED criteria on a validated eating disorder screen (Stice et al., 2000) and 26 women who endorsed few eating disorder syndrome criteria on this screen served as non-eating disordered controls. Data of two other women who participated (one each from BED-symptomatic and control groups) were excluded due to motion artifacts during their scans (>2.5 mm). Furthermore, we elected to exclude data from two men who also met all BED criteria from the main analyses, given the sharp gender disparity. Respondents ranged in age between 18 and 23 years (M = 19.65 years, SD = 1.27) and were predominantly of Han majority ethnicity (82.6%). The mean BMI of respondents was 19.80 (SD = 1.97, Range: 16.16–24.14). None of the BED-symptomatic women had a BMI lower than 17.5 (range: 17.62–24.14). Exclusion criteria included metallic implants, claustrophobia, current psychopharmacological medication, current or past psychiatric diagnosis aside from BED and presence of a current medical condition. All participants were right-handed non-smokers with normal or corrected-to-normal vision. No group differences in menstrual cycle phase were found. Written informed consent was obtained from each participant before entry into the study, which was approved by the human research ethics committee of Southwest University, China.

Stimuli

Digital color images depicting high-caloric foods (e.g., French fries, ice cream, cake, chips), low-caloric foods (e.g., cucumbers, carrots), and neutral images (i.e., cars) were used in this study. Each category included 90 different images. Complexity, brightness, and color composition were matched among the three categories based on recent related work (Jackson et al., 2014).

Questionnaire Measures

Eating Disorder Diagnostic Scale (EDDS; Stice et al., 2000)

This 22-item self-report scale was based on DSM-IV criteria for Anorexia Nervosa, BN and BED and was used to identify participants endorsing all BED criteria as well as ruling out a BED diagnosis among those in the non-disordered control group. The scale has excellent reliability, a high level of stability over 2 weeks, and excellent concordance with diagnoses based on structured interviews and self-report measures of disordered eating (Stice et al., 2000, 2004).

The EDDS has also been used extensively in identifying eating disorder-symptomatic adolescents and young adults in large mainland Chinese samples (Jackson and Chen, 2007, 2010; Chen and Jackson, 2008) and has high positive correlations with weight-based body image disturbances and eating disorder risk factors in Chinese adolescents and young adults of each gender (Jackson and Chen, 2008, 2010, 2011, 2014, 2015). The full EDDS was used as a screen to identify BED-symptomatic and non-disordered control subgroups. Participants also completed the five EDDS items assessing binge-eating criteria during the formal experiment to confirm the status as BED-symptomatic or control group members. The alpha coefficient for these five items was $\alpha = 0.75$ in the final sample.

Uncontrolled Eating Scale (UES; Karlsson et al., 2000)

The nine-item UES of the Three-Factor Eating Questionnaire-R18 was included as an additional continuous measure of binge eating to evaluate the distinctiveness of BED-symptomatic women vs. non-disordered controls. Items were rated on a four-point likert scale and summed to derive total scores. The measure has sound reliability and validity in past studies (e.g., Karlsson et al., 2000; de Lauzon et al., 2004). Its alpha was $\alpha = 0.88$ in this sample.

Image Pleasantness

After the fMRI scans, participants rated each image on a 9-point scale assessing pleasantness (1 = not at all, 9 = very much). For each image type (high calorie food, low calorie foods, cars) ratings were summed into total scores.

Demographics

Age, gender, ethnicity (Han majority vs. ethnic minority), menstrual cycle phase and objective measures of height and weight were assessed.

Behavior Measures

The amount of a popular, name brand chocolate consumed by each participant during the post-fMRI assessment was measured on the basis of total weight in grams (g).

Procedure

The day before their scan, participants were instructed to consume regular meals, but refrain from drinking caffeinated beverages for 12 h and to avoid eating for at least 3 h before the experiment. BED-symptomatic women and controls were randomly assigned to unpleasant and less unpleasant stressor control conditions. Women in the unpleasant stressor condition completed two CPT trials. Specifically, they were asked to immerse their non-writing hand in ice water maintained at 3.5°C for as long as possible but to remove the hand when it became too uncomfortable. There was a 4 min time limit for each trial with a 3 min break between trials. The CPT has been widely used as a stress test (Kelly and Cooper, 1998) and produces stronger cortisol responses in BED groups (Gluck et al., 2014). BED-symptomatic women and controls did not differ in hand immersion durations averaged across the two CPT trials, $t = -0.31$, $p = 0.97$ ($M = 37.00$ s, $SD = 40.30$ vs. $M = 37.58$ s, $SD = 44.75$). Because negative performance feedback is also widely used in stress-induction paradigms, and reliably induces negative affect and anxiety (Stroud et al., 2002; Bogdan and Pizzagalli, 2006), after each trial, all women in this condition were also told that they had done poorly compared to most others who previously performed the task.

Women in the less unpleasant stressor condition were asked to complete two "temperature detection" task trials (2 min immersions in room temperature water with a 3 min break between trials). To better ensure the task was perceived as less unpleasant, all participants in this condition were also given positive performance feedback indicating that they performed better than most other people who had previously completed the task.

As a manipulation check after each task, women in each stressor condition rated how: (1) stressful; (2) painful; and (3) unpleasant the task was on 11-point (0–10) scales with *0 = Not at all* and *10 = Very much so* as anchors. Supporting the integrity of the experimental manipulations, women in the unpleasant stressor condition rated their task to be more stressful ($M = 4.61$, $SD = 2.04$ vs. $M = 2.70$, $SD = 2.11$), $F_{(1,44)} = 9.73$, $p = 0.003$, painful ($M = 6.98$, $SD = 2.21$ vs. $M = 1.13$, $SD = 1.45$), $F_{(1,44)} = 112.47$, $p < 0.001$, and unpleasant ($M = 5.52$, $SD = 1.97$ vs. $M = 3.91$, $SD = 2.36$), $F_{(1,44)} = 6.31$, $p = 0.016$, than did their peers in the control condition.

Following stress inductions, fMRI scans were undertaken. Each scan comprised two runs, including three blocks of each image type (high calorie food, low calorie food, cars), respectively. Each block included 15 images presented for 2 s with an inter-stimulus interval (ISI) of 0.5 s. Before and after each block, a white fixation cross was presented in the middle of the screen for 16 s. Participants were told to simply watch every image on the screen.

After their scans, participants were taken to a waiting room to have their height/weight assessed and complete the measures described above. All self-report measures had been back-translated previously for use in Chinese samples (Jackson et al., 2014). The waiting room was standardized to include bowls of brand-name milk chocolate and bottled water. To enhance this environment as one conducive to food consumption, standardized baskets with empty chocolate wrappers and water bottles, presumably from previous participants, were on display. After handing a participant the post-task research measures, the first author stated, "If you'd like, you can help yourself to snacks and water while you're finishing these", and left her alone for

20 min. After participants completed the experiment and left, amounts of chocolate consumed were assessed and newly empty chocolate wrappers were replaced before the arrival of the next participant.

Prior to debriefing, the women were asked to guess the main research purpose; none of them identified binge eating, different stressor conditions, or amount of chocolate consumed as the foci of the experiment. Debriefing followed and featured a description of the general research purposes and time to answer participant queries. Typically, the experiment took 50 min to complete. Sixty yuan was paid as compensation.

fMRI Data Acquisition

Scans were performed with a Siemens TIM Trio 3T MRI system equipped with a standard 12 channel head coil (Siemens Magnetom Trio TIM, Erlangen, Germany). An echo-planar imaging (EPI) sequence was used with 432 T2*-weighted images recorded per run (TR = 2000 ms; TE = 30 ms; flip angle = 90°; FOV = 192 × 192 mm^2; matrix size = 64 × 64; voxel size = 3 × 3 × 3 mm^3; interslice skip = 0.99 mm; Slices = 32). T1-weighted images were acquired with a total of 176 slices at a thickness of 1 mm and in-plane resolution of 0.98 × 0.98 mm^2 (TR = 1900 ms; TE = 2.52 ms; flip angle = 9°; FOV = 250 mm^2 × 250 mm^2).

Design and Data Analysis

Demographic and Behavioral Measures

All analyses were performed using SPSS Version 20. Group differences on demographics (age, ethnicity, education, BMI) were assessed via chi-square analysis or one-way analysis of variance (ANOVA). Significant demographics were to be included as covariates in subsequent behavior and fMRI analyses. One way ANOVAs assessed group differences on continuous measures of bingeing behavior, uncontrolled eating, image pleasantness and post-fMRI chocolate consumption, controlling for any group differences on demographics. Bonferroni-adjusted *post hoc* tests assessed specific group differences when F′s were significant.

fMRI Data

fMRI data were analyzed in the context of General Linear Modeling (GLM) on a voxel by voxel basis via SPM8 (Friston et al., 1994) in MATLAB (Mathworks, Inc., Sherborn, MA, USA; Worsley and Friston, 1995). Data were normalized to the Montreal- Neurological-Institute template in 3 mm^3 × 3 mm^3 × 3 mm^3 voxel sizes, and smoothed with a 6-mm kernel full-width-at-half-maximum. Image types (high-calorie foods, low calorie foods, cars) were modeled by a function convolved with a hemodynamic response function (HRF) in the GLM. Six movement parameters applied by the realignment procedure were introduced as covariates in the first-level GLM. The time course of brain activation was modeled with a boxcar function convolved with the canonical HRF and a temporal derivative function. A first order autoregressive model was also implemented to correct for autocorrelations in error terms of the fMRI model.

Following associated research (Schienle et al., 2009), a two-stage analysis procedure was used within a mixed-effects design. At the first level, fMRI data from each woman generated statistical contrasts for comparing brain activation to: (1) High-calorie food vs. car (HiCal-Car) images; (2) Low-calorie food vs. car (LoCal-Car) images; and (3) high-calorie food vs. low-calorie food (HiCal-LoCal) images. These contrasts were then entered into second level analyses to compare each BED-symptomatic group with controls within the same stressor condition. Contrasts between conditions of interest were assessed with t statistics.

Pre-specified ROIs noted above were based on past reviews (Born et al., 2010; Bruce et al., 2010; Martin et al., 2010). Those related to reward sensitivity/motivation and food reward included the OFC, putamen, caudate, vmPFC, nucleas accumbens, amygdala and insula while ROIs reflecting behavioral inhibition and cognitive control were the DLPFC, SFG, MFG, IFG, vlPFC, hippocampus and ACC. ROI masks were generated using the AAL-atlas (Tzourio-Mazoyer et al., 2002) as implemented in the WFU-pickatlas toolbox (Maldjian et al., 2003). Corrected p values were reported for exploratory analyses ($p < 0.05$, false discovery rate corrected, FDR) while uncorrected p values ($p < 0.001$) were reported for ROI effects based on associated recent peer-reviewed studies (Stice et al., 2010; van der Laan et al., 2014; Coveleskie et al., 2015; García-García et al., 2015). The minimum cluster size threshold was set to $k = 5$, also following related peer-reviewed work (Schienle et al., 2009; Jovanovic et al., 2013; Jackson et al., 2014). Finally, within the whole sample, we assessed associations between ROIs that significantly differentiated groups in response to visual food cues and post-fMRI chocolate consumption levels.

RESULTS

Behavioral Data

No group differences were found on ethnicity ($\chi^2 = 4.17$, $p > 0.05$) or age, education and BMI (**Table 1**). One-way ANOVAs indicated each BED-symptomatic subgroup reported more binge-eating behavior and uncontrolled eating than control subgroups did, supporting the distinctiveness of these subgroups. Only one group difference was found *vis a vis* image pleasantness ratings: control group women in the unpleasant stressor condition rated high-calorie food images to be less pleasant than peers in any of the other conditions ($p < 0.05$). Finally, for post-fMRI chocolate consumption, BED-symptomatic women in the unpleasant stressor condition ate significantly more chocolate than women in each of non-disordered control group did (p's < 0.05), and marginally more chocolate than BED-symptomatic women in the control condition ($p = 0.051$).

fMRI Data

Activation Differences in the Unpleasant Stressor Condition

BED-symptomatic women in the unpleasant stressor condition showed significantly less IFG, insula and hippocampus activation

TABLE 1 | Group differences on demographics, self-report measures and chocolate consumption.

	BED-HI ($n = 9$)	BED-LO ($n = 9$)	CON-HI ($n = 12$)	CON-LO ($n = 14$)	F	Group differences
Age (years)	19.22 (0.44)	19.89 (1.54)	20.00 (1.41)	19.43 (1.34)	0.87	no differences
Education (years)	13.00 (0.00)	13.33 (0.71)	13.17 (0.58)	13.21 (0.58)	0.58	no differences
BMI	20.80 (1.48)	20.72 (2.34)	19.19 (1.52)	19.22 (2.16)	2.34	no differences
Binge behavior	3.78 (0.97)	3.11 (1.05)	1.25 (1.06)	0.64 (0.63)	28.31***	BED-HI > CON-HI, CON-LO***; BED-LO > CON-HI, CON-LO***
UES	24.00 (3.04)	23.44 (4.67)	17.00 (4.08)	18.43 (4.60)	6.46***	BED-HI > CON-HI, CON-LO**; BED-LO > CON-HI, CON-LO**
High-calorie food pleasantness	6.26 (0.82)	6.58 (1.28)	4.49 (1.33)	6.39 (1.17)	8.34***	BED-HI > CON-HI**; BED-LO > CON-HI***; CON-LO > CON-HI***
Low-calorie food pleasantness	5.01 (1.74)	4.86 (1.93)	4.96 (1.19)	5.63 (0.77)	0.51	no differences
Car pleasantness	4.99 (1.87)	4.58 (1.61)	4.70 (0.79)	5.36 (1.46)	0.56	no differences
Chocolate consumed (grams)	43.56 (37.98)	21.78(21.13)	15.17 (17.36)	7.00 (14.27)	4.85***	BED-HI > CON-HI, CON-LO**; BED-HI > BED-LO†

Note: BED-HI, binge eating disorder symptomatic group in unpleasant stressor condition; BED-LO, binge eating disorder symptomatic group in less unpleasant stressor condition; CON-HI, non-eating disordered control group in unpleasant stressor condition; CON-LO, non-eating disordered control group in less unpleasant stressor condition; BMI, body mass index; UES, uncontrolled eating scale; $^{\dagger}p < 0.10$; $^{**}p < 0.01$; $^{***}p < 0.001$. All p-values were based on two-tailed significance tests.

than controls did during exposure to high calorie food images relative to low calorie food or car images (**Table 2**). The former group also experienced less hippocampus activity in the low calorie food vs. car contrast. Finally, BED-symptomatic women showed comparatively less hippocampus, IFG and amygdale activation in the HiCal-LoCal contrast condition (see **Table 2**, **Figure 1**).

Activation Differences in the Less Unpleasant Stressor Condition

BED-symptomatic women in the less unpleasant stressor condition showed less ACC and SFG activation than controls did in HiCal-Car and LoCal-Car contrasts. BED-symptomatic women also had less parahippocampal gyrus activity in the HiCal-LoCal contrast. Contrary to predictions, the former group also showed comparatively weaker rather than stronger activity in select hypothesized reward ROIs (left OFC, putamen) for HiCal-Car and LoCal-Car contrasts, respectively (see **Table 2**).

Correlations Between ROIs and Chocolate Consumption

Finally, we examined relations between ROIs that differentiated BED subgroups from controls in between-groups analyses and post-fMRI amounts of chocolate eaten within entire sample. No outliers (3 SDs ± mean) were found for total chocolate consumed. No significant ROIs from HiCal-Car or LoCal-Car contrasts were related to chocolate consumption (all p's > 0.05). However, supporting its role as an inhibitory control region, less hippocampus activation in the HiCal-LoCal contrast condition predicted more subsequent chocolate consumed in the sample [MNI coordinates x, y, z: -21, -39, 9; $r = -0.37$, $p = 0.012$] (see **Figure 2**). Even after dichotomizing chocolate consumption subgroups (no chocolate eaten vs. chocolate eaten), reduced hippocampus activity was related to eating rather than not eating chocolate, $t = -2.46$, $p = 0.012$.

DISCUSSION

Exposure to acute stressors and increases in unpleasant affect can precipitate binge-eating episodes among BED-symptomatic persons, but neural responses underlying related experiences have never been examined within this literature. Towards illuminating this issue, we assessed the extent to which reward area activity increases and decreased inhibitory region responsiveness to external food cues contributed to this pattern in BED-symptomatic women vs. non-eating disordered controls.

Regarding links between BED symptomatology, unpleasant stressors and post-fMRI chocolate consumption levels, results supported hypothesized group differences. As expected, BED-symptomatic women and controls randomly assigned to the unpleasant stressor condition experienced more pain, stress, and unpleasant affect in response to associated tasks than did peers in the less unpleasant stressor control condition. Supporting tenets of the affect regulation perspective (Haedt-Matt and Keel, 2011; Leehr et al., 2015), BED-symptomatic women exposed to unpleasant stressors also ate more chocolate following their scans than did non-disordered controls or complementary groups in the control stressor condition. In fact, the former group consumed more than twice as much chocolate, on average, than did BED-symptomatic women in the control condition ($p = 0.051$) and about 300–600% more chocolate than non-eating disordered subgroups did. While researchers have linked stress and daily hassles to binge-eating (Crowther et al., 2001; O'Connor et al., 2008), the pattern observed here underscored how exposure to unpleasant stressors triggers more eating in women who report previous binge-eating than non-binge eaters or binge-eaters not exposed to such triggers (Hawkins

TABLE 2 | Regions of interest (ROI) reaching significance ($p < 0.001$) in Group × Stressor condition analyses.

Condition contrast and region	BA	Hem	Cluster Size	*x*	*y*	*z*	*t*
Unpleasant stressor							
CON > BED							
High-caloric food vs. car							
Inferior Frontal Gyrus	47	R	6	30	27	−9	3.00
Insula	13	L	56	−33	6	−9	3.68
Hippocampus	–	L	9	−30	−36	0	3.44
Low-caloric food vs. car							
Hippocampus	–	L	13	−30	−36	0	4.22
High- vs. Low-caloric food							
Hippocampus	–	R	13	39	−15	−18	4.12
Inferior Frontal Gyrus	–	R	5	27	24	−9	3.63
Amygdala	–	L	11	−27	−6	−12	3.79
Less unpleasant stressor							
CON > BED							
High-caloric food vs. car							
ACC	32	L	10	−3	36	6	3.58
Superior Frontal Gyrus	6	R	18	24	9	48	3.93
Low-caloric food vs. car							
Putamen	–	R	13	21	3	−3	3.75
ACC	–	R	7	15	39	12	3.42
Superior Frontal Gyrus	6	R	16	21	12	45	3.55
High- vs. Low-caloric food							
Parahippocampal Gyrus	–	L	7	−21	−30	−21	3.88

Note: BED, binge eating disorder symptomatic; CON, non-eating disorder control; IFG, Inferior Frontal Gyrus; ACC, anterior cingulate cortex.

and Clement, 1984). Reassuringly, Laessle and Schulz (2009) previously observed this pattern in obese samples but the current findings indicate this facet of the affect regulation model extends to average weight BED-symptomatic women as well. Taken together, these studies suggest that reported history of binge-eating rather than weight status or the synergy of binge-eating and obesity may be the more critical influence on overeating following unpleasant stressors in BED-symptomatic samples.

Hypothesized ROI activation differences reflecting response inhibition also received support. Specifically, within the unpleasant stressor condition, BED-symptomatic women experienced less activation than controls did in one inhibitory ROI, the hippocampus, across all three image contrast conditions. Critically, reduced hippocampus activation during exposure to high calorie images also predicted significantly more post-fMRI chocolate consumption within the entire sample. Past work has found neurohormonal signals related to meal initiation (e.g., ghrelin), meal cessation (e.g., cholecystokinin), and feedback on the status of energy stores (e.g., leptin, insulin) all have receptors in the hippocampus (Lathe, 2001); these appear to modulate hippocampal-dependent learning and memory processes (Zhao et al., 2004; Diano et al., 2006; Harvey et al., 2006). Aside from its possible role in appetitive processes, the hippocampus is involved in regulating neuroendocrine stress responses (Pruessner et al., 2008; Ulrich-Lai and Herman, 2009). For example, fMRI evidence from Pruessner et al. (2008) indicated hippocampus activity was curtailed in response to a stressor, in turn, contributing to hypothalamic-pituitary-adrenal axis initiation and stress hormone release.

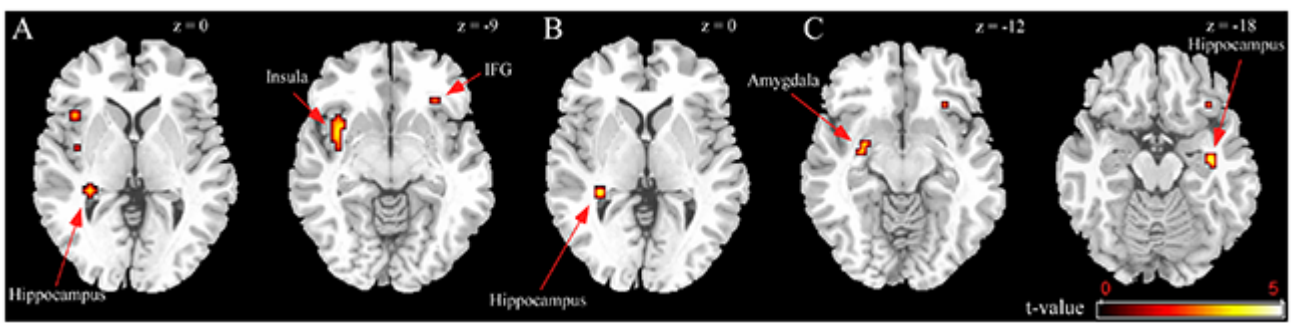

FIGURE 1 | Group differences in unpleasant stressor condition. (A) High-caloric food vs. car contrast; **(B)** Low-caloric food vs. car contrast; **(C)** High-caloric food vs. Low-caloric food contrast.

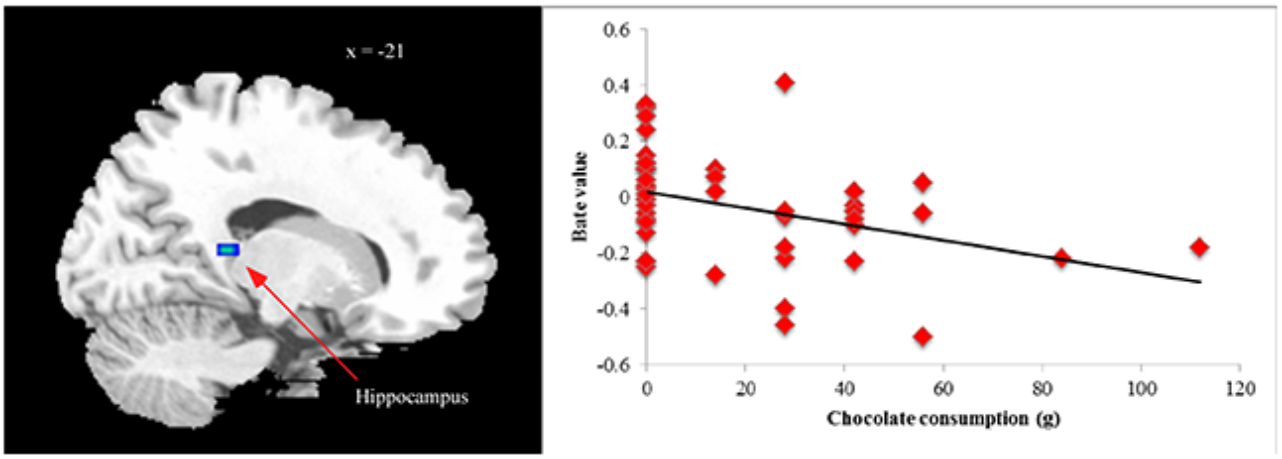

FIGURE 2 | Correlation between chocolate consumption and hippocampus activation [MNI coordinates *x*, *y*, *z*: −21, −39, 9] in high- vs. low-calorie food image contrast.

Animal researchers have found rats with hippocampal lesions have general learning deficits that require suppression of previously learned responses (Winocur, 1980) in addition to increased food intake, weight gain, appetitive behavior, metabolic activity, and difficulty inhibiting responses to external food cues when compared to intact controls (Tracy et al., 2001; Davidson et al., 2009). On this basis, Davidson et al. (2009) argue that impaired hippocampal activity underlying such deficits interferes with the ability to use conditional cues evoked by food or food-related environmental cues and interoceptive satiety signals in suppressing appetitive and consumption responses. Hippocampus damage in humans can also interfere with the capacity to use intero- and extroceptive cues in satiety signaling (Born et al., 2010). One fMRI study found obese and formerly obese people had lower hippocampal blood flow than did lean controls after consuming a liquid meal to satiation (DelParigi et al., 2004). Extending such work, the present results underscored how reduced hippocampus responsiveness to *external* food cues following an unpleasant stressor differentiated a non-obese BED-symptomatic group from non-obese controls and helped to explain the higher post-fMRI chocolate consumption level of the former group.

In contrast to this difference in inhibitory region responsiveness, there was no evidence that *increased* responsiveness in reward/motivation ROIs discriminated BED-symptomatic women from controls exposed to unpleasant stressors. In fact, the former group showed significantly less activation during exposure to high calorie food images in the putamen, an area implicated in elevated reward motivation as well as the insula which has links with taste (Carnell et al., 2012). Explaining the functional significance of these structures on this basis would suggest exposure to the more unpleasant tasks corresponded to less activation of reward/taste regions in response to high calorie food images among BED-symptomatic participants.

However, such an interpretation seems problematic because pleasantness ratings for high calorie food images and chocolate consumption levels were elevated among BED-symptomatic participants compared to controls in the unpleasant stressor condition. The putamen and insula have multiple functions, aside from those reflecting reward and taste. Consistent with behavior results and the interpretation of hippocampus findings, the putamen has also been identified as a key inhibitory region in separate meta-analyses of structures involved in response inhibition during go/no-go tasks (Buchsbaum et al., 2005; Simmonds et al., 2008). In addition, insula activity differences were not localized to the anterior region associated with taste but rather the left posterior insula (BA13); increased activity in this area corresponds to the anticipation and experience of negative affect (Richieri et al., 2011; Aupperle et al., 2012) in addition to pain processing (Rainville, 2002). In this light, reduced BA13 responsiveness among BED-symptomatic women exposed to unpleasant stressors might have reflected comparatively less aversive affect evoked by high calorie food rather than neutral images.

In the less unpleasant control condition, the hypothesis that BED-symptomatic and non-disordered groups would have fewer ROI activation differences was not supported. Instead, BED-symptomatic women in this condition also showed relatively lower activation to food images in hypothesized ROIs reflecting cognitive control (i.e., the SFG, ACC, parahippocampal gyrus), albeit none of these predicted later chocolate consumption levels in the sample. Comparatively reduced putamen activity in this group mirrored results for the other BED-symptomatic subgroup, though the effect emerged for low- rather than high calorie images. Also paralleling results for the unpleasant stressor condition, BED-symptomatic women exposed to the less unpleasant stressors did not show enhanced reward ROI responsiveness to food images. Rather, this subgroup had lower ACC activity than did controls in the high-calorie food-

car contrast. Reduced anterior cingulate metabolism has been linked to impaired inhibitory control and risk for overeating among healthy obese persons (Volkow et al., 2009), but this study may be the first to implicate these areas in average BMI persons with binge-eating concerns. Taken together, the present results and those of Volkow et al. (2009) suggest implications of these activation differences should be explored in future longitudinal work on risk for later weight gain and obesity.

Notwithstanding possible implications of this study, several limitations should be noted as foundations for extensions. First, while the sample *N* was more than twice as large as those used in 26 of 29 fMRI studies included in a review of responses to visual food cues (van der Laan et al., 2011), within cell n's were quite modest; therefore extensions of the current paradigm to larger samples with more power may aid in detecting subtle neural responses. Second, even though results for BED-symptomatic women were based on endorsing all DSM-IV BED criteria from a validated diagnostic screen, BED diagnosis can be established only from structured interviews with follow-up probes. Eating disorder assessment via anonymous surveys can increase candor (Keel et al., 2002) and the EDDS has excellent concordance with diagnostic interviews (e.g., Stice et al., 2000) but interview-based assessment should be considered in extensions to determine how well findings apply to women with a definitive BED diagnosis.

Third, standardized procedures and random assignment were employed to control for potential stressor condition differences in background functioning. However, the inclusion of pre-experiment hunger ratings would have been preferable in assessing and ruling out group differences in hunger as an influence on results. Fourth, in relation to external validity, a considerable subset of women with BED and binge-eating tendencies may not be obese (Jackson and Chen, 2010; Kessler et al., 2013; Tong et al., 2014) and assessment of average weight samples here underscored links of reported BED symptoms with hypothesized behavior and neural activation responses independent of obesity. However, obesity is also common in BED and BED-symptomatic samples (Grucza et al., 2007; Kessler et al., 2013). Furthermore, findings may not generalize to predominantly male samples, given evidence of gender can affect ability to inhibit brain activation elicited by food stimuli (Wang et al., 2011). Extensions to obese groups, men, and samples from non-Asian contexts can elucidate relative contributions of neural responses associated with reward and cognitive control more fully. Finally, possible causal links between acute stressors, neural responses to food images, and subsequent chocolate consumption were examined on a single occasion lasting less than 1 h. As a result, inferences about long-term consequences of such associations are necessarily tentative and warrant consideration in extensions based on more costly prospective designs.

CONCLUSION

In sum, this experiment supported key assumptions of the affect regulation perspective of binge-eating. In line with tenets of the model, BED-symptomatic women exposed to unpleasant stressors subsequently consumed more chocolate following exposure to external food images relative to non-eating disordered controls or BED-symptomatic women exposed to less unpleasant stressors. Imaging results for the unpleasant stressor condition indicated reduced activation to food cues in one inhibitory ROI, the hippocampus, differentiated the BED-symptomatic group from controls and predicted higher chocolate consumption levels in the entire sample. While BED-symptomatic women in the less unpleasant control stressor condition also showed reduced inhibition ROI activity relative to controls, there was no evidence that enhanced reward ROI responsiveness differentiated BED-symptomatic groups from controls in either stressor condition or predicted post-fMRI chocolate consumption. As such, this study provides initial evidence suggesting reduced hippocampal responsiveness to external food cues helps to explain why exposure to unpleasant stressors magnifies vulnerability to later overeating among the BED-symptomatic.

AUTHOR CONTRIBUTIONS

TJ designed the project. ZL performed the experiment and analyzed the data. ZL and TJ wrote the manuscript.

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frontiers in Behavioral Neuroscience

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Western Diet Chow Consumption in Rats Induces Striatal Neuronal Activation While Reducing Dopamine Levels without Affecting Spatial Memory in the Radial Arm Maze

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Rats fed high fat diets have been shown to be impaired in hippocampal-dependent behavioral tasks, such as spatial recognition in the Y-maze and reference memory in the Morris water maze (MWM). It is clear from previous studies, however, that motivation and reward factor into the memory deficits associated with obesity and high-fat diet consumption, and that the prefrontal cortex and striatum and neurotransmitter dopamine play important roles in cognitive performance. In this series of studies we extend our research to investigate the effect of a high fat diet on striatal neurochemistry and performance in the delayed spatial win-shift radial arm maze task, a paradigm highly reliant on dopamine-rich brain regions, such as the striatum after high fat diet consumption. Memory performance, neuronal activation and brain dopaminergic levels were compared in rats fed a "Western" (21% fat, 0.15% cholesterol) chow diet compared to normal diet (6% fat, 0.15% cholesterol)-fed controls. Twelve weeks of dietary manipulation produced an increase in weight in western diet-fed rats, but did not affect learning and performance in the delayed spatial win-shift radial arm maze task. Concurrently, there was an observed decrease in dopamine levels in the striatum and a reduction of dopamine turnover in the hippocampus in western diet-fed rats. In a separate cohort of rats Fos levels were measured after rats had been placed in a novel arena and allowed to explore freely. In normal rats, this exposure to a unique environment did not affect neuronal activation. In contrast, rats fed a western diet were found to have significantly increased Fos expression in the striatum, but not prefrontal cortex or hippocampus. Our study demonstrates that while western diet consumption in rats produces weight gain and brain neuronal and neurotransmitter changes, it did not affect performance in the delayed spatial win-shift paradigm in the radial arm maze. We conclude that modeling the cognitive decline-obesity relationship is complex with considerations, of type of memory, behavioral task and dietary intervention (fat, fat and sugar, sugar, and cafeteria diets) all adding to our overall understanding.

Keywords: western diet, high fat diet, neuronal activation, spatial memory, cognition, striatum, dopamine

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INTRODUCTION

The rapid rise of obesity rates has been attributed to the increasing availability of unhealthy diets (that is over-consumption of food and beverages with a high content of fats, sugars and salt) and physical inactivity (WHO, 2015). The presence of overweight and obesity contributes to significant health impairments with large increases in the risk of cardiovascular disease, type 2 diabetes and cancer (McGee, 2005; Adams et al., 2006). The incidences of mild cognitive impairment (Elias et al., 2005; Jeong et al., 2005; Hassing et al., 2010), dementia (Whitmer et al., 2005; Anstey et al., 2011), and Alzheimer's disease (Solfrizzi et al., 2004; Whitmer et al., 2005; Gustafson et al., 2012; Besser et al., 2014) are similarly increased with obesity.

Rats fed high fat diets have been shown in some studies to be cognitively impaired compared to those fed a normal chow diet. Much emphasis has been placed on hippocampal-dependent behavioral tasks (Molteni et al., 2002; Wu et al., 2003; Goldbart et al., 2006; Pathan et al., 2008; Stranahan et al., 2008; Xia et al., 2015). In the Morris water maze (MWM), a number of studies have shown that high fat fed animals took longer to learn the location of a submerged platform relative to their control counterparts (Wu et al., 2003; Molteni et al., 2002; Goldbart et al., 2006; Pathan et al., 2008; Stranahan et al., 2008; Xia et al., 2015). These studies used varying levels of fat ranging from 21 to 58 kcal% and different lengths of diet consumption, with the general consensus that high fat diets consumed long-term can impair spatial learning and memory in the MWM.

The western diet (WD) model of obesity is a subtype of HFD-induced obesity that mimics the so-called "Western" diet by feeding rats a WD chow (containing 22% w/w fat equivalent to 40 kcal% fat) or a control chow diet (containing 6% total fat). The WD was formulated to represent a typical HFD typically consumed in developed "Western" countries and is equivalent to the *Harlan Teklad* TD88137 or *Research Diets* Western Diet D12079B that have previously been used to accelerate and enhance hypercholesterolemia and atherosclerotic plaque formation (Febbraio et al., 2000; Ascencio et al., 2004; Yang et al., 2006). We have previously shown that 12 week WD feeding causes a significant change in metabolic measures including increasing body weight, blood pressure and serum triglycerides (Kosari et al., 2012).

Dopamine (DA) has a well-recognized role in cognition including motivation, reward, punishment, and working memory (Cools, 2008). Recent research has discovered the involvement of DA with obesity (Volkow et al., 2012, 2013). It has been postulated that in individuals with a hypo-responsive mesocorticolimbic pathway there is an increased risk of the development of obesity (Davis et al., 2004). In humans sensitivity to reward has been associated with emotional overeating, preference for high fat foods, binge eating and food cravings (Loxton and Dawe, 2001; Davis et al., 2004, 2007; Franken and Muris, 2005). In a rodent study lentivirus-mediated knockdown of striatal DA D2-receptors resulted in the onset of compulsive-like food seeking in rats with extended access to palatable high-fat food and also decreased responsiveness of the brain reward system, consist with some of the evidence from humans (Johnson and Kenny, 2010). Moreover, a high fat cafeteria style diet also lowers both basal levels of DA and DA release in response to food or amphetamine (Geiger et al., 2009).

Given that we see a cognitive deficit in our animals after WD consumption (Kosari et al., 2012), along with important markers observed with weight gain and metabolic syndrome (Kosari et al., 2012), and the reported involvement of DA in obesity and reward, we hypothesized that the WD diet would produce modifications to the brain dopaminergic system and that this might lead to cognitive deficits. In this investigation we therefore examined cognitive function using the delayed-win shift (DWSh) task in the radial arm maze (RAM) (Jarrard, 1993; Floresco et al., 1997) in adult rats made obese from consuming the WD. This requires rodents to hold spatial information for food reward location during task performance, and across a delay (Seamans et al., 1995). Similar to the MWM, in that the rodent acquires, retains and uses trial-unique information, the DWSh task exploits food reward as a motivator. As such it can be suggested to be less stressful when compared to the MWM which relies on the rodents' swimming ability (Seamans et al., 1995). Furthermore, the task has a reliance on DA-rich regions, such as the medial prefrontal cortex (PFC) (Taylor et al., 2003), hippocampus (HPC) (Jarrard, 1993), and ventral striatum (Floresco et al., 1997; Jarrard, 1993). We then examined the effects of WD on neuronal activation within these regions along with DA content to assess how WD impacts on the ability to modulate these learning-associated brain regions to facilitate memory.

MATERIALS AND METHODS

Animals

Male Wistar hooded rats (University of Adelaide, Australia) were housed at RMIT University animal facility, a controlled environment (20 $\pm$ 1°C) with 12-h light/dark cycle (lights on at 07:00 h) in groups of 4, with food and water *ad libutum* in the home cage. Behavioral tests were performed from 9:00 to 19:00 h in a dedicated animal behavior room. All experiments were performed in accordance with the Prevention of Cruelty to Animals Act 1986 and with approval from the RMIT University Animal Ethics Committee.

Dietary Manipulation

Upon delivery, all animals were allowed to acclimatize for at least 1 week before commencement of dietary manipulation. Rats were randomly assigned to either a control diet (CON, Standard AIN93G rodent diet, 6% total fat including 1.05% total saturated fatty acids; Specialty Feeds, Perth, Australia) or WD (SF00-219, 21% total fat including 1.80% total saturated fats and 0.15% cholesterol; Specialty Feeds, Perth, Australia) and remained on this diet for 12 weeks.

EXPERIMENT 1

Food Restriction

One week prior to the start of DWSh task, rats ($N = 10$ per group) were food restricted with their respective CON or WD. Body weight was monitored twice weekly to ensure rats do not

fall below 85% of their free-feeding weight. Food restriction was maintained for the entire duration of behavioral testing.

Delayed Win-Shift Task in the Radial Arm Maze

Testing was carried out in an eight-arm radial maze (Lafayette Instrument, USA), consisting of an octagonal central platform (34 cm diameter) and eight equally spaced radial arms (87 cm long, 10 cm wide). At the end of each arm was a food well (2 cm in diameter and 0.5 cm deep). At the start of each arm was a clear Perspex door that controlled access in and out of the central area. Each door was controlled by a computerized control box enabling the experimenter to control access to the arms. Salient visual cues of different geometric shapes were placed around the maze on the walls of the room.

On the first 3 days of testing, rats were habituated to the RAM in two sessions per day lasting 10 min each. After the final habituation session of the day, rats were returned to their home cages and given approximately 20 grain reward pellets (45 mg, Bio-Serv, USA). Following habituation, rats underwent a total of 12 training sessions with 2 sessions performed per day. This consisted of a 5 min training phase, 5 min inter-trial interval where the rat was returned to the home cage and a 5 min test phase. Before the training phase, 4 arms were pseudo-randomly chosen and blocked, with the following rule that no more than 2 adjacent arms could be closed in any trial. The remaining arms that were not blocked were baited with grain reward pellets. The training phase involved allowing the rat 5 min to enter and retrieve the grain pellet rewards from all the baited arms. After a 5 min inter-trial interval, the test phase occurred where all 8 arms were opened and the previously blocked arms are baited with grain reward pellets. The rat was then placed back inside the maze and the number of arm entries was recorded.

For analysis purposes, 2 training/test sessions were grouped into a single block. An arm entry was recorded when the animal fully moved off the central platform into the arm. Two types of errors were recorded: within phase error (working memory error, re-entry of an arm that has been baited and has been visited) and across phase error (reference memory error, entry into a training phase baited arm).

Removal of Epididymal Adipose Tissue

Once rats were culled with pentobarbital sodium (1 mg/kg), epididymal adipose tissue located within 10 mm from the epididymis (proximal) and within 10 mm from the distal end of the epididymal fat depot (distal) were harvested and weighed.

HPLC Sample Preparation

Randomly selected rats from the RAM cohort ($N = 5$ per group) were killed by 0.5 ml i.p. injection of pentobarbital sodium (1 mg/kg). Brains were snap frozen in iso-pentane cooled to −35°C by dry ice then stored at −80°C. Whole striata, hippocampi and prefrontal cortices were dissected on ice with the use of the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007).

Prefrontal cortices, striata and hippocampi were assessed for DA and dihydroxyphenylacetic acid (DOPAC; DA metabolite) levels. Samples were homogenized in extraction buffer (4 M perchloric acid, 0.008 M sodium metabisulphate, 0.002 M disodium ethylenediaminetetra-acetic acid (EDTA) and MilliQ water to bring volume to 100 ml) and sonicated to rupture vesicular membranes. Samples were then spun at 10,500 g for 5 min, and the supernatant transferred to a fresh tube. The samples were spun a further two times, to ensure all debris was eliminated. Samples were stored at −80°C until required.

Forty μl of sample was transferred to a HPLC recovery vial. Standards for DA and DOPAC were made in the same extraction buffer used for sample preparation. The mobile phase was composed of 70 mM monopotassium phosphate, 0.5 mM EDTA disodium salt, 8 mM octane sulfonic acid sodium salt, 170 ml HPLC grade methanol, to a final volume of 1000 ml and pH 3. The flow rate was 500 μl/min with reverse phase C18 columns. HPLC analysis was conducted on PFC, striatal and HPC samples from rats fed either CON diet or WD for 12 weeks for total (intracellular and extracellular) DA and DOPAC levels. Standards of known concentrations for dopamine and DOPAC were used to quantify and identify the peaks on the chromatographs.

EXPERIMENT 2

Exposure to Novel Environment

A separate cohort of rats ($N = 6$–7 per group, 12 weeks CON or WD dietary manipulation) was assessed for activated Fos expression. Rats were placed into a novel arena, in our case a Y-maze (three-arm maze with equal angles between all arms which were 50 cm long × 17 cm wide × 32 cm high. Rats were allowed to move around this novel environment for 30 min. Rats were then returned to their home cages for 90 min in a dark, quiet room. This manipulation was to reduce exposure to other stimuli that might evoke Fos production. Immediately after this 90 min quiet period rats were deeply anesthetized with pentobarbitone sodium (1 mg/kg) and perfused transcardially with 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS).

Home Cage Controls

In a further cohort of rats ($N = 6$ per group), underwent the identical dietary manipulation as the cohort above. These home cage control rats, run at a different time to the cohort above, remained untouched until culled when they were also deeply anesthetized with pentobarbital sodium (1 mg/kg) and perfused transcardially with 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M PBS.

Brain Preparation

After transcardial perfusion heads were removed with a purpose built rat guillotine and brains removed and postfixed for 4 h in the 4% paraformaldehyde in PBS before placing them in 30% sucrose in PBS solution (4°C) until sectioning. Following fixing of brains, serial coronal sections (30 μm) were cut on a cryostat (Leica CM1950, Leica Microsystems, Germany) at −16°C and placed in cyroprotectant [30% (w/v) sucrose, 30% (w/v) ethylene glycol,

0.01% (w/v) polyvinyl pyrolidine in 0.1 M PBS (pH 7.4) solution] and stored at −20°C to later undergo immunohistochemistry.

Fos Immunohistochemistry

Sections were washed and transferred to 0.3% hydrogen peroxide in 0.1 M PBS containing 0.2% Triton X-100 (PBST) for 10 min to inhibit endogenous peroxidase and then washed several times with PBST. Sections were incubated in PBST containing Fos rabbit polyclonal antibody (1:5000; Ab-5; Oncogene Science, UK) for 48 h at 4°C with periodic rotation. Sections were then washed with PBST and incubated in biotinylated goat anti-rabbit secondary antibody (diluted 1:200 in PBST; Vectastain; Vector Laboratories, USA) and 1.5% normal goat serum for 2 h at room temperature on a rotator. Sections were then washed and processed with avidin-biotinylated horseradish peroxidase complex in PBST (Elite Kit; Vector Laboratories, USA) for 1 h at room temperature, again with constant rotation. Sections were washed again in PBST and then in 0.05 M Tris buffer. The reaction was then visualized using 3′, 3′–diaminobenzidine intensified with nickel chloride. Sections were mounted and allowed to dry overnight before being dehydrated via a graded series of alcohol washes and coverslipped.

Image Analysis

Photomicrographs of immunolabelled brain sections were captured at 10x objective using a BX60 microscope (Olympus, Japan) and RTKE SPOT camera (Diagnostic Instruments, USA) interfaced to a PC computer with SPOT imaging software. Counts of stained nuclei were carried out using the public domain Image J program (National Institutes of Health, USA). Images were digitized into gray scale where a threshold, set above the mean value ± S.E.M. of the background, was applied for background correction. Inside each region, the number of particles above the threshold was automatically calculated. There were no observed rostrocaudal differences in all brain regions analyzed.

Regions of Interest

A total of 7 regions were analyzed with sites selected because they have been implicated previously in memory processes. All of the sites from which it was decided *a priori* to count Fos-positive cells are presented. For each brain region analyzed, counts were taken from a minimum of four alternate coronal sections. Cytoarchitectonic subfields within the hippocampal formation consisting of the cornu ammonis area 1 (CA1), cornu ammonis area 2/3 (CA2/3) and dentate gyrus (DG) of the HPC were investigated. Hippocampal counts were taken at interaural 5.28 mm and bregma −3.72 mm in Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007). Fos immunoreactive cells were counted in the prelimbic area (PrL), cingulate cortex (Cg), and infralimbic cortex (IL) corresponding to interaural 12.00 mm and bregma 3.00 mm (Paxinos and Watson, 2007). The striatum were counted at levels corresponding to interaural 11.04 mm and bregma 2.04 mm (Paxinos and Watson, 2007). Three areas within each striatal section were sampled using a 1 × 1 cm square generated the imaging program and a single value was obtained by averaging the 3 counts.

Statistical Analysis

All data are presented as mean ± S.E.M. A p-value of < 0.05 was considered statistically significant. Statistical comparisons were made between groups by repeated measures two-way ANOVA for DWSh task performance using GraphPad Prism version 6.00 (GraphPad Software, USA).

Two-way ANOVA was used for comparing HPLC data (neurotransmitter level x group). Basal and activated Fos counts were analyzed separately. Both the PFC and HPC counts were analyzed by two way ANOVA (subregion × group). Unpaired t-tests assessed basal and activated Fos counts separately for the striatum data. Further analysis by a *post-hoc* Bonferroni's t-test was performed if a significant effect was detected by the ANOVA.

RESULTS

Effect of Diet on Metabolic Measures

Rats fed a WD were observed to be heavier than rats fed a CON diet [Week 12, CON: 415.7 ± 9.8 g; WD: 458.2 ± 11.6 g; $F_{(1, 22)} = 7.2$ $p < 0.05$]. Both groups increased weight over time [time: $F_{(12, 264)} = 0332.7$, $p < 0.0001$] while WD rats increased their body weight at a more pronounced rate than CON, [group × time: $F_{(12, 264)} = 3.5$, $p < 0.01$]. *Post-hoc* analysis showed significant body weight differences starting from week 8 until week 12. WD consumption was shown to increase epididymal adipose tissue weight (CON: 8.2 ± 0.3 g; WD: 11.5 ± 0.6 g, $p < 0.001$).

Experiment 1-Radial Arm Maze and Neurotransmitter Changes

Training Phase Performance in the Delayed Win-Shift Task

Both CON and WD groups learnt to complete the DWSh task in the RAM, had fewer errors and entered more correct arms before an error was recorded as training progressed. Performances of rats in the training phase of the delayed win-shift radial arm maze procedure are shown in **Figure 1**. A repeated measures ANOVA was conducted and revealed that both CON and WD rats entered more arms as training progressed [$F_{(5, 90)} = 29.86$, $p < 0.0001$], however there was no group [$F_{(1, 18)} = 0.63$, $p = 0.44$] nor group x block effect [$F_{(5, 90)} = 1.40$, $p = 0.23$; **Figure 1A**]. During training, rats steadily increased the number of correct arm choices over blocks [block effect: $F_{(5, 90)} = 36.34$, $p < 0.0001$] but no group or group x block effect was observed (both $F < 1$, **Figure 1B**). As training progressed both CON and WD animals became more proficient in the task as the animals made more correct arm choices before an error was recorded [block effect: $F_{(5, 90)} = 27.78$, $p < 0.0001$; **Figure 1C**].

Test Phase Performance in the Delayed Win-Shift Task

During the test phase, WD animals did not show any evidence of cognitive impairment relative to CON. The number of correct arm choices before an error was made steadily increased as training progressed [block effect: $F_{(5, 90)} = 10.41$, $p < 0.0001$; **Figure 2A**] and there was an overall effect of block to influence

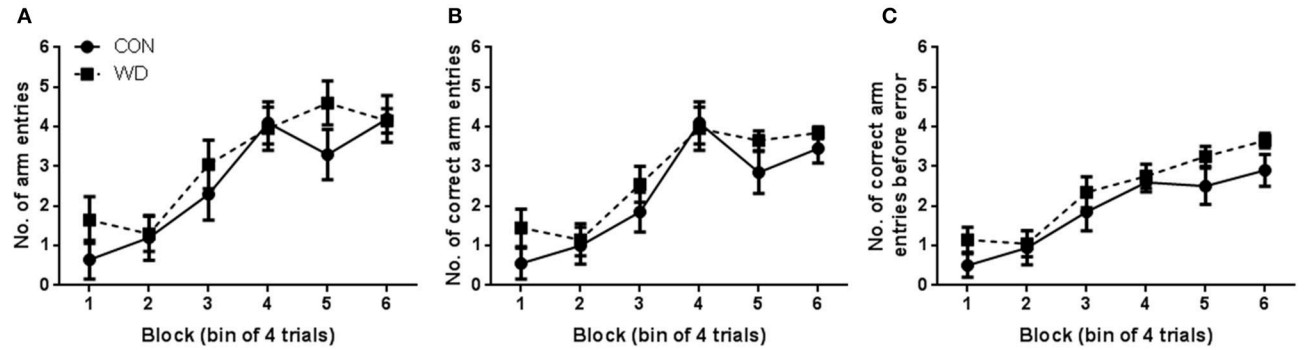

FIGURE 1 | Performance in training phase of DWSh task. (A) Number of arm entries in each session of training. **(B)** Number of correct arm entries in each session of training. **(C)** Number of correct arm entries before error in each session of training. $n = 10$ per group.

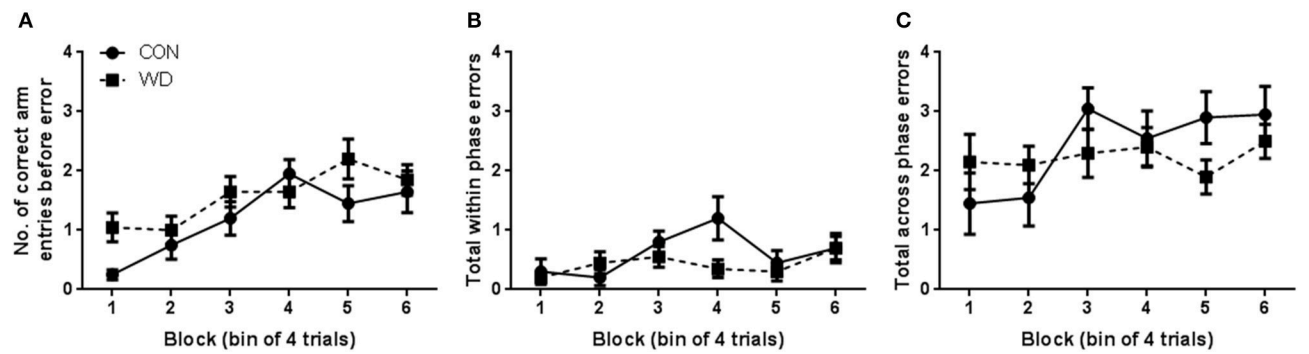

FIGURE 2 | Performance in test phase of DWSh task. (A) Number of correct arm choices before error in each session of training. **(B)** Total number of within phase errors in each session of training. **(C)** Number of across phase errors in each session of training. $n = 10$ per group.

total within phase errors [$F_{(5, 90)} = 2.79$, $p = 0.02$; **Figure 2B**] but there was no other significant differences in any other measure including group (**Figure 2C**).

HPLC Neurotransmitter Analysis

In the PFC, WD consumption did not alter neurotransmitter levels (DA CON 34.01 ± 14.66 pmol/mg vs. WD 30.17 ± 3.66 pmol/mg and DOPAC CON 25.97 ± 11.24 pmol/mg vs. WD 19.43 ± 3.55 pmol/mg, $p > 0.05$; **Figure 3A**). No group or group x neurotransmitter effect was observed (all $F < 1$). DA turnover was also found not to be different between diet groups (CON 0.75 ± 0.06 vs. WD 0.65 ± 0.11, $p > 0.05$; **Figure 3D**).

WD feeding was observed to change DA and DOPAC levels in the striatum relative to CON with a significant effect of group [$F_{(1, 16)} = 10.63$, $p = 0.0049$], differing amounts of neurotransmitter [$F_{(1, 16)} = 112.1$, $p < 0.0001$] which was attributable to a significantly higher amount of DA than DOPAC (DA CON 1651.59 ± 85.61 pmol/mg vs. WD 1370.88 ± 99.45 pmol/mg; DOPAC (CON 822.54 ± 52.02 pmol/mg vs. WD 615.23 ± 49.83 pmol/mg) but not group x neurotransmitter interaction ($F < 1$). *Post-hoc* analysis showed a marked reduction in DA levels in the striatum relative to CON ($p < 0.05$; **Figure 3B**), but not DOPAC levels ($p > 0.05$). The DA turnover rate in the striatum was not seen to be influenced by WD consumption (CON 0.50 ± 0.02 vs. WD 0.46 ± 0.05, $p > 0.05$; **Figure 3E**).

In the HPC no differences were observed in neurotransmitter levels [$F_{(1, 16)} = 4.3$, $p = 0.06$], nor group x neurotransmitter ($F<1$), while overall group effect was observed [$F_{(1, 16)} = 4.9$, $p = 0.04$]. However, with *post-hoc* analysis no individual differences were seen with DA (CON 13.22 ± 3.81 pmol/mg vs. WD 9.51 ± 1.34 pmol/mg, $p > 0.05$) or DOPAC (CON 9.88 ± 2.81 pmol/mg vs. WD 2.68 ± 0.27 pmol/mg, $p > 0.05$) levels compared to CON (**Figure 3C**). WD animals did have significantly reduced DA turnover relative to control (CON 0.78 ± 0.14 vs. WD 0.32 ± 0.07, $p < 0.05$; **Figure 3F**).

Experiment 2-Fos Immunohistochemistry

Home Cage Control Fos Counts

Table 1 shows the expression of basal Fos in the regions analyzed after 12 week WD consumption. In the PFC subregions a two way ANOVA showed there was no effect of diet on home cage control Fos expression in the Cg, IL, and PrL regions group [$F_{(1, 30)} = 0.95$, $p = 0.34$]. Analysis of home cage control Fos in the striatum revealed no significant effect of diet ($p = 0.59$). The initial analysis of the HPC involved separate counts taken across subfields (CA1, CA2/3, and DG). Overall, there was no observed

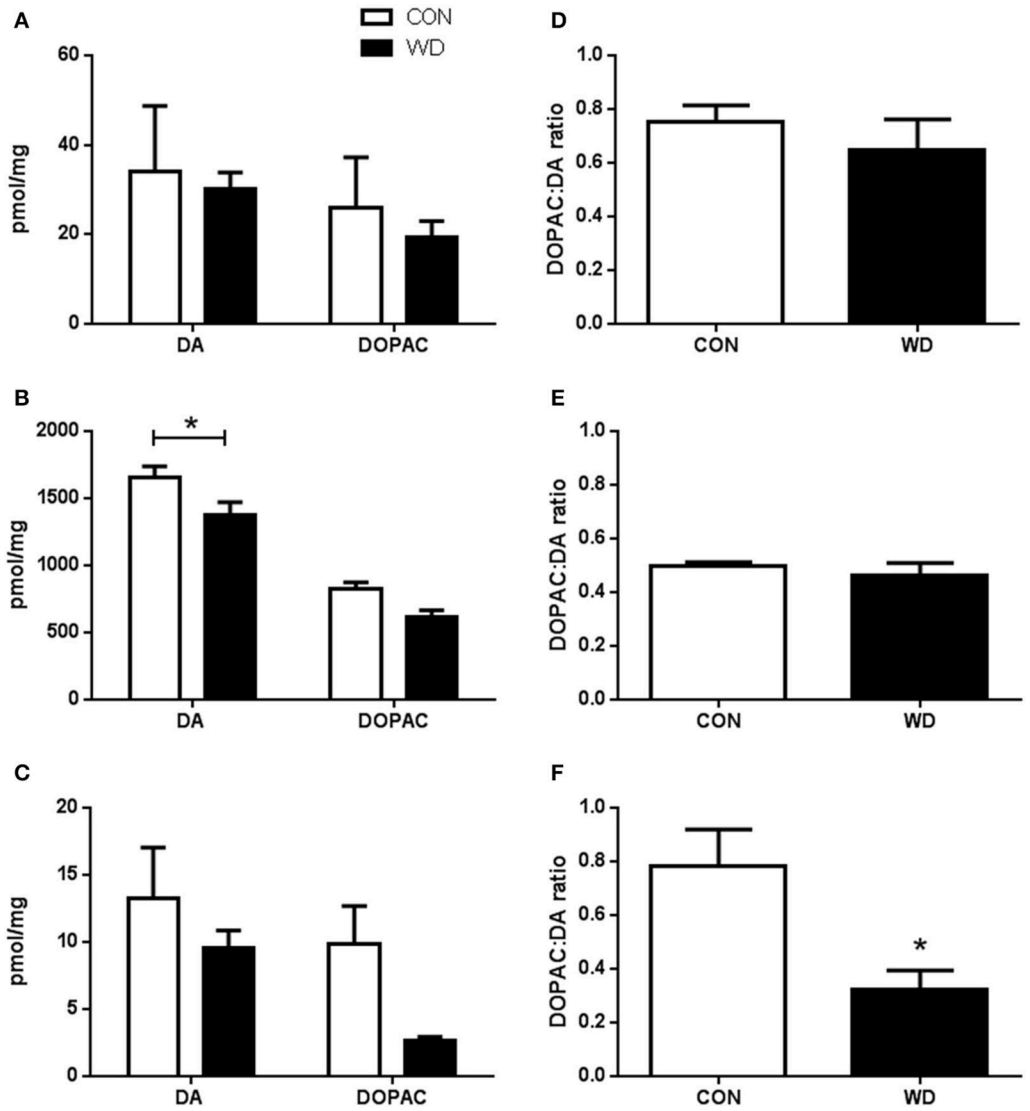

FIGURE 3 | HPLC analysis determination of DA, DOPAC, and DA turnover in rats fed a WD compared to CON in the (A) prefrontal cortex. **(B)** striatum and **(C)** hippocampus. **(D–F)** HPLC analysis of DOPAC to DA ratio in the prefrontal cortex, striatum and hippocampus, respectively. $n = 5$ per group. *Significantly different to CON $p < 0.05$.

difference in home cage control Fos expression (group effect: $[F_{(1, 30)} < 01, p = 0.99]$.

Activated Fos Counts

Expression of activated Fos following novel environment stimulation in our CON and WD was examined. In the PFC subregions there was no effect of diet on activated Fos expression in the Cg, IL, and PrL regions group $[F_{(1, 33)} = 2.36, p = 0.13$; **Figure 4A**]. Analysis of stimulated neuronal activation in the striatum demonstrated a significant increase in Fos expression in this region in the WD-fed rats compared to controls ($p< 0.05$; **Figure 4B**). In the HPC subfields (CA1, CA2/3, and DG), there was no observed difference in activated Fos expression [group effect: $F_{(1, 30)} = 3.67$, $p = 0.65$; **Figure 4C**].

DISCUSSION

In this study we investigated the effect of WD on cognitive performance using the DWSh task in the RAM in adult male Wistar hooded rats. Our findings indicate that WD consumption caused a significant increase in body weight and epididymal adipose weight compared to controls, but did not affect learning and performance in the DWSh task, a PFC, HPC, and striatal-dependent memory task. Concurrently, there was an observed decrease in DA levels in the striatum and a reduction of DA turnover in the HPC in WD fed rats. In a further series of animals WD consumption was observed to be associated with an increase in activated Fos expression in the striatum after exposure to a novel environment.

TABLE 1 | Number of positively stained Fos immunoreactive cells of home cage control fed CON or WD.

BRAIN REGION	CON ($n = 6$)	WD ($n = 6$)
PREFRONTAL CORTEX		
Cingulate gyrus (Cg)	12.04 ± 2.03	12.83 ± 0.82
Infralimbic cortex (IL)	17.92 ± 2.77	15.46 ± 2.11
Prelimbic cortex (PrL)	18.88 ± 4.59	16.63 ± 1.86
STRIATAL REGION		
Striatum	117.50 ± 17.84	102.50 ± 19.78
HIPPOCAMPUS		
Cornu Ammonis area 1 (CA1)	61.70 ± 12.86	57.79 ± 19.01
Cornu Ammonis area 2/3 (CA2/3)	209.95 ± 55.71	196.13 ± 48.25
Dentate gyrus (DG)	116.70 ± 22.26	134.25 ± 44.27

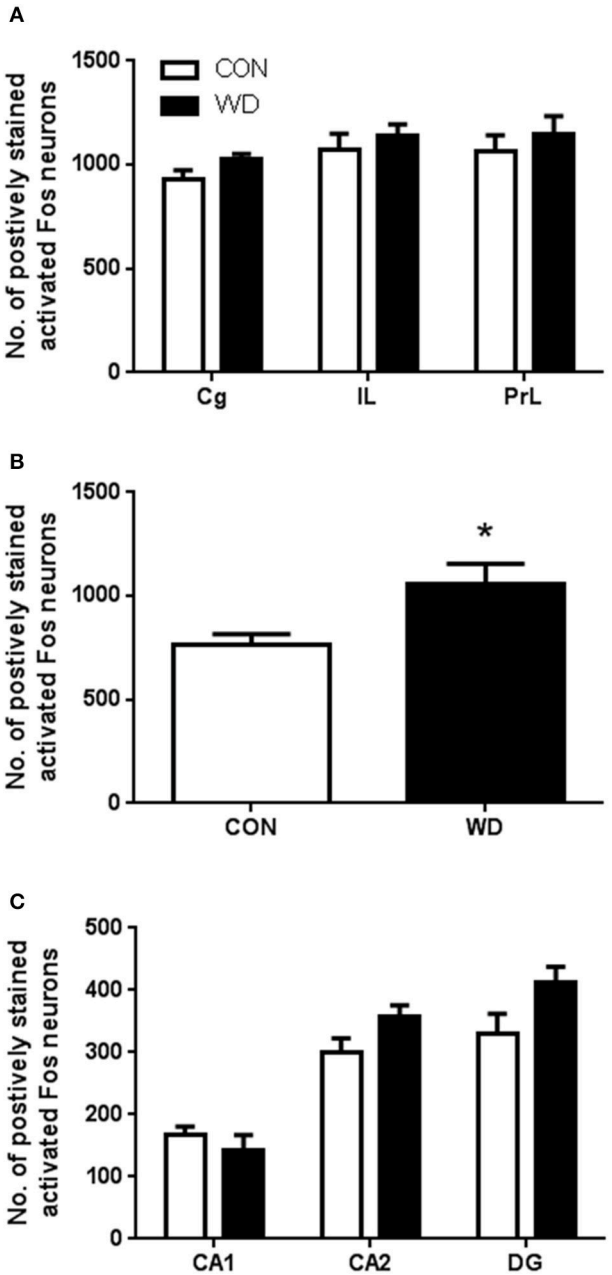

FIGURE 4 | Number of positively stained activated Fos neurons in the (A) prefrontal cortex, **(B)** striatum and **(C)** hippocampus. $n = 6$-7 per group. *Significantly different to CON $p < 0.05$.

Using the DWSh task, we failed to detect any impairment after WD consumption. Similar acquisition rates and spatial memory ability were observed in WD rats as the CON rats. This is in contrast to our previous study with this dietary manipulation where we showed impairment in spatial memory in the Y-maze, a one trial-one test procedure (Kosari et al., 2012). In the MWM, female Fisher 344 rats fed a diet with higher fat (approximately 39 kcal%) and similar sugar content to that in our study for 2 months displayed an impairment of spatial reference memory (Molteni et al., 2002). The radial arm water maze is known for combining the simplicity of results analysis from the RAM with the rapid and strong motivation observed in the MWM without the food deprivation (Alamed et al., 2006). Using the radial arm water maze, Alzhoubi et al. illustrated that male Wistar rats fed a similar WD for 3 months produced an impairment of short and long term spatial memory (Alzoubi et al., 2013). Researchers have also reported impairments of spatial memory following high fat consumption using other one trial-one test behavioral tasks. Arnold et al. reported spatial memory impairments using the T-maze spontaneous alternation task in C57BL/6J mice fed 45 kcal% fat diet for 8 weeks (Arnold et al., 2014), whilst a 60 kcal% fat diet for 27 weeks, produced an impairment of spatial reference memory in the object location task (Heyward et al., 2012). Of note between these studies, where a memory deficit was observed, and our present one, is a variation in food. Our DWSh paradigm prompts the animals to solve the task using food pellets as a reward, and for both WD and CON animals we used the same "control" grain pellets. A consequence of this is that WD animals, if they solved the task successfully, consumed approximately sixteen 45 mg pellets/day during habituation and testing that were not of WD composition. Moreover, throughout the task animals were on food restriction, albeit of their WD or CON food, meaning that for the final 15 days all rats received less of their specific diet than the previous 12 weeks of *ad lib* feeding. These two methodological points may have resulted in our WD animals becoming normalized, and thus attributable to the lack of deficit in this task.

We demonstrated that 12 week WD consumption increased activated Fos expression in the striatum in response to a novel environment. Other brain regions involved in memory and learning were also investigated with no comparable differences in activated Fos expression in the PFC or HPC between control and WD animals. C57BL/6J mice fed a 58 kcal% fat diet for 15 weeks display increased expression of basal Fos in the lateral hypothalamus (Lin and Huang, 1999; Xin et al., 2000), dorsal medial hypothalamus (Xin et al., 2000; Lin and Huang, 1999), and paraventricular nuclei (Wang et al., 1999). A study in female Long-Evans rats fed a 40 kcal% fat diet for 12 weeks also demonstrated an increase of activated Fos expression in

the paraventricular nuclei induced by introduction into a novel environment (Ressler et al., 2015). In a further study, acute high fat diet (21 kcal% fat for 2 h) consumption in C57BL/6J mice elicited an increase of activated Fos expression not only in the lateral hypothalamus but also the ventral tegmental area, nucleus accumbens, and central amygdala (Valdivia et al., 2014), suggesting that acute high fat consumption recruits the mesolimbic system. This finding is corroborated by Del Rio et al. who demonstrated that the dorsal medial PFC selectively showed increased activated Fos immunoreactivity in response to acute high fat diet consumption (Del Rio et al., 2015).

Whilst the stimulus used to induce activation of Fos expression was the introduction of the animal into a novel environment, our group has not observed any differences of exploration time in novel environments with this model (Kosari et al., 2012). We would suggest that this indicates that the increase of neuronal activation is due to the diet manipulation and not the stimulus, in this case new surroundings. However, it should be considered that there may also be an interaction between the experience of stress and the western diet. In the water maze RAM study (Alzoubi et al., 2013), the combination of stress and western diet resulted in the strongest impairment in the memory test, suggesting that stress may exacerbate the effect of diet.

The presented results also demonstrate that WD consumption for 12 weeks causes a dysregulation of the dopaminergic system in the striatum and HPC, as reflected by the decrease of DA levels in the striatum by 20% and DA turnover in the HPC by 40% in WD rats compared to controls. Our findings parallel data observed by Ma et al. who also showed a decrease of DA levels and no change in DA turnover in the striatum of rats fed a 60 kcal% HFD for 13 weeks (Ma et al., 2015). Results by Baladi et al. using chronoamperometry also indicated a decrease in DA turnover in the striatum though interestingly this was independent to any observed changes to body weight in high fat diet fed rats (Baladi et al., 2015).

Multiple studies have also shown that obese animals have a decrease of dopaminergic levels in other limbic areas of the brain and/or decreased DA receptor expression. A consistent finding is the reduction of DA levels in the nucleus accumbens in animal models of obesity observed in *ob/ob* mice (Fulton et al., 2017), diet-induced obesity rats (Pothos et al., 1998; Geiger et al., 2009), cafeteria diet model (Geiger et al., 2008) and high fat diet exposed mice (Carlin et al., 2013). Mice fed a 60 kcal% fat diet for 12 weeks also had a decrease of DA levels and increased DA turnover in the PFC (Carlin et al., 2013). High fat diet exposure, even as short as 5 days, has been shown to reduce basal DA levels in the nucleus accumbens (Rada et al., 2010). The reduction in DA levels at least in the nucleus accumbens is suggested to be due to reduced stimulated DA release and vesicle size (Pothos et al., 1998; Geiger et al., 2008). Additionally, DA reuptake has been observed to decrease independent of DA transporter protein gene expression in rats fed a high fat diet, thought to be due to interference in DA transporter trafficking or maturation (Petrovich et al., 2007).

Striatal DRD2 receptor expression has been previously shown to be decreased after consumption of a HFD in both mice and rat models of diet-induced obesity (Huang et al., 2006; Johnson and Kenny, 2010; van de Giessen et al., 2012). One study also found a clear inverse association between body weight and striatal DRD2 receptor expression suggested to be due to a down regulation of postsynaptic striatal DRD2/3 receptors (Huang et al., 2006; Johnson and Kenny, 2010; van de Giessen et al., 2012). Thus, it could be hypothesized that WD consumption alters DRD2 receptor expression which can lead to the neuroadaptive response to decrease DA levels in the striatum and DA turnover in the HPC. It is known that DRD2 receptor plays an inhibitory role in dopaminergic transmission in the mesolimbic dopaminergic system (Nestler, 1994). Previous observations have shown an inverse correlation between adiposity and striatal D2 binding in HFD fed mice (Huang et al., 2005), rats (Johnson and Kenny, 2010) and obese humans (Davis and Fox, 2008).

The mechanisms of how a WD, or in a broader context high fat diets, can produce memory impairments is still under much scrutiny. Our lab has previously considered the possibility that the cholinergic system is associated with spatial deficits caused by WD consumption (Kosari et al., 2012). Nonetheless using immunohistochemistry we reported no change in acetylcholinesterase activity, the enzyme responsible for the metabolism of acetylcholine, in the HPC and striatum after WD consumption (Marco et al., 2013). WD consumption was not observed to affect spatial working and reference memory using the DWSh task in the RAM. The present study is the first to demonstrate that WD consumption increases Fos expression in the striatum following a novel environment stimulus. We also show that WD consumption induces a reduction in DA levels in the striatum and DA turnover in the HPC. This suggests that WD consumption induces central changes in the striatum and HPC through neuronal activation which could be mediated through DA activity. However, these changes are independent to impairments in spatial working or reference memory in WD fed rats.

In conclusion our present results expand on the known relationship between obesity and central effects. We have shown that a major neurochemical component of cognition and reward, dopamine, is reduced after WD consumption in our rats, an effect that has been largely observed in rodents following a more palatable food intake, suggesting that the type of diet, that is sugary and appealing, is not specific to producing neurochemical changes in higher order brain regions. We have also extended the previous neuronal activation data largely focused around the hypothalamus to show that WD-manipulation in the rat produces specifically an upregulation of striatal neuronal activation. As this data was collected from a novel environment paradigm of much interest would be to expand this to assess neuronal activation during memory tests where a cognitive deficit is observed. Whether WD manipulation, or indeed fat manipulation, is the ideal model to assess obesity-associated cognitive decline is still contentious; indeed our Fos data, and the reduction in striatal dopamine content, is independent of a deficit in memory using the radial arm maze task. While we have previously shown cognitive deficits along with metabolic changes with this model (Kosari et al., 2012) it is not universal; indeed no deficit was observed in working memory in the novel object recognition task (Kosari et al., 2012), as no deficiency was observed here. It is clear that the

biological contribution to obesity in humans involves numerous factors beyond fat, including the more palatable sugar, inactivity, along with broader factors, such as genes and mood, and these are yet to be considered or produced in a single animal model.

AUTHOR CONTRIBUTIONS

JN with TJ, SK, SA, and OW were involved in the conception and design of the studies. Data collection was performed by JN, SA, and SK; Data analysis and interpretation by JN, SS, and TJ. The article was drafted by JN, ASK, and TJ with critical revision and final approval of the version to be published from all authors.

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frontiers in Behavioral Neuroscience

MINI REVIEW
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Gut to Brain Dysbiosis: Mechanisms Linking Western Diet Consumption, the Microbiome, and Cognitive Impairment

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Consumption of a Western Diet (WD) that is high in saturated fat and added sugars negatively impacts cognitive function, particularly mnemonic processes that rely on the integrity of the hippocampus. Emerging evidence suggests that the gut microbiome influences cognitive function via the gut-brain axis, and that WD factors significantly alter the proportions of commensal bacteria in the gastrointestinal tract. Here we review mechanisms through which consuming a WD negatively impacts neurocognitive function, with a particular focus on recent evidence linking the gut microbiome with dietary- and metabolic-associated hippocampal impairment. We highlight evidence linking gut bacteria to altered intestinal permeability and blood brain barrier integrity, thus making the brain more vulnerable to the influx of deleterious substances from the circulation. WD consumption also increases production of endotoxin by commensal bacteria, which may promote neuroinflammation and cognitive dysfunction. Recent findings also show that diet-induced alterations in gut microbiota impair peripheral insulin sensitivity, which is associated with hippocampal neuronal derrangements and associated mnemonic deficits. In some cases treatment with specific probiotics or prebiotics can prevent or reverse some of the deleterious impact of WD consumption on neuropsychological outcomes, indicating that targeting the microbiome may be a successful strategy for combating dietary- and metabolic-associated cognitive impairment.

Keywords: neuroinflammation, insulin, gut bacteria, sugar, fat, endotoxin, hippocampus

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INTRODUCTION

Substantial evidence has linked consumption of a Western Diet (WD), defined here as diets consisting of both high levels of fat (35–60% total kcal) and added sugars, with cognitive dysfunction (Molteni et al., 2002, 2004; Kanoski et al., 2007, 2010; Kanoski and Davidson, 2011; Davidson et al., 2012; Francis and Stevenson, 2013; Baym et al., 2014; Beilharz et al., 2014, 2016a,b; Noble et al., 2014; Hsu et al., 2015; Khan et al., 2015b; Noble and Kanoski, 2016). The hippocampus, a brain region associated with the control of certain learning and memory processes, is particularly vulnerable to the deleterious effects of WD intake (Kanoski and Davidson, 2011; Baym et al., 2014; Davidson et al., 2014). The mechanisms through which WD consumption

impacts the brain are not completely understood, however emerging research has implicated the gut-brain axis as playing a critical role. The gut microbiome (the collective genome of microbes residing in the gastrointestinal tract) has a substantial impact on brain function (Bercik et al., 2011; Davari et al., 2013; Hsiao et al., 2013; Bruce-Keller et al., 2015). Moreover, the gut microbiome is profoundly affected by dietary factors (de La Serre et al., 2010; David et al., 2014; Noble et al., 2017). In this review we raise the hypothesis that the microbiome is a critical link between WD consumption and neurocognitive dysfunction. Putative mechanisms connecting WD consumption, microbiota alterations, and cognitive impairment include barrier integrity (gastrointestinal tract, neurovascular), neuroinflammation, and impaired insulin signaling. Though each topic is considered individually, it is likely that these and other biological outcomes associated with WD consumption work in concert to impact brain function.

WESTERN DIET, HIPPOCAMPAL FUNCTION, AND GUT MICROBIOTA

Extensive evidence from rodent (reviewed in Kanoski and Davidson, 2011) and human studies (Kalmijn et al., 2004; Francis and Stevenson, 2011; Baym et al., 2014) reveals that consumption of a WD is linked with impaired hippocampal-dependent learning and memory function. Due to the obesity-promoting nature of WD, it is difficult to discern the relative contribution of dietary factors and obesity on cognitive outcomes. However, while obesity *per-se* is associated with reduced hippocampal volume (Jagust et al., 2005) and impaired hippocampal function (Li et al., 2002; Winocur et al., 2005; Khan et al., 2015a), evidence shows that a WD negatively impacts hippocampal function independent of obesity. For example, hippocampal-dependent spatial memory impairments have been reported after only 3 (Kanoski and Davidson, 2010) or 9 (Murray et al., 2009) days of consuming a WD, despite similar body weights between animals compared to standard chow-fed controls. Similar to WDs, high fructose diets can also impair hippocampal-dependent learning and memory in rodents independent of obesity (Hsu et al., 2015; Agrawal et al., 2016; Meng et al., 2016; Noble and Kanoski, 2016). Together these data suggest that dietary factors common in a WD have the capacity to impart cognitive dysfunction after only a brief exposure, and independent of severe metabolic impairments.

While much progress has been made in elucidating the neurobiological mechanisms underlying WD-associated cognitive impairment (reviewed in Kanoski and Davidson, 2011; Beilharz et al., 2015), few reports consider the gut microbiome, which consists of an estimated 100 trillion microorganisms that reside in the host GI tract (Bäckhed et al., 2005). The gut microbiome has emerged as a major contributor to cognitive health (Gareau et al., 2011; Bajaj et al., 2012; Bruce-Keller et al., 2015; Desbonnet et al., 2015; Fröhlich et al., 2016) and is affected by dietary factors (Daniel et al., 2014; David et al., 2014; Bruce-Keller et al., 2015; Magnusson et al., 2015; Noble et al., 2017). For example, consumption of a WD reduces populations in the phylum *Bacteroidetes* and increases *Firmicutes* and *Proteobacteria* (Hildebrandt et al., 2009; Zhang et al., 2012) in adult rodents. Importantly, these shifts have been associated with cognitive impairments. Magnusson et al., observed that both high fat (45% kcal from fat) and high sugar (70% kcal from carbohydrate) diets elevated levels of *Clostridiales* (Phylum: *Firmicutes*) and reduced levels of *Bacteroidales* (Phylum: *Bacteroidetes*) in rodents, changes that correlated to poor cognitive flexibility (Magnusson et al., 2015). Recent evidence supports a functional link between the gut microbiome and WD-induced cognitive dysfunction. Bruce-Keller and colleagues revealed that fecal/cecal transplantation from adult mice fed a WD to antibiotic pre-treated mice fed a control diet increased anxiety and stereotypic activity and impaired contextual fear conditioning (Bruce-Keller et al., 2015). While the bacterial species or combination of species responsible for the behavioral effects was not identifiable due to the whole microbiome transfer approach, the bacteria *Akkermansia muciniphila* were substantially (5.4-fold) reduced by the WD, whereas *Bilophila* sp. were elevated in the WD group and barely detectable in the control group (Bruce-Keller et al., 2015). Notably, *A. muciniphila* promotes insulin sensitivity and reduces metabolic endotoxemia in mice (Shin et al., 2014) and is negatively associated with metabolic disease, intestinal inflammatory diseases, and autism in humans (reviewed in Derrien et al., 2016). Conversely, *Bilophila* are positively associated with inflammatory intestinal diseases (Devkota et al., 2012; Jia et al., 2012) and may contribute to neurocognitive abnormalities by promoting inflammation, though this has not been directly tested.

Recent studies examined the individual contributions of particular macronutrients from a WD on the gut microbiome. For example, a high-fat, carbohydrate-free diet (72% kcal from fat) reduces *Bifidobacteria* (Cani et al., 2007, 2008), which have been shown modulate intestinal barrier function and reduce endotoxin levels in the gut (Griffiths et al., 2004; Wang et al., 2006). Conversely, Jena and colleagues found that a low-fat chow diet supplemented with a 65% w/v fructose solution had no effect on levels of *Bifidobacteria* (Jena et al., 2014). However, using more moderate concentrations that model commonly consumed sugar sweetened beverages, recent data from our group reveal that *Bifidobacteria* were elevated following free access to the sugar solutions (and chow and water) relative to controls that were not given sugar (Noble et al., 2017), suggesting that sugar-induced gut microbiome alterations are dependent on the carbohydrate concentration. Data from our recent study further show that consuming the sugar solutions altered gut bacteria at every phylogenetic level, including significant group effects in ∼25% of gut bacteria at the family level. Similar to animals on very high doses of fructose solution (Jena et al., 2014), our data revealed that sugar consumption elevated *Enterobacteriaceae,* which are associated with gut (Lupp et al., 2007) and brain inflammation, and poor cognition in hepatic encephalopathy (Bajaj et al., 2012; Ahluwalia et al., 2016). Surprisingly, rodents consuming 11% concentrations of sugar solutions have elevated levels of *Lactobacilli* (Noble et al., 2017), which are anti-inflammatory, whereas rodents

consuming much higher concentrations of sugar solutions (Jena et al., 2014) or dietary fat (Lecomte et al., 2015) have reduced levels of *Lactobacilli*. Notably, *Lactobacilli* facilitate short chain fatty acid transport (Kumar et al., 2015). Short chain fatty acids (SCFAs) alter human health and their reduced absorption may be one of the mechanisms by which diet impacts cognitive health via the gut microbiome, a concept reviewed below.

SHORT CHAIN FATTY ACIDS

SCFAs such as acetate, propionate, and butyrate are produced in the gut by microbial-mediated fermentation of indigestible carbohydrates, such as resistant starch and non-starch polysaccharides from cereals, vegetables, and fruits (MacFarlane and MacFarlane, 2011). The production of SCFAs is significantly reduced in humans within days following a dietary change from a complex carbohydrate rich plant-based diet to an animal based diet high in saturated fat and low in complex carbohydrates (David et al., 2014). Similarly, rodents fed a WD had reduced levels of SCFAs, including acetic, propionic, isobutyric, and isovaleric acids (Ojo et al., 2016) as well as butyric and valeric acids (Berger et al., 2014), compared with controls fed a low-fat chow diet. When taken in context with previously discussed studies demonstrating that short-term consumption of a WD significantly impacts cognitive function (Kanoski and Davidson, 2010; Beilharz et al., 2014, 2016a,b), the rapid alteration of SCFAs as a putative contributor to WD-induced cognitive dysfunction is temporally feasible.

While the majority of the SCFAs in portal circulation are metabolized by the liver, SCFAs produced in the distal colon bypass portal circulation and reach the brain through circulation (reviewed in MacFabe, 2012). In the brain, SCFAs have a neuroprotective effects (Sun et al., 2015), for example, the salt of the SCFA butyric acid, sodium butyrate, promotes cell proliferation and differentiation in the dentate gyrus, increases the expression of brain-derived neurotrophic factor (BDNF) and glia-derived neurotrophic factor (GDNF), and improves memory performance in the novel object recognition task (Wu et al., 2008; Stefanko et al., 2009; Intlekofer et al., 2013; Yoo et al., 2015). These neuroprotective effects of sodium butyrate potentially occur through the inhibition of histone deacetylase (HDAC), which is known to prevent the transcription of BDNF and GDNF (Wu et al., 2008). Butyrate also has anti-inflammatory actions in the gut and brain by preventing the induction of the inflammatory cytokine TNFα by the endotoxin lipopolysaccharide (LPS) via the suppression of nuclear factor κB (Segain et al., 2000). Taken together, SCFAs produced by gut bacteria (whose levels are reduced by WD consumption; Berger et al., 2014) may affect brain health directly via HDAC inhibition in the brain, or indirectly by reducing systemic inflammation in the gut. Butyrate has also been shown to stabilize hypoxia-inducible factor (HIF; Kelly et al., 2015), which is critical for maintaining gut barrier integrity and protecting against the influx of potentially harmful toxins, a topic discussed in more depth below.

GUT AND NEUROVASCULAR BARRIER INTEGRITY

Emerging research is revealing that gut microbiota have potent effects on gut permeability (Cani et al., 2007, 2008, 2009; Lam et al., 2012; Pendyala et al., 2012; Tulstrup et al., 2015; Maffeis et al., 2016; Mokkala et al., 2016; Müller et al., 2016) and blood-brain barrier integrity (Braniste et al., 2014), both of which are negatively impacted by WD intake and proposed mechanisms underlying WD induced cognitive impairments (Kanoski et al., 2010; Davidson et al., 2012; Hsu and Kanoski, 2014; Ouyang et al., 2014; Hargrave et al., 2016; Stranahan et al., 2016). Several studies discussed here support a causal relationship between WD-mediated gut microbiota alterations, the gut/neurovascular barrier integrity, and hippocampal function.

The gut barrier consists of a specialized, semi-permeable mucosal, and epithelial cell layers that are reinforced by tight junction proteins. Among other functions, this barrier serves to regulate nutrient and water entry and prevents the entry of harmful compounds into extra-luminal tissues (for review see Turner, 2009). WD consumption impairs gut permeability, which in turn allows for the influx of adverse substances and may ultimately contribute to the development of metabolic disorders, and cognitive dysfunction. For example, in humans there is a strong association between obesity, gut permeability, and systemic inflammation (Maffeis et al., 2016; Rainone et al., 2016). In rodents, WD intake decreases levels of the tight junction protein ZO-1 and transepithelial resistance in the proximal colon, both markers of gut barrier dysfunction (Lam et al., 2012). A compromised gut barrier makes the intestinal tract potentially vulnerable to the gram-negative bacteria-derived LPS, which upon excess entry into circulation promotes endotoxemia and systemic inflammation (Griffiths et al., 2004; Cani et al., 2007, 2008; Tsukumo et al., 2007). Indeed, mice maintained on a WD for 4 weeks exhibit a ∼three-fold increase in circulating LPS levels with concurrent increased intestinal permeability, as reflected by reduced mRNA expression of tight junction proteins ZO-1 and occludin, as well as elevated plasma levels of a gavaged fluorescent molecule (FITC-dextran) that is typically unable to cross the gut barrier (Cani et al., 2008). This study further demonstrated that antibiotic treatment attenuated obesity-induced endotoxemia, thus providing potential physiological links between WD, the gut microbiome, and gut barrier integrity (Cani et al., 2008).

The blood-brain barrier (BBB) consists of a structural complex of endothelial cells, pericytes, and glial cells that encompass microvasculature networks within the central nervous system. It serves as a critical regulator for the entry of blood-derived nutrients and compounds required for healthy brain function, while simultaneously precluding the entry of potentially harmful blood-derived toxins. Importantly, WD intake is associated with BBB damage, which may be causally related to WD-induced cognitive dysfunction (Kanoski et al., 2010; Freeman et al., 2011; Davidson et al., 2012; Freeman and Granholm, 2012; Pallebage-Gamarallage et al., 2012; Hargrave et al., 2016; Stranahan et al., 2016). For example, in a study from Kanoski et al. (2010), rats maintained on a WD for 90 days exhibited a

leaky BBB in the hippocampus and reduced mRNA expression of the tight junction proteins claudin-5 and claudin-12. These negative BBB outcomes were accompanied by impairments in hippocampal-dependent memory tasks, suggesting that WD-induced BBB damage may be causally related to cognitive deficits. Davidson and colleagues extended this work (Davidson et al., 2012) by showing that rats prone to obesity are more susceptible to WD-induced BBB damage and cognitive impairment compared to obesity resistant animals. Moreover, the magnitude of BBB damage and memory impairment depends on both obesity susceptibility and the duration of WD exposure (Hargrave et al., 2016). Collectively, these data provide strong evidence linking WD intake, BBB integrity, and hippocampal dysfunction.

Braniste et al. (2014) illuminate an association between gut microbiome perturbation and impaired BBB integrity. Infrared-labeled immunoglobulin antibody (IgG2b; normally precluded from brain parenchyma) injected into pregnant germ-free (microbiome-free) mouse dams was abundant in the brains of their mouse embryos compared to the embryos of pathogen-free (microbiome-intact) dams, suggesting that maternal gut microbiome has strong influences on the offspring's BBB integrity. Moreover, compared to adult pathogen-free mice, adult germ-free mice exhibited impaired BBB integrity evidenced by increased brain uptake of tail-vein injected radio labeled ligand [11C] raclopride and increased presence of Evans blue dye (normally precluded from BBB penetration) in brain parenchyma following circulatory injections. Interestingly, transfer of pathogen-free fecal matter to germ-free mice attenuated BBB damage, reflected by the increased expression of tight junction proteins. These data implicate an important role for the gut microbiome in regulating BBB integrity. Whether gut microbiome perturbations associated with WD consumption are causally related to WD-associated barrier dysfunction and memory impairments require further investigation.

NEUROINFLAMMATION

Rodent studies have consistently shown that chronic consumption of a WD elevates levels of neuroinflammatory markers, which are associated with impaired cognition (Pistell et al., 2010; Puig et al., 2012; Herculano et al., 2013; Camer et al., 2015; Hsu et al., 2015; Ledreux et al., 2016). In conjunction with cognitive impairments, rats fed a WD have increased neuroinflammation in both the hippocampus (Puig et al., 2012; Herculano et al., 2013; Hsu et al., 2015; Ledreux et al., 2016) and in the cortex (Pistell et al., 2010; Camer et al., 2015). Moreover, clinical reports implicate a positive association between circulating inflammatory factors and cognitive decline in humans (Sweat et al., 2008; Sellbom and Gunstad, 2012). A WD may impact neuroinflammation and cognitive outcomes in part via altering levels of gut bacteria, as certain gut bacteria stimulate the innate immune system to elevate inflammatory cytokines in the brain (for review see Sankowski et al., 2015).

One putative mechanism through which WD influences gut bacteria and imparts hippocampal dysfunction involves elevated levels of endotoxin and accompanying inflammatory cytokines. WD consumption elevates levels of inflammatory endotoxins such as LPS (Cani et al., 2007; Amar et al., 2008; Bruce-Keller et al., 2015), and elevated levels of microbiome-derived LPS in circulation stimulate inflammatory pathways. Additionally, gut microbiota directly stimulate the production of the proinflammatory cytokines IL-1β and TNFα (Heumann et al., 1994), which have been shown to impair hippocampal-dependent memories in rodents (Rachal Pugh et al., 2001; Goshen et al., 2007; Hein et al., 2010).

In addition to elevating levels of endotoxin producing bacteria, a WD may affect neuroinflammation by reducing levels of anti-inflammatory commensal gut bacteria. Bruce-Keller et al. (2015) demonstrated that WD fecal/cecal transplant recipient mice were normal weight, yet had elevated levels of endotoxin and neuroinflammatory markers accompanied by impaired cognitive function. The bacterial species *A. muciniphila* was reduced by the diet (Bruce-Keller et al., 2015); a species that is negatively associated with inflammation (Schneeberger et al., 2015). Similarly, anti-inflammatory *Lactobacilli* are reduced by WD factors (Jena et al., 2014; Lecomte et al., 2015) and supplementation with *Lactobacillus helveticus* prevents spatial memory impairment in WD-fed mice lacking the anti-inflammatory cytokine IL-10 (Ohland et al., 2013). One method by which members of the *Lactobacilli* family and other commensal bacteria may reduce systemic inflammation is by improving insulin sensitivity (Simon et al., 2015), a concept reviewed in the following section.

INSULIN

Insulin, produced in pancreatic beta cells and released in response to conditioned cephalic cues or circulating metabolites, crosses the BBB via a saturable transporter, and insulin receptors are present in neurons and primarily localized to synapses (Zhao and Alkon, 2001). Levels of insulin receptor are particularly concentrated in the hippocampus (Havrankova et al., 1978) where insulin signaling improves cognitive function and neuronal plasticity (Biessels et al., 1998; Kamal et al., 2000; Grillo et al., 2009, 2015; Biessels and Reagan, 2015). WD-induced peripheral insulin resistance is associated with impaired cognitive function and synaptic plasticity in rats (Elias et al., 1997; Grodstein et al., 2001; Hiltunen et al., 2001; Yaffe et al., 2004; Stranahan et al., 2008; Pavlik et al., 2013; Gao et al., 2015) and the risk for developing Alzheimer's disease or dementia in humans (Ott et al., 1999; Arvanitakis et al., 2004; Luchsinger et al., 2004; Cukierman et al., 2005; Rönnemaa et al., 2008).

Insulin impacts neurological health via multiple mechanisms. One of the functions of CNS insulin is to phosphorylate α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) receptors, which leads to increased hippocampal long-term potentiation (LTP; Adzovic and Domenici, 2014). Another mechanism through which insulin may improve cognitive function is by reducing neuroinflammation. For example, intracerebroventricular injection of insulin attenuates LPS-induced elevations in IL-1β and improves spatial memory impairment in young rats (Adzovic et al., 2015). Insulin has a

similar anti-inflammatory function in the periphery, where it has been shown to reduce the systemic inflammatory response to endotoxin (Jeschke et al., 2004). Thus, if gut microbiota impact cognitive function in part via the modulation of inflammatory responses, or by elevating levels of peripheral or central endotoxin, then insulin may provide protection against gut microbiome-mediated cognitive dysfunction in insulin sensitive individuals. Given that long-term exposure to *ad libitum* fructose (15% w/v in water) impairs insulin receptor function in the hippocampus and reduced hippocampus-dependent spatial memory (Agrawal et al., 2016), a harmful synergy may occur through which WD intake both increases neuroinflammation (as reviewed above) and impairs central insulin sensitivity, thereby preventing insulin from attenuating inflammatory responses and associated adverse neuronal outcomes.

Recent findings directly link gut microbiota and CNS/peripheral insulin sensitivity. In humans, an intra-duodenal microbiome transfer from lean healthy donors to individuals with impaired insulin sensitivity improves insulin sensitivity in the recipients (Vrieze et al., 2012). Interestingly, transferring the fecal microbiome from obese or lean discordant human twin pairs to mice resulted in impaired glucose metabolism in the mouse if the transfer came from an obese twin (Ridaura et al., 2013). In mice, antiobiotic-induced microbiome depletion improves peripheral insulin sensitivity caused by a WD (Suárez-Zamorano et al., 2015). The effect of gut microbiota on peripheral insulin sensitivity begins at the level of the intestinal mucosa, as WD-induced insulin resistance is prevented by blocking live intestinal bacteria from translocating into the blood and tissues where they generate an inflammatory response (Amar et al., 2011). Bacterial translocation preceded WD-induced insulin resistance, and required functioning Nod1 and CD14 receptors, which bind to gram-negative bacteria. Furthermore, the translocation of bacteria and the insulin resistance were preventable when animals were treated with the probiotic *Bifidobacterium Animalis*, which specifically prevents translocation of *Enterobacteriaceae* (Amar et al., 2011). Taken together, commensal bacteria may alter peripheral insulin sensitivity in a mechanism that likely involves inflammatory signaling and/or bacterial translocation from the gut into the periphery.

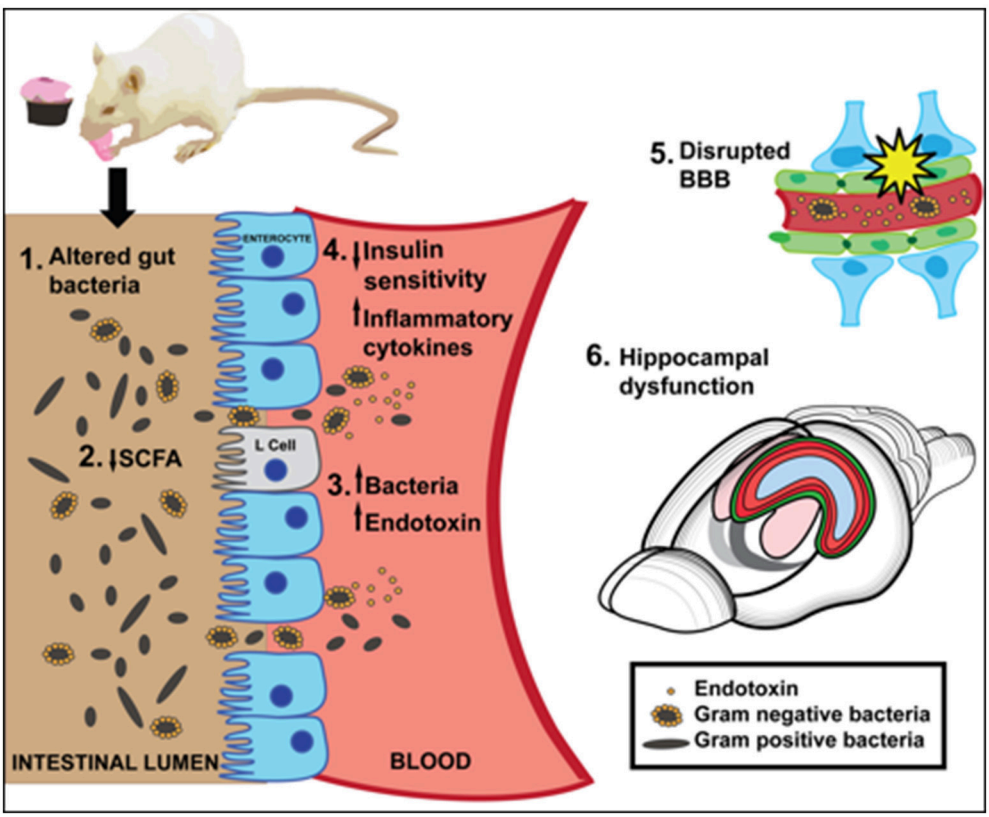

FIGURE 1 | A summary of putative mechanisms linking Western Diet (WD) consumption, the gut microbiome, and cognitive dysfunction. [1] A high fat/high sugar WD diet alters gut bacteria [2] WD reduces short chain fatty acids (SCFA), which may impair neuroprotection or anti-inflammatory effects in the gut. SCFAs affect insulin signaling by stimulating L cell production of GLP-1. [3] WD may impair intestinal barrier and promote translocation of endotoxin-producing gram negative bacteria into the blood. [4] Inflammatory cytokines and/or reduced insulin sensitivity caused by WD-induced gut bacteria may negatively affect hippocampal function and memory. [5] A WD impairs BBB integrity, which may be caused in part by altered gut microbiota. [6] WD consumption significantly impairs hippocampal dependent learning and memory.

Whether consuming a WD promotes cognitive dysfunction through modifying gut microbiota that impair insulin receptor signaling remains to be determined. However, one study revealed that probiotic treatment normalized spatial memory deficits and improved hippocampal LTP in a streptozocin rat model of diabetes (Davari et al., 2013), suggesting that gut microbiota can improve cognitive dysfunction due to reduced insulin production. Interestingly, evidence suggests that commensal gut bacteria may enhance insulin sensitivity and cognitive function by modulating the production and/or secretion of the incretin hormones glucagon-like peptide—1 (GLP-1). SCFAs, such as butyrate, produced by commensal gut bacteria act on G protein coupled receptors to stimulate GLP-1 secretion (Tolhurst et al., 2012). Hwang and collegues showed that antibiotics reduced the proportion of *Bacteriodetes* and *Firmicutes* in mice, which resulted in attenuated pancreatic islet hypertrophy and improved insulin and glucose tolerance through a GLP-1 signaling pathway (Hwang et al., 2015). Importantly, GLP-1 signaling promotes hippocampal neural plasticity and improved memory function (During et al., 2003; McClean et al., 2010; Li et al., 2012). Taken together, these collective data suggest that commensal gut bacteria modulate insulin sensitivity via multiple mechanisms, which may be related to WD-induced hippocampal dysfunction.

CONCLUDING REMARKS

Several neurobiological mechanisms link WD consumption with gut microbiome alterations that potentially contribute to WD-mediated cognitive dysfunction, including reduced SCFA production, compromised barrier integrity, neuroinflammation, and peripheral and/or central insulin receptor resistance (**Figure 1**). Consuming a WD promotes endotoxemia, which is linked with memory impairment, either via translocation of gram-negative bacteria into circulation, and/or by impairing the permeability of the gut barrier. Both gut microbiota and WD intake have been shown to impair the permeability of the BBB, however mechanistic studies linking WD intake, BBB integrity, and the gut microbiome are required. WD-associated microbiota alterations impair peripheral insulin sensitivity, which is strongly linked with central insulin resistance and hippocampal dysfunction. In addition, insulin protects against peripheral inflammatory responses to endotoxin, and may prevent the deleterious effects imparted by WD-mediated bacterial production of endotoxins in insulin sensitive individuals.

Overall we present multiple pathways through which WD-induced microbiome alterations can impact neurocognitive function. Mechanistic studies examining these putative gut brain axis pathways may facilitate the development of therapies that target the microbiome (probiotics, prebiotics, antibiotics, or microbiota transfer) to treat neurobiological and cognitive dysfunction associated with WD intake and associated metabolic disorders.

AUTHOR CONTRIBUTIONS

All three authors contributed to the idea for the manuscript. EN and TH wrote and edited the manuscript. SK edited the manuscript, provided vital input to shape the manuscript, and contributed to the writing. All three authors approved the final manuscript.

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Adolescent Maturational Transitions in the Prefrontal Cortex and Dopamine Signaling as a Risk Factor for the Development of Obesity and High Fat/High Sugar Diet Induced Cognitive Deficits

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Adolescence poses as both a transitional period in neurodevelopment and lifestyle practices. In particular, the developmental trajectory of the prefrontal cortex (PFC), a critical region for behavioral control and self-regulation, is enduring, not reaching functional maturity until the early 20 s in humans. Furthermore, the neurotransmitter dopamine is particularly abundant during adolescence, tuning the brain to rapidly learn about rewards and regulating aspects of neuroplasticity. Thus, adolescence is proposed to represent a period of vulnerability towards reward-driven behaviors such as the consumption of palatable high fat and high sugar diets. This is reflected in the increasing prevalence of obesity in children and adolescents as they are the greatest consumers of "junk foods". Excessive consumption of diets laden in saturated fat and refined sugars not only leads to weight gain and the development of obesity, but experimental studies with rodents indicate they evoke cognitive deficits in learning and memory process by disrupting neuroplasticity and altering reward processing neurocircuitry. Consumption of these high fat and high sugar diets have been reported to have a particularly pronounced impact on cognition when consumed during adolescence, demonstrating a susceptibility of the adolescent brain to enduring cognitive deficits. The adolescent brain, with heightened reward sensitivity and diminished behavioral control compared to the mature adult brain, appears to be a risk for aberrant eating behaviors that may underpin the development of obesity. This review explores the neurodevelopmental changes in the PFC and mesocortical dopamine signaling that occur during adolescence, and how these potentially underpin the overconsumption of palatable food and development of obesogenic diet-induced cognitive deficits.

Keywords: dopamine, adolescence, obesity, high fat diet, sucrose, prefrontal cortex, hippocampus, striatum

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INTRODUCTION

Adolescence, Fast Food and Adverse Psychological Conditions

Adolescence represents a transitional developmental period between childhood and adulthood in the human (and animal) lifespan. In humans, the World Health Organisation (WHO) identifies adolescence as the period in human growth and development that occurs after childhood and before adulthood, approximately between ages 10–19 (WHO), although it is typically defined as the period from puberty to legal adulthood, encompassing the teenage years (13–19). Longitudinal neuroimaging studies have demonstrated that the brain, in particular the prefrontal cortex (PFC), continues to mature until ∼24 years old (Giedd et al., 1999; Sowell et al., 2001; Casey et al., 2008b; Wahlstrom et al., 2010; Mills et al., 2014). Brain maturation processes during adolescence are influenced by sex steroids, which increase during puberty (Sisk and Foster, 2004). Sex hormones augment neuronal myelination (Martini and Melcangi, 1991) and modulate the development of neurocircuitry that subserves high-order cognition, reward and emotional processing (Dahl, 2001; Zhou et al., 2002; Peper et al., 2011). Neurodevelopmental changes and cortical reorganization are needed for the occurrence of adult behaviors, however the adolescent brain is highly susceptible to neural insults, which may disturb the natural course of brain maturation and key processes of brain development. Thus, adolescence is also the time when symptoms of a variety of mental illnesses often manifest, including mood disorders, eating disorders and schizophrenia (Spear, 2000; Sisk and Foster, 2004; Paus et al., 2008).

Excessive consumption of highly palatable sugar and fat laden foods, often in the form of "junk" or "fast" foods plays a central role in the development of obesity in humans (Malik et al., 2013). Of greatest concern is that the prevalence of obesity is increasing among children and adolescents. In the last 30 years obesity has more than doubled in children and quadrupled in adolescents, with now more than one third of children and adolescents in the developed world classified as overweight or obese (Ogden et al., 2014). Dramatic modifications in lifestyle patterns, such as making independent food choices, occur during adolescence (Nielsen et al., 2002; Story and French, 2004). Fast foods are laden with refined sugars and saturated fats and due to their convenience and low cost are readily accessible to young people (Davis and Carpenter, 2009). Adolescents and young adults consume more fast foods in comparison to older adults (Nielsen et al., 2002). Reports indicated that North American college students aged 18–24 ate at fast food restaurants 1–3 times weekly (Morse and Driskell, 2009) and 75% of school age children consumed fast foods once a week (French et al., 2002). Furthermore, dietary intake of refined sugar is greatest in adolescents than any other age group (Bremer and Lustig, 2012).

Epidemiological studies have identified associations between obesity in young people and psychological conditions including impulsivity, anxiety, drug abuse and attention deficit hyperactive disorder (ADHD; Waring and Lapane, 2008; Pagoto et al., 2009; Cortese and Vincenzi, 2012). ADHD and obesity are proposed to share a similar underlying neurobiological dysfunction of the dopaminergic system. In particular, a high incidence of comorbidity between ADHD and obesity has been observed (Altfas, 2002; Cortese et al., 2008). The behavioral manifestation of impulsivity in ADHD potentially contributes to weight gain via dysregulation of eating patterns (Erhart et al., 2012). A significant link between ADHD in adolescents and excessive consumption of "Western" junk food diets has been observed, indicating that adolescents with ADHD symptoms had a significantly higher intake of dietary fat, sugar and sodium than a traditional healthy diet (Howard et al., 2011). The co-occurrence of ADHD and obesity may be due to a common genetic and neurobiological pathway. Reports indicate that ADHD coincided with the expression of certain obesity-related genes in the pathways of dopaminergic neurocircuitry, such as fat mass–and obesity-associated variant (FTO; Albayrak et al., 2013) and melanocortin-4 receptor (MC4R; Agranat-Meged et al., 2008). Polymorphisms of these genes have been linked with the incidence of obesity and dysregulated eating behaviors in humans (Frayling et al., 2007; Gerken et al., 2007; Cecil et al., 2008; Peng et al., 2011; Yilmaz et al., 2015). In particular the MC4R rs17782313 polymorphism is implicated in emotional eating and food cravings (Yilmaz et al., 2015) and the FTO rs1558902 polymorphism is associated with binge eating in adolescence (Micali et al., 2015).

Palatable Food as a Rewarding Substance

Consumption of palatable high fat and high sugar foods leads to activation of the brain's reward neurocircuitry, the mesocorticolimbic dopamine system (Del Parigi et al., 2003; Avena et al., 2006, 2008; Kenny, 2011), resulting in the extracellular release of dopamine in regions including the nucleus accumbens and PFC. These regions receive long axon dopamine projections originating from the ventral tegmental area that form the mesocorticolimbic reward system. Dopamine acts within and across limbic, striatal and frontal neurocircuitry to promote and regulate motivated behavior, especially food seeking (Depue and Collins, 1999). Excessive consumption of sugar and fat rich foods evokes enduring changes in dopamine signaling within regions involved in reward processing, cognitive functions and motivation, including the nucleus accumbens (Rada et al., 2005; Sharma et al., 2013), the hippocampus (Kaczmarczyk et al., 2013; Krishna et al., 2015) and the PFC (Wakabayashi et al., 2015).

Maturation of the Dopaminergic Reward System During Adolescence

Adolescence has been described as a period of heightened affective reactivity characterized by an increased sensitivity to natural rewards (Van Leijenhorst et al., 2008, 2010; Somerville et al., 2010; Crone and Dahl, 2012), including palatable foods (Spear, 2000; Wilmouth and Spear, 2009; Friemel et al., 2010). Age-dependent changes in fronto-striatal cortical maturation may underlie the increased sensitivity to palatable food rewards

during adolescence (Friemel et al., 2010; Crone and Dahl, 2012). The rapid growth spurt that occurs in puberty and adolescence provides partial protection against diet-induced obesity, but a large tendency for hyperphagia (Spear, 2000; Labouesse et al., 2013). As such, chronic stimulation of the still maturing mesocorticolimbic dopamine system during adolescence by excessive consumption of palatable foods is hypothesized in this manuscript to increase vulnerability to psychiatric disorders (Zametkin et al., 2004), pathological eating behaviors (Smith and Robbins, 2013) and cognitive dysregulation (Liang et al., 2014).

Studies using rodents and non-human primates provide neurochemical, structural and electrophysiological evidence signifying that the reward-signaling dopaminergic innervation originating from the ventral tegmental area to the PFC and nucleus accumbens matures during adolescence (Tseng and O'Donnell, 2007b; Benoit-Marand and O'Donnell, 2008; Brenhouse et al., 2008; Wahlstrom et al., 2010; Mastwal et al., 2014; Palm and Nylander, 2014). These observations have significant implications for adolescent-onset drug addiction (Palmer et al., 2009), but also may elucidate why reward driven behaviors such as excessive overeating leading to obesity is increasingly abundant in young people (Lee and Gibbs, 2013).

Dopamine Release During Adolescence

The dopaminergic innervation of the PFC peaks during adolescence in rats and monkeys (Kalsbeek et al., 1988; Rosenberg and Lewis, 1994, 1995; Tarazi and Baldessarini, 2000) and neurochemical changes evoked by psychostimulants are distinct during this stage (Andersen et al., 2001; Tirelli et al., 2003). Primate and rodent studies have indicated increased levels of functionally available dopamine during adolescence, though differences exist with respect to the neuroanatomical regions and aspects of the dopamine system affected between species. Increased cortical and subcortical tissue concentrations of dopamine are observed during juvenile and adolescent periods in monkeys (Goldman-Rakic and Brown, 1982; Irwin et al., 1994; Caballero et al., 2016). In rodents, including mice and rats, adolescence, comprising the pubertal period, extends across postnatal days (P) 28–42, however functional alterations occur into late adolescence (P56) including maturation of the rodent homolog of the primate PFC and changes in frontostriatal dopamine signaling (Spear, 2000; Caballero et al., 2016; Hunt et al., 2016). Dopamine levels in the rodent brain increase in the striatum during adolescence (Teicher et al., 1993; Andersen et al., 1997), this is proposed to result from a reduced basal rate of dopamine release in adolescents relative to adults (Stamford, 1989; Andersen and Gazzara, 1993). However, when stimulated by environmental or pharmacological challenges, dopaminergic neurons in the adolescent brain release more dopamine than adults measured by microdialysis (Laviola et al., 2001). This indicates that during adolescence rewarding events such as consuming palatable foods may result in larger dopamine release in comparison to adulthood (Laviola et al., 2003).

Dopamine Receptors During Adolescence

Dopamine receptors are overproduced and then pruned during adolescence in fronto-striatal regions (Teicher et al., 1995). Postmortem analysis of human brain tissue has reported developmental declines in dopamine receptor populations in striatal regions during adolescence, with approximately one-third to one-half of the dopamine receptor 1 (D1R) and dopamine receptor 2 (D2R) present in the striatum of children being lost by adulthood (Seeman et al., 1987; Palacios et al., 1988). Similarly, in monkeys, cortical and subcortical D1R and D2R density peaks in childhood, and decreases across adolescence into adulthood (Seeman et al., 1987; Lidow and Rakic, 1992). Rodent anatomical studies demonstrated that D1R and D2R density peaks in adolescence and then declines across adulthood in the striatum and PFC (Teicher et al., 1995; Andersen et al., 2000; Tarazi and Baldessarini, 2000; Brenhouse et al., 2008). Autoradiography studies have indicated that pruning of about one-third of D1R and D2R in the dorsal striatum and nucleus accumbens during adolescence (Teicher et al., 1995; Tarazi et al., 1998, 1999), and PFC D1R density and associated second messenger activity rises dramatically between P25 (juvenile) and P40 (adolescence), with a subsequent reduction by P100 (full adulthood) in rats as shown in **Figure 1** (Teicher et al., 1995; Andersen et al., 2000). Anatomical studies have shown that dopaminergic innervation of the PFC increases progressively until P50–60 (Verney et al., 1982; Kalsbeek et al., 1988; Benes et al., 2000) and D2R/D4R expression reaches a stable adult level at P35 (Tarazi et al., 1998; Tarazi and Baldessarini, 2000). This pinpoints adolescence as a period of substantial change in the dopaminergic reward pathways and cortico-accumbal neural connectivity. The heightened expression of D1R on cortico-accumbal projections further supports increased sensitivity to environmental events and addictive behaviors during adolescence (Laviola et al., 2003; Brenhouse et al., 2008).

Development of Inhibitory Neurotransmission Across Adolescence

D1R stimulation is a major modulator of synaptic plasticity in the PFC, hence may regulate synaptic connectivity (Huang et al., 2004). During adolescence, dopamine is critical in controlling balance between excitatory and inhibitory neurotransmission in the PFC (Tseng et al., 2007;Tseng and O'Donnell, 2007a). In the postnatal mammalian brain, γ-aminobutyric acid (GABA) is the principle inhibitory neurotransmitter and glutamate is the principle excitatory neurotransmitter. Excitatory and inhibitory neurotransmission balance in the mature PFC is critical in cognition and the control of behavior (Yizhar et al., 2011; Lewis et al., 2012; Nelson and Valakh, 2015). Neurochemical evidence indicates that GABAergic neurotransmission, particularly within the PFC, remains under construction during adolescence (Lewis, 1997; Crews et al., 2007; Tseng and O'Donnell, 2007a; Caballero and Tseng, 2016). This delayed development of GABAergic neurotransmission may underpin behaviors such as increased risk-taking in adolescence (Van Leijenhorst et al., 2010; Schindler et al., 2016) and susceptibility to development of psychiatric disorders during this period (Caballero and Tseng, 2016).

The dopaminergic innervation of PFC glutamatergic pyramidal neurons and GABAergic interneurons and their

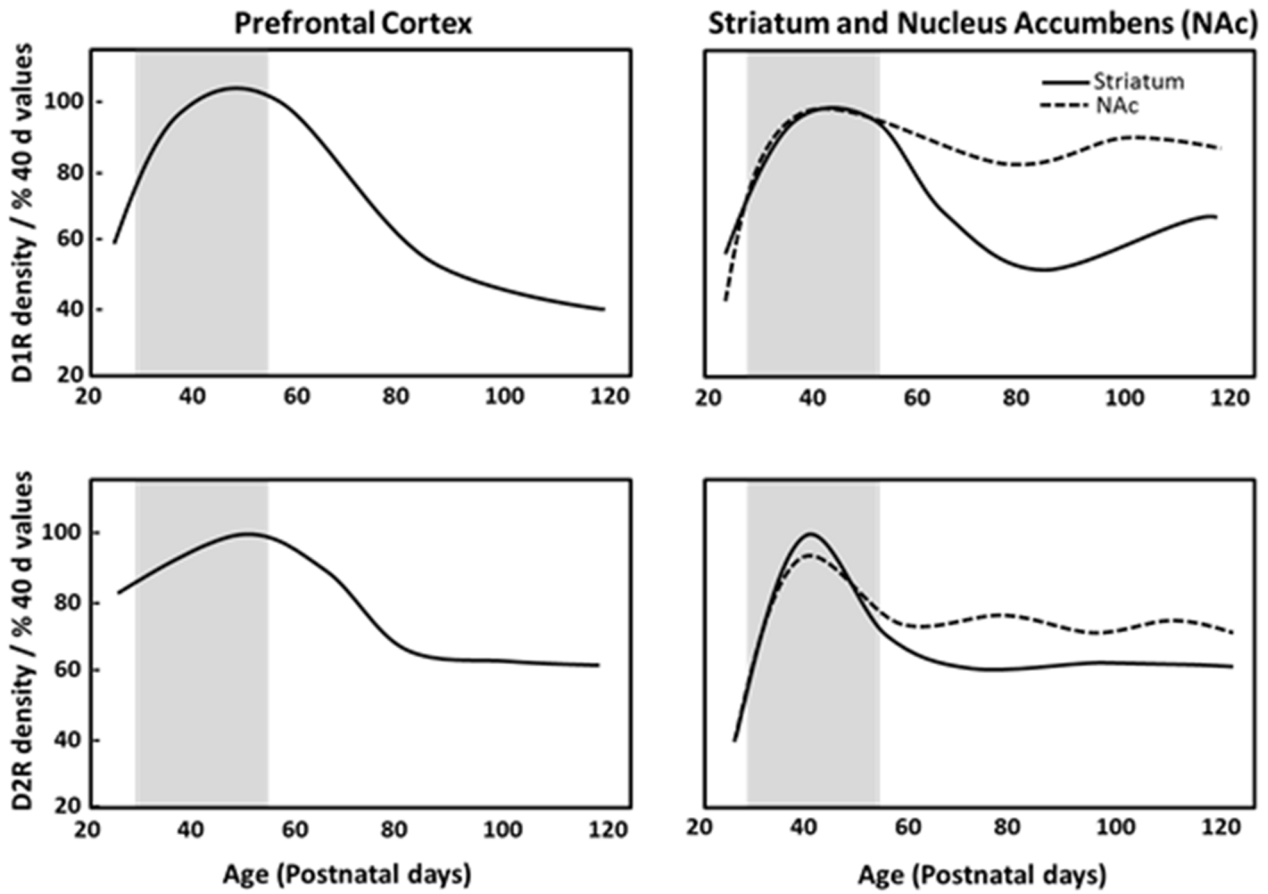

FIGURE 1 | Density of dopamine D1 (D1R) and D2 receptors (D2R) in the prefrontal cortex (PFC), striatum and nucleus accumbens of male Sprague Dawley rats at specific postnatal days (P), based on Andersen et al. (2000). Gray region depicts adolescence as P28–56.

interaction matures during adolescence (Tseng and O'Donnell, 2007a) and controls aspects of reward processing. Parvalbumin expressing neurons are a major class of GABAergic interneurons. A recent study indicated reduced parvalbumin immunoreactivity in the PFC and dorsal hippocampus of rats that consumed a high sucrose diet for 28 days across adolescence (Reichelt et al., 2015). This suggests that high sucrose diets may reduce GABAergic inhibition in these brain regions, potentially underpinning diet-evoked cognitive deficits manifesting as dysregulation of behavioral control (Reichelt et al., 2015), however further studies are needed to establish whether these effects are specific to adolescent diet-exposure. Sonntag et al. (2014) utilized virally mediated elevation of PFC D1R on glutamatergic neurons in adult rats (CamKII.D1) to recapitulate the increased PFC D1R levels in adolescence (see **Figure 1**, Andersen et al., 2000). The lentiviral induced elevation of D1R functionally resulted in greater consumption of ascending concentrations of sucrose (0, 0.25, 0.5 and 1%) or 0.1% saccharin using a two-bottle choice task between the solutions and water, indicative of increased sensitivity to the rewarding properties of sweet liquids in CamKII.D1 rats (Sonntag et al., 2014). Furthermore, a delayed discounting task was conducted in a T-maze where the animals had the choice between an immediate small reward or waiting across a delay period for a large reward (1 or 4 pieces of Reece's Pieces respectively), increased choices for the small reward rather than waiting for the larger reward was observed in the CamKII.D1 rats, indicating increased impulsivity (Sonntag et al., 2014). Elevation of cortical D1R also resulted in decreased D2R expression in the nucleus accumbens (Sonntag et al., 2014), which is known to be a risk marker for obesity (Wang et al., 2004). The up-regulation of D1Rs in the PFC may therefore render adolescents selectively vulnerable to overeating compared with a normal adult population, and provides a potential mechanism for why reduced D2Rs are observed in the nucleus accumbens of obese subjects.

Development of other PFC Reward-Processing Neurotransmitter Systems During Adolescence

Multiple neurotransmitter systems are also developing during adolescence, particularly those projecting to the PFC. This includes the acetylcholinergic system that is critical for reward processing and cognitive processes. Neuronal nicotinic acetylcholine receptors (nAChRs) nAChRs exhibit distinct

patterns of expression that parallel key developmental events within the cholinergic system and are critical regulators of brain maturation from prenatal development through adolescence (Dwyer et al., 2009). In rodents, expression and binding at α4β2 and α7 nAChR subtypes is higher in many brain regions in adolescents than in adults (Adriani et al., 2003; Doura et al., 2008). Neuronal nAChRs centrally regulate signaling in reward pathways (Dani and Balfour, 2011); and nAChR activation modulates dopamine release, which is strongly implicated in reward processing and reinforcement (Gotti et al., 2006; Albuquerque et al., 2009). Thus, in the adolescent brain, reduced regulation of dopamine by nAChRs may exacerbate reward-seeking, including palatable food consumption.

Furthermore, the endocannabinoid system has recently emerged as a regulator of PFC plasticity and undergoes age-dependent changes that directly impact PFC activity. CB1 receptor expression increases during postnatal development, with levels peaking at mid-adolescence (Schneider et al., 2008; Klugmann et al., 2011), which is accompanied by relative changes in the concentrations of endocannabinoids (Berrendero et al., 1998). Recent studies in rats indicate that repeated cannabinoid agonist administration during adolescence evokes neuronal deficits are at the level of PFC GABAergic circuitry, however this does not occur when cannabinoid agonist treatment occurs in adulthood, indicating that stimulation during adolescence hinders the appropriate development of the PFC (Cass et al., 2014). Notably, alterations to CB1 receptor expression has been observed in dietary obese rats (Bello et al., 2012), mice (South and Huang, 2008) and humans (Bordicchia et al., 2010). However, the exact mechanisms by which CB1 receptor signaling enables PFC maturation remain to be determined, as is whether adolescent diet induced alterations in CB1 receptors alter PFC maturation.

Summary—Neurodevelopmental Impact of Reward System Stimulation by Palatable Foods

Following prolonged consumption of high fat and/or high sugar diets neuroadaptive effects have been observed in reward processing regions. Obesity is associated with deficits in dopamine neurotransmission, which may drive the overconsumption of palatable foods (Geiger et al., 2009). This manifests as down-regulation of striatal D2R density in obese adult rats (Johnson and Kenny, 2010), and humans (Wang et al., 2001; Stice et al., 2008). Stimulation of the mesocorticolimbic dopaminergic system during adolescence by the consumption of highly palatable foods may potentially impact on cortico-striatal maturation, altering the age-associated pruning of frontostriatal D1R/D2R (Andersen et al., 2000), dysregulating PFC function and altering reward-processing. However, currently no studies have directly compared D1R and D2R densities in the forebrain between adolescent and adult animals exposed to high fat and/or high sugar diets. From drug addiction literature it is proposed that reduced top-down dopaminergic innervation from the PFC to the nucleus accumbens and striatum may increase the motivation to procure rewards (Volkow et al., 2012). In drug addiction, the enhanced incentive value of the drug in the reward, motivation, and memory circuits overcomes the inhibitory control exerted by the PFC, promoting consumption of the drug due to enhanced activation of motivational neurocircuitry (Volkow et al., 2012). Therefore, in the setting of reward-driven eating behavior, the reduced inhibitory control from the PFC may permit the overconsumption of palatable foods observed in adolescents (Tomasi and Volkow, 2013; Volkow et al., 2013).

BEHAVIORAL AND COGNITIVE CHANGES FOLLOWING ADOLESCENT HIGH FAT/HIGH SUGAR DIET CONSUMPTION

Excessive consumption of palatable high fat/high sugar foods may exacerbate cognitive deficits including impulsivity and impaired decision-making (Crews et al., 2007), evoke enduring reward processing alterations that continue into adulthood, and promote the development of addiction-like behaviors and obesity (Volkow et al., 2008; Johnson and Kenny, 2010; de Weijer et al., 2011; Tuominen et al., 2015). The following sections discuss experimental studies that have functionally examined the resultant behavioral and cognitive changes that are evoked by adolescent palatable high fat and/or high sugar diet consumption, and experiments that pinpoint adolescence as a period of diminished behavioral control. These studies support the hypothesis that adolescence is a period of vulnerability to diet induced cognitive and behavioral alterations.

Impact of Adolescent High Fat and/or High Sugar Diets on Learning and Memory Processes

A growing body of experimental literature has indicated that adolescence may be a critical period for the development of pronounced and enduring cognitive and behavioral alterations following exposure to alcohol (Nasrallah et al., 2009; Gass et al., 2014; Schindler et al., 2014), psychostimulants (Sherrill et al., 2013; Hammerslag et al., 2014) cannabinoids (Schneider et al., 2008) and high fat or high sugar diet consumption (Boitard et al., 2012, 2014, 2015; Reichelt et al., 2015). Exacerbated cognitive deficits in young rats and mice are reported following consumption of diets rich in saturated fats and/or refined sugars commencing in early life, as juveniles or adolescents, when compared to animals that commenced exposure to the same diets during adulthood.

Studies have characterized diet-induced learning deficits in hippocampal-dependent long term memory formation (assessed in the radial-arm maze or Morris water maze) when high fat diet consumption began during the juvenile/adolescent period in both rats (Greenwood and Winocur, 2005) and mice (Valladolid-Acebes et al., 2011). This memory impairment was not observed when diet access started in 8 week old rodents (P56; Mielke et al., 2006; White et al., 2009; McNeilly et al., 2011), which is considered to be the end of adolescence in rodents.

Studies have reported consumption of high fat and/or high sugar diets leads to cognitive deficits in PFC mediated behaviors,

particularly those requiring modulation of attention towards stimuli. McNeilly et al. (2011) measured working memory in animals fed a high fat diet in an operant delayed match to position test shown to be sensitive to PFC function (Sloan et al., 2006). In this task animals had to respond to one lever "sample" then after a 5 s delay, a choice phase of the presentation of two levers which was correct if the original lever was depressed "match to position". High fat diet fed rats were impaired at this task, however these deficits were further exacerbated when the task was switched to a delayed non-match to position task, whereby the correct lever was the different lever to the sample "non-match to position". This indicated a deficit in behavioral flexibility in high fat diet fed rats, in particular, reversing a learned contingency.

Prepulse inhibition (PPI), which is the ability to filter out sensory information and is subserved by the PFC and striatum, can be measured by an acoustic startle response. Typically, the reduction of the startle response amplitude to an intense stimulus is observed if there by a preceding weak, non-startling stimulus. This blunted startle response was shown to be impaired in adult mice fed a high fat diet (Labouesse et al., 2013; Wakabayashi et al., 2015) and these deficits in PPI were linked to decreased PFC D1R in high fat diet fed mice (Wakabayashi et al., 2015). Furthermore, the ability to reduce, or block, learning about a stimulus when it is paired in compound with another stimulus already associated with an outcome depends on the PFC (Fletcher et al., 2001) and midbrain dopamine signaling (Waelti et al., 2001). An appetitive blocking task was conducted in adult rats that had consumed sucrose in a binge-like manner for 28 days (Sharpe et al., 2016), the sucrose exposed animals, but not controls, approached a cue signaling food delivery that is usually blocked by prior learning, an effect dependent on dopaminergic prediction-error signaling in the midbrain. It appeared that disruption of PFC dopaminergic signaling following consumption of sucrose supplemented diets contributed to the behavioral alterations observed. In particular, intraventricular infusion of the D2R agonist quinpirole restored PFC-mediated cognitive control of learning about food cues in adult rats with a history of sucrose binging. This provides evidence of diet-induced alterations to D2R signaling mechanisms in the PFC, potentially due to a down-regulation of D2R (Sharpe et al., 2016).

Functional connectivity between the hippocampus and PFC is essential for many executive cognitive functions (Floresco et al., 1997; Thierry et al., 2000). Disruption of hippocampal–PFC synchrony is associated with the cognitive deficits that occur in neuropsychiatric disorders such as schizophrenia (Dickerson et al., 2010, 2012; Sigurdsson et al., 2010), and D2R-dependent control of glutamatergic NMDAR neurotransmission has been particularly implicated in the regulation of hippocampal-PFC functional connectivity (Banks et al., 2015). More so, presynaptic D2Rs in the hippocampus modulate long-term depression and aspects of long-term potentiation expression and functionally regulate hippocampally dependent learning and memory performance (Rocchetti et al., 2015). Thus, alterations to D2R signaling by excessive consumption of high fat and/or high sugar diets may underpin aspects of diet-induced cognitive deficits.

Highly controlled studies using animal models have revealed that consumption of high fat diets during the juvenile and adolescent period of life negatively impact memory function to a greater degree than adult consumption of the same diets. Rats exposed to a high fat diet (45% kcal from fat) from weaning (i.e., 3 weeks of age) as juveniles, but not adult rats, were observed to be impaired at spatial memory retention and spatial reversal learning (Boitard et al., 2014). Similarly, mice fed a high fat diet for 11 weeks post-weaning were impaired at memory flexibility assessed in a two-stage radial arm maze concurrent spatial discrimination task, whereas adults exposed to the same high fat diet beginning at 12 weeks old (P84) and for the same duration of diet exposure did not show cognitive deficits on this task (Boitard et al., 2012). Adolescent mice aged 5 weeks old at commencement of diet access were impaired in the hippocampal dependent novel location recognition task and these cognitive deficits continued after 5 weeks of diet restriction, whereas mice that were given the diet at 8 weeks old were not impaired (Valladolid-Acebes et al., 2013). Similarly, rats that consumed sugar supplemented diets in the form of either 11% sucrose solution or 11% high fructose corn syrup (HFCS) solution for 30 days across adolescence had hippocampal dependent learning impairments, which were not observed in adult rats consuming the same high sugar diets (Hsu et al., 2015). Protein expression of the pro-inflammatory cytokines IL-1β and IL-6 were increased in the hippocampus of adolescent HFCS consuming rats, but this was not observed in adult rats (Hsu et al., 2015). This indicates a particular vulnerability of the adolescent hippocampus to neuroinflammation following consumption of high sugar diets.

Adolescence is also considered a period of enhanced emotional reactivity to stressful and arousing events. Long term emotional memories are underpinned by limbic regions including the basolateral amygdala (BLA; McGaugh, 2004), and influenced by function of the hypothalamic–pituitary–adrenal (HPA) axis (Roozendaal et al., 2006). Obesity induced alterations to the HPA axis is observed in humans (Pasquali et al., 2006) and rodent models (Sharma et al., 2013), leading to an exaggeration of emotional responses. In rats and mice, emotional responses can be measured by fear conditioning preparations, whereby a stimulus such as a tone is presented prior to a mild footshock. Following conditioning, animals will elicit fear responses in the form of freezing when the tone is presented, indicative of learning an association between the stimulus and the aversive event. Furthermore, emotional responses can also be assessed by pairing the consumption of an odorized water solution with lithium chloride induced malaise. Animals will then avoid consuming the odorized solution as they associate it with unpleasant malaise. Emotional memories were examined in rats that consumed a high fat diet across adolescence, but not in adulthood. It was observed that a high fat diet consumed across adolescence enhanced the expression of emotional memories assessed by odor-malaise, the rats that consumed high fat diets as adolescents formed a stronger aversion to the malaise-paired solution in comparison to adult rats that consumed high fat diets, and age-matched control animals (Boitard et al., 2015). Furthermore, freezing

to the shock paired stimulus was increased in adolescent rats that consumed a high fat diet, whereas rats that consumed the high fat diet as adults showed similar levels of freezing to control diet rats (Boitard et al., 2015). This demonstrated exacerbated emotional memory learning in high fat diet consuming adolescent, but not adult rats (Boitard et al., 2015). The PFC regulates fear behavior by modulating the activity of the amygdala (Paré et al., 2004; Chan et al., 2011), therefore the enhanced emotional memories observed in adolescent may be underpinned by reduced regulation of the BLA by the immature adolescent PFC. This indicated a potential vulnerability of adolescence to the development of increased emotional learning that may lead to the development of anxiety disorders.

Reduction of Hippocampal Neurogenesis Following Adolescent High Fat/High Sugar Diet Consumption

Hippocampal neurogenesis, and more specifically the integration of adult-born neurons into the hippocampal circuitry, is important in learning and memory processes (Koehl and Abrous, 2011) and is shown to be reduced in high sugar (Van der Borght et al., 2011; Reichelt et al., 2016b) and high fat diet consuming rodents (Park et al., 2010; Boitard et al., 2012). Neurogenesis occurs at higher levels in the hippocampus during adolescence compared to adulthood (Crews et al., 2007). Chronic alcohol consumption during adolescence induced long-term changes persisting into adulthood such as reduced neurogenesis marker expression in the dentate gyrus and depressive-like behaviors (Briones and Woods, 2013), and impaired object recognition memory (Vetreno and Crews, 2015). Similarly, mice that consumed a high fat diet across adolescence for 11 weeks had reduced levels of hippocampal neurogenesis measured by doublecortin immunoreactivity in the dentate gyrus compared to controls, but doublecortin immunoreactivity did not differ between high fat diet consuming adult mice and age-matched controls (Boitard et al., 2012).

In summary, the adolescent brain appears particularly vulnerable to the neurobiological impact of high fat and/or high sugar diet consumption. Collectively these studies strongly suggest that neural substrates of learning and memory, particularly the hippocampus, in the adolescent brain are susceptible to persistent neurobiological changes caused by overconsumption of palatable high fat and/or high sugar diets, and this may be due to an enduring reduction in neurogenesis, or increased neuroinflammatory reactions.

Impact of Adolescent High Fat/High Sugar Diets on Reward-Directed Behaviors

Developing dopaminergic neurotransmission, particularly in the PFC, is associated with reward-seeking behavior during adolescence (Wahlstrom et al., 2010). An important question is whether consumption of palatable foods impacts the developing adolescent brain in a different way and with longer-lasting consequences compared to the mature adult brain. The affective components of rewards such as palatable high fat/high sugar foods can be broken down into *wanting* and *liking* (Berridge and Robinson, 1998; Barbano and Cador, 2007; Castro and Berridge, 2014), whereby wanting refers to the motivational component of reward, and liking refers to the hedonic component of reward. Thus, the affective impact of rewarding substances during development can be examined separately by how motivated an animal is to procure the substance, that is, the incentive salience attributed the reward, and the hedonic appraisal of the substance. Neuroimaging studies in humans demonstrate greater reactivity within the nucleus accumbens in adolescents following food reward deliveries relative to young children and adults (Galvan et al., 2006; Geier et al., 2010), indicating a particular sensitivity to rewards in adolescents. This increased sensitivity to the rewarding properties of palatable foods and drinks observed in adolescents may promote hyperphagia (Bernheim et al., 2013), and due to this increased "dose" of rewarding foods consumed, alterations to neuronal processes may also be more pronounced. Under experimental conditions this hyperphagic behavior is supported by reports of enhanced consumption of palatable sucrose solutions by adolescent rats as a factor of body weight in comparison to adult animals (Kendig et al., 2013) and may be underpinned by an increased positive taste responsivity in adolescent rats (Wilmouth and Spear, 2009).

Motivation to procure an outcome typically depends on the strength, or reinforcing efficacy, of the reward, which can be measured by progressive ratio schedules (Richardson and Roberts, 1996). Under progressive ratio schedules the requirements (i.e number of lever presses) to earn a reinforcement delivery are increased systematically, usually after each reinforcer. It was demonstrated that male rats that had their diet supplemented with 5% sucrose solution continuously in their homecages during adolescence, but not in adulthood, were less motivated to perform lever press responses on a progressive ratio schedule for palatable food rewards when tested as adults (Vendruscolo et al., 2010), indicating sucrose consumption during adolescence evoked long term changes in reward processing. However, another recent study indicated that observation of reduced motivation measured by progressive ratio following adolescent sucrose consumption may be specific to male rats. (Reichelt et al., 2016a). In this study, female rats that consumed 10% sucrose for 2 h a day across adolescence showed increased motivation measured by an increased breakpoint of lever presses to procure palatable rewards when tested as adults, however male rats showed reduced breakpoints on a progressive ratio schedule (Reichelt et al., 2016a). It should also be noted that in both the studies by Vendruscolo et al. (2010) and Reichelt et al. (2016a), behavioral alterations were observed without differences in body weight between the experimental groups despite increased overall energy intake in sucrose consuming rats, thus behavioral changes cannot simply be attributed to altered motivation state due to different baselines of hunger during behavioral testing. Naneix et al. (2016) recently assessed the effect of sucrose consumption across adolescence in adult male rats on the hedonic impact of sweet rewards (sucrose and saccharin) by affective reactions, measured by orofacial reactions to intraoral infusions. This study indicated that daily sucrose intake during adolescence led to decreased positive

orofacial responses to sweet tastes when the rats were assessed as adults (Naneix et al., 2016) and this hedonic deficit was associated with lower c-Fos expression levels in the nucleus accumbens. Thus, the hedonic appraisal of both caloric and non-caloric sweet rewards was lessened following adolescent sucrose consumption, suggesting a long-lasting lower hedonic state that may contribute to the development of reward-related disorders in adulthood (Naneix et al., 2016), which may potentiate the overconsumption of palatable foods (Johnson and Kenny, 2010). In a human setting, the hedonic response to palatable sweet tastes of ascending concentrations of sucrose solution measured by a self report questionnaire was associated with elevated sensitivity to the mood altering effects of sweet foods and impaired control over eating sweets, with a greater preference for concentrated sucrose observed in women (Kampov-Polevoy et al., 2006).

Conditioned place preference (CPP) studies pair one discriminable context with the administration of a rewarding substance, and another with no reward, so that the animals come to prefer the reward paired context paired when presented with a choice between the two environments. Rats and mice prefer and approach environmental cues that are associated with consumption of a palatable food reward (Perello et al., 2010). Through repeated pairings of access to palatable foods in a certain environment, animals will elicit an approach response to the food-rewarded environment in comparison to a control, non-food paired environment. Consumption of a high fat diet across adolescence (P21–40), but not as adults (P61–80), evoked long lasting impairments in CPP for a palatable food (Cheetos) in male rats (Privitera et al., 2011). This suggests that rats that consumed palatable food during adolescence learned less about the environment that the food reward was presented in. This may be indicative that the adolescent high fat diet consuming rats found the palatable food less rewarding, or that they showed deficits in encoding the environmental features associated with the food rewards.

These studies indicate that high fat or high sugar diets across adolescence evoked alterations in both motivation and hedonic appraisal of food rewards in adulthood, processes subserved by reward-processing regions including the striatum and nucleus accumbens. This may increase the risk of developing neuropsychiatric disorders, including depression, eating disorders and addiction, as well as obesity (Blundell and Finlayson, 2004; Marmorstein et al., 2014), which commonly emerge during adolescence (Pine et al., 1998; Paus et al., 2008).

Adolescence as a Period of Impaired Behavioral Control

Instrumental behavior, such as performing a lever press action that is reinforced by a palatable outcome, is controlled by two discrete behavioral and neuronal systems: a stimulus-response habit mechanism and a goal-directed (action-outcome) process (Adams and Dickinson, 1981; Balleine and Dickinson, 1998; Balleine and O'Doherty, 2010). Habitual behaviors are generally inflexible, while the action-outcome is a dynamic process with a continuous and flexible feedback over performance of actions to acquire outcomes allowing behavior to adapt to changing environments (Adams and Dickinson, 1981; Balleine and Dickinson, 1998). In rats, the capacity to detect changes in action–outcome contingencies is governed by a neural circuit including the prelimbic PFC (Balleine and Dickinson, 1998) and the posterior dorsomedial striatum (pDMS; Yin et al., 2005). Furthermore, this capacity depends on dopamine signaling in the pDMS (Lex and Hauber, 2010a,b; Braun and Hauber, 2012). Performance of instrumental actions in rats is initially goal-directed and therefore sensitive to changes in reward value. However, after extended training stimulus-response habits emerge that are no longer goal-directed and are insensitive to changes in incentive value or action-outcome contingencies. Habitual responses are subserved by the infralimbic PFC (Balleine and Dickinson, 1998; Killcross and Coutureau, 2003) and dorsolateral striatum (DLS; Yin et al., 2004). Dopamine is known to play a role in the development of habits, whereby sensitization of the dopamine system in adult rats by chronic d-amphetamine treatment prior to instrumental training leads to an acceleration of habit formation (Nelson and Killcross, 2006), and dopamine depleting 6-OHDA lesions of the nigrostriatal dopamine system decreases habit formation (Robbins et al., 1990; Faure et al., 2005).

A large body of literature demonstrates that the adolescent period affords vulnerability to the higher-order control of behaviors. The following sections discuss experimental evidence demonstrating that adolescence is a distinctive period characterized by altered behavioral regulation of actions and stimuli associated with obtaining food rewards. The control of goal-directed instrumental actions is proposed to depend on the dopamine system (Balleine and O'Doherty, 2010). Evidence for contingency learning comes from demonstrations that instrumental performance is sensitive not only the probability of contiguous reward but also to the probability of unpaired rewards (Balleine and Dickinson, 1998; Braun and Hauber, 2012). Rats are initially trained to press on two separate levers for a reward that is delivered in a contingent manner—the action leads to the outcome. However, during a contingency degradation test, responding on one lever becomes degraded, as rewards are delivered randomly in a non-contingent manner to the action performed, and the rat should typically cease to respond to the lever, but maintain responding to the lever that responses are contingent to outcome deliveries. It was observed that adolescent rats failed to adapt their response to changes of action–outcome relationships, however these rats adapted to the contingency degradation protocol once adults, which paralleled the maturation of the cortical dopamine system (Naneix et al., 2012). This failure to update action-outcome contingencies in adolescent rats is potentially due to a diminished ability to encode the alteration in contingency due to immaturity of the PFC, as action-outcome contingencies has been shown to depend on dopamine signaling in the prelimbic PFC and DMS (Lex and Hauber, 2010b). The immaturity of the PFC and delayed development of the mesocortical dopamine pathway that projects to the PFC during adolescence is proposed to

underpin sub-optimal decision making in the selection and execution of actions according to their predicted consequences (Naneix et al., 2012, 2013). Furthermore, treatment with the D2R agonist quinpirole across adolescence impacts on the developing dopamine system, decreasing dopamine fiber density, dopamine tissue concentration and dopamine receptors expression in the PFC (Naneix et al., 2013). The behavioral consequence of D2R stimulation was that adult rats treated with quinpirole showed behavioral deficits in updating actions during a contingency degradation test (Naneix et al., 2013), mimicking the deficits observed in adolescent rats (Naneix et al., 2012).

Poor behavioral control, lack of inhibition and impulsivity contribute to the propensity for adolescents to engage in risk-taking behaviors. Inhibiting a response can be assessed by a differential reinforcement of low-rate (DRL) schedule. This task requires animals to withhold a food-procuring response over a set period of time, before the response will be reinforced. Adolescent male rats were less sensitive to both the extinction of a learned response to obtain a palatable chocolate milk reward and to withhold a response on a DRL schedule indicating impaired behavioral inhibition in adolescent animals compared to adults. (Andrzejewski et al., 2011).

Reaction time tasks, such as the five-choice serial reaction time task (5-CSRTT) can be used to assess attention in rodents. Animals are required to attend a visual array that briefly presents a cue (illuminating a light across a 5 light array) to which responding is reinforced with a palatable reward, whilst inhibiting responses during the inter-trial period (Robbins, 2002). This task is shown to be dependent on dopaminergic neurotransmission within the medial PFC (Burton and Fletcher, 2012). Adolescent rats were trained to obtain a food pellet reward on a two-choice serial reaction time task (2-CSRTT), adolescents performed more impulsive actions in the form of premature responses in comparison to adults (Burton and Fletcher, 2012).

Studies examining choice behavior for large and small magnitudes of food rewards are used to recapitulate aspects of human gambling tasks, such as probability discounting tasks. These have noted a preference for large rewards in adolescent rats despite reductions in the probability in the delivery of the large reward (Zoratto et al., 2013). Choice performance can also be studied by requiring an animal to wait for a large reward over varying delays, or choose a small, immediate reward. Delay discounting describes the decrease in preference for a reward as a function of the delay to receiving it, so recapitulating elements of delayed gratification. Adolescent rats display more delay discounting, as they switch to the smaller reward associated response more rapidly than adult rats, indicative of increased impulsivity and inability to tolerate delays during adolescence (Doremus-Fitzwater et al., 2012).

A recent study examined the impact of high sugar diets on decision making in accordance to the presentation of discriminable stimuli that direct behavior. Consumption of 10% sucrose during adolescence reduced contextually appropriate responding to stimulus compounds in a biconditional discrimination task requiring the use of context as a task-setting cue (Reichelt et al., 2015). Rats first acquired two instrumental conditional discrimination in distinct contexts, one auditory (i.e A1→ R1, A2 → R2) in context 1 (C1; grid floor), and one visual (i.e V1→ R1, V2 → R2) in a different context (C2; bar floor). At test rats received compound stimuli that either comprised the auditory and visual elements that signaled the same lever response (congruent—A1V1, A2V2) or signaled different lever responses (incongruent—A1V2, A2V1) during training. During conflict (incongruent) trials, correct lever selection by control animals followed the stimulus element that had previously been trained in that same test context, whereas animals that had previously consumed sucrose failed to disambiguate the conflicting response cues. This task is sensitive to mPFC dysfunction (Haddon and Killcross, 2006; Reichelt et al., 2013b) and changes to dopaminergic signaling (Haddon and Killcross, 2011; Reichelt et al., 2013a), indicating a functional impact of sucrose-binging on decision making tasks.

Goal-directed behavioral control of instrumental responding can be assessed by conducting outcome devaluation by specific-satiety. This procedure provides experimental animals with the food outcome, or chow as a control, freely prior to the test session. During testing, animals are presented with the levers in extinction, that is, responses do not result in reward deliveries. It was observed that rats with a history of consuming a palatable food intermittently showed insensitivity to the devaluation treatment, and continued to lever press to procure the devalued outcome, indicative of stimulus-response habit formation, whereas control animals reduced lever pressing demonstrating goal-directed responding as the outcome is no longer valued (Furlong et al., 2014). However, whether consumption of high sugar diets during adolescence in comparison to maturity has a more pronounced behavioral impact on goal-directed instrumental responding has not been observed, as both sucrose-exposed adolescent rats and control adolescent rats showed reduced sensitivity to outcome devaluation by specific-satiety procedures (Kendig et al., 2013). This contrasts an earlier study demonstrating that adolescent rats were able to adapt their actions to changes in reward value following devaluation by sensory-specific satiety, but showed impairments in contingency degradation (Naneix et al., 2012).

As such, experimental studies with rats indicate adolescents are less able to regulate behavioral control over food reinforced actions, and in a human setting, this may predispose a tendency to consume palatable foods when posed with negative consequences such as weight gain and obesity, and intolerance to delayed gratification. Human studies have further identified that obese participants demonstrate risky patterns of decision-making on the Iowa Gambling Task (Brogan et al., 2010) and exhibited more impulsive patterns of choice for monetary outcomes than non-obese participants on delay discounting tasks (Lawyer et al., 2015).

Increased Stimulus-Directed Behavior in Adolescence

It has been suggested that adolescents may engage in more reward-seeking behaviors because their responses are biased

towards stimulus-driven processes as opposed to the incentive value of an outcome (Ernst et al., 2011). This suggests that external, environmental cues or stimuli are more likely to direct attention and as such evoke a response (i.e., eating) which is independent of internal state (i.e., satiety/hunger; Ernst et al., 2011). In the laboratory setting, the accelerated development of stimulus-directed habitual behavior in adolescent rats has been observed (Hammerslag and Gulley, 2014). In this study, the interaction between age and sex on the expression of stimulus-directed behavior was assessed in rats using a Pavlovian conditioned approach paradigm. An auditory conditioned stimulus (CS+) was paired with delivery of a sucrose solution (unconditioned stimulus; US) to a food trough. The conditioned response (CR) to presentation of the CS+ measured by food trough entries was assessed during daily training sessions, following the devaluation of the reward by specific-satiety, and during periods of extinction and reacquisition. In this case, adolescent rats were less sensitive to outcome devaluation by specific-satiety, and exhibited a greater degree of reacquisition to an extinguished cue-reward association (Hammerslag and Gulley, 2014). The enhanced development of habitual responses to a food-associated stimulus is proposed to contribute to the vulnerability of adolescents to develop compulsive behaviors, such as binge eating (Hammerslag and Gulley, 2014).

In a human setting, adolescence has been noted as a period of particular reactivity to food-associated cues. Epidemiological studies have shown that adolescent obesity has tripled over the last three decades in the setting of food advertising directed at children (Flegal et al., 2016; Ogden et al., 2016). Studies have shown that the televised presentation of food-related advertisements increases food intake in children (Halford et al., 2004, 2007, 2008; Andreyeva et al., 2011; Kemps et al., 2014), and the increase in overweight and obese children has been linked to TV advertising of sugar and fat-dense junk foods (Lobstein and Dibb, 2005; Andreyeva et al., 2011; Lee et al., 2014). Advertising for food and beverages communicates food cues, priming the consumption of unhealthy foods and beverages (Harris et al., 2009; Lee et al., 2014). Adolescent youth appear especially sensitive to cue associated with rewards, as evidenced by exaggerated neural responses when exposed to them, specifically within structures innervated by mesolimbic dopamine (Galvan et al., 2006; Casey et al., 2008a; Hare et al., 2008; Bruce et al., 2010; Ernst et al., 2011). Obese adolescents displayed exaggerated neural responses measured by functional magnetic resonance imaging (fMRI) in striatal and limbic pathways upon exposure to high calorie food images vs. non-food images (Jastreboff et al., 2014). This observation suggested that obese adolescents show greater responsivity in reward related brain regions than lean adolescents to visual food stimuli, such as those commonly depicted in food advertising (Jastreboff et al., 2014).

Sucrose Craving in Adolescence

Sucrose is a highly rewarding substance, and sweet foods are often subject to cravings (Avena et al., 2008). Following prolonged withdrawal, or forced abstinence from a rewarding substance such as a drug of abuse, responding for that substance, or a cue associated with the substance, will increase when made available again, an effect referred to as "incubation of craving" (Lu et al., 2004; Pickens et al., 2011; Wolf, 2016). This effect has been observed in rats trained to lever press to receive deliveries of sucrose solution. The animals then undergo an "abstinence" period of days or weeks where they do not receive access to the training chambers where they learned to lever press for sucrose, which may or may not be preceded by extinction training where the sucrose is no longer delivered when a lever press response is made. Following the abstinence period, the rats typically reinstated lever press responding when returned to the training chambers, indicating that sucrose is subject to craving (Grimm et al., 2005, 2007; Uejima et al., 2007). Despite evidence of adolescent drug use resulting in an increased vulnerability to addiction (Palmer et al., 2009), studies in adolescent rats have indicated a diminished "craving", measured by significantly lower rates of responding when returned to the instrumental chambers after the forced abstinence period compared to adult rats. This has been demonstrated after adolescent and adult rats were trained to self-administer cocaine following a 30-day abstinence period (Li and Frantz, 2009), heroin, following a 12-day abstinence period (Doherty et al., 2013) and under cue-induced sucrose-seeking conditions following a 21-day abstinence (Counotte et al., 2014). The subsequent attenuation of lever pressing elicited in adolescent rats contrasts the hypothesis that if adolescence affords an increased risk of addiction—craving should be greater, and the adolescent rats should perform more lever presses than adult rats following the enforced abstinence period. However, the reduced reinstatement of responding for sucrose solution adolescent rats is in-keeping with observations of diminished incubation of craving following self-administration of drugs of abuse (Li and Frantz, 2009; Doherty et al., 2013).

Summary—Adolescence as a Period of Impaired Behavioral Control

Experimental studies specify that adolescence is a period of enhanced responsiveness to rewards and reward-associated stimuli and responses. This is potentially due to increased risk-taking to gain rewards (Zoratto et al., 2013), the expression of behavioral responses that are insensitive to the contingency between actions and outcomes, and in some cases responses that are insensitive to the value of an outcome (Naneix et al., 2012; Hammerslag and Gulley, 2014). Furthermore, human neuroimaging studies have indicated increased neural responsivity to food-associated cues (Galvan et al., 2006; Casey et al., 2008a; Hare et al., 2008; Bruce et al., 2010; Ernst et al., 2011). These behaviors are underpinned by fronto-striatal regions and dopaminergic signaling mechanisms. Hence the impaired regulation of behavior observed in adolescents may potentiate the overconsumption of foods, driving the development of obesity.

CONCLUSIONS AND IMPLICATIONS

A range of animal and human experimental literature specifies that adolescence is a period of vulnerability to engage

in rewarding, yet potentially risky, behaviors, including overconsumption of high fat and high sugar foods. This food preference and hyperphagia is partially underpinned by the still developing PFC and mesocorticolimbic dopamine system. The immature adolescent PFC impedes self-regulation during this life stage (Casey et al., 2008a; Blakemore and Robbins, 2012). More so, maturational changes occurring within the mesocorticolimbic dopamine system alters the sensitivity to the rewarding properties of palatable foods and drinks. Overconsumption of high fat and high sugar foods during adolescence may therefore impact the development of the PFC and mesocorticolimbic dopamine, leading to the pronounced behavioral alterations observed in tasks that rely on these systems when compared to adults consuming high fat and high sugar foods. Events that occur during sensitive periods such as adolescence may derail the normal maturation process and evoke a different trajectory of development, leading to an enduring predisposition towards certain behaviors (Andersen and Teicher, 2008; Paus et al., 2008). It is known that elevated PFC D1R play a significant role in increased motivational salience during adolescence (Brenhouse et al., 2008). In the setting of junk food diets, this may promote excessive consumption and increased reactivity to palatable food associated cues (Jastreboff et al., 2014), driving overconsumption during adolescence that extends into adulthood.

The physiological consequence of increasing global consumption of diets laden in fat and sugar are not simply the increasing prevalence of obesity, but also cognitive dysfunction, memory deficits and increased risk of developing psychiatric disorders in a younger population. Adolescence offers a period to identify key developmental processes that are amenable to intervention. This can create opportunities to identify and intervene in high-risk youth during periods where neural systems are more amenable to change, averting some of the destructive negative behavioral and cognitive spirals that may originate in adolescence. Thus, addressing the prevalence of high fat and high sugar diets in adolescents is vital, and further research should be undertaken to determine age-related cognitive effects of these diets and tailored intervention strategies.

AUTHOR CONTRIBUTIONS

ACR wrote and developed the manuscript.

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Short-Term High-Fat Diet (HFD) Induced Anxiety-Like Behaviors and Cognitive Impairment Are Improved with Treatment by Glyburide

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Obesity-associated comorbidities such as cognitive impairment and anxiety are increasing public health burdens that have gained prevalence in children. To better understand the impact of childhood obesity on brain function, mice were fed with a high-fat diet (HFD) from weaning for 1, 3 or 6 weeks. When compared to low-fat diet (LFD)-fed mice (LFD-mice), HFD-fed mice (HFD-mice) had impaired novel object recognition (NOR) after 1 week. After 3 weeks, HFD-mice had impaired NOR and object location recognition (OLR). Additionally, these mice displayed anxiety-like behavior by measure of both the open-field and elevated zero maze (EZM) testing. At 6 weeks, HFD-mice were comparable to LFD-mice in NOR, open-field and EZM performance but they remained impaired during OLR testing. Glyburide, a second-generation sulfonylurea for the treatment of type 2 diabetes, was chosen as a countermeasure based on previous data exhibiting its potential as an anxiolytic. Interestingly, a single dose of glyburide corrected deficiencies in NOR and mitigated anxiety-like behaviors in mice fed with HFD-diet for 3-weeks. Taken together these results indicate that a HFD negatively impacts a subset of hippocampal-independent behaviors relatively rapidly, but such behaviors normalize with age. In contrast, impairment of hippocampal-sensitive memory takes longer to develop but persists. Since single-dose glyburide restores brain function in 3-week-old HFD-mice, drugs that block ATP-sensitive K^+ (K_{ATP}) channels may be of clinical relevance in the treatment of obesity-associated childhood cognitive issues and psychopathologies.

Keywords: glyburide, anxiety, cognition, high-fat diet, oxidative stress

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Abbreviations: DA, dopamine; DHEA, dehydroepiandrosterone; DIO, diet induced obesity; DOPAC, 3, 4-dihydroxyphenylacetic acid; EZM, elevated zero maze; FBG, fasting blood glucose; GSH, glutathione; GSSG, glutathione disulfide; HFD, high fat diet; HFD-mice, high-fat diet mice; HVA, homovanillic acid; IDO, indoleamine 2, 3 deoxygenase; LFD, low fat diet; LFD-mice, low-fat diet mice; NEFA, non-esterified fatty-acids; NOR, novel object recognition; OFT, open field test; OLR, object location recognition; SOD, superoxide dismutase.

INTRODUCTION

With over one billion overweight and obese individuals afflicted, world-wide overnutrition is a significant threat to human health (Kelly et al., 2008). Obesity is associated with increased susceptibility to various comorbidities like depression (Leckie and Withers, 1967; Luppino et al., 2010), type 2 diabetes (Wannamethee and Shaper, 1999), cardiovascular disease (Wannamethee et al., 1998), and cancer (Vainio and Bianchini, 2002). One of the root causes of obesity is attributed to ingestion of dietary fat (Lissner and Heitmann, 1995) which parallels the global permeation of a "western diet" (Cordain et al., 2005), where nearly 33% of total energy derives from fat. Predictably, extension of the obese phenotype into childhood is associated with added risks including diminished cognition and executive function (Liang et al., 2014). Additionally, obese children are susceptible to certain psychological complications like attention deficit hyperactivity disorder (ADHD), impulsivity, inattention and anxiety (Daniels et al., 2005; Kalarchian and Marcus, 2012). Current work demonstrates that childhood and adolescent obesity is disadvantageous, especially as psychological illness and/or cognitive impairment persists even when consumption of a high-fat diet (HFD) is well in the past (Wang et al., 2015). Given the projected prevalence of childhood obesity in the next 30 years (Ogden et al., 2014) and the magnitude of associated co-morbidities, identifying therapeutics to address this accelerating health concern is crucial.

Cognitive impairment and psychological abnormalities described in mouse models of diet-induced obesity (DIO; Buettner et al., 2012) are often connected to a reduction in molecules associated with neurogenesis and/or learning/memory such as brain derived neurotrophic factor (BDNF; Molteni et al., 2002), dopamine (DA; Kaczmarczyk et al., 2013) and inflammatory bioactives (Pistell et al., 2010). Recent studies demonstrate that DIO-associated brain dysfunction is not solely attributable to these mechanisms because hippocampal-based memory impaired by dietary fat occurs without significant change in BDNF (Heyward et al., 2013). Along similar lines, prolonged HFD-feeding does not upregulate brain-based IL-1β, TNF-α or IL-6 mRNA, even though sickness-like behaviors suggestive of brain inflammation are observed (Lavin et al., 2011). In juvenile animals, however, a HFD can impact working through pathways tied to glucocorticoids, neurogenesis and leptin (Boitard et al., 2012, 2014, 2015; Valladolid-Acebes et al., 2013), and it was found that this exposure impacted cognition in adulthood (Boitard et al., 2012). Although the brain-based complications of obesity are phenotypically well-described in animals, pharmacologic interventions that overcome such morbidities that are easily translated into humans are lacking.

The second-generation sulfonylurea, glyburide, has long been used in the treatment of type 2 diabetes (Kolterman et al., 1984; Groop, 1992). Glyburide inhibits sulfonylurea receptor 1 (Sur1) preventing K_{ATP} channel function (Ashcroft, 2005). It is this action on pancreatic β-cells that results in insulin release and improved blood glucose homeostasis (Niki et al., 1989; Zini et al., 1991). In turn, glyburide shows promise as is a promising brain therapeutic due to its ability to cross the blood-brain barrier (Simard et al., 2012). Since it has been explored as a countermeasure for traumatic brain injury (TBI), stroke, and spinal cord injury, it may be a suitable agent for other injuries and diseases as well (Kunte et al., 2007; Simard et al., 2009a,b; Patel et al., 2010). While glyburide has been explored as a mitigating agent in Alzheimer's disease (Lavretsky and Jarvik, 1992), neuroinflammation (Lamkanfi et al., 2009; Koh et al., 2011) and oxidative stress-associated brain injury (Nazaroglu et al., 2009), little is known about the impact of sulfonylureas on the mental function of the disease it was originally designed to treat. To determine whether the sulfonylurea, glyburide, positively impacts HFD-induced cognitive impairment and anxiety-like behaviors in young mice, such behaviors were examined in animals 1, 3 and 6 weeks post-weaning fed with a low-fat or HFD. Thus, this pre-clinical study examines the role of glyburide as a deployable countermeasure to combat brain dysfunction associated with the early-life ingestion of a HFD.

MATERIALS AND METHODS

Animals

The use of animals was in accordance with the Institutional Animal Care and Use Committee (IACUC) approved protocols at the University of Illinois Urbana, IL, USA. C57BL/6J male mice (3–4 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Mice were placed on experimental diet 1 week after (between 4–5 weeks of age prior to puberty) arrival to allow for acclimation. Mice were group-housed (8 per cage), unless otherwise noted, in shoebox cages (length 46.9 cm; width 25.4 cm; height 12.5 cm) and allowed free access to food and water. Housing temperature (72°F) and humidity (45–55%) were controlled as was a 12/12 h reversed dark-light cycle (light = 1000–2200 h). All behavioral and biochemical experiments were performed in the dark cycle and separate cohorts to eliminate repeated measures as a factor. Individual mice were used in a single behavioral test. Total number of mice used was 396.

Diets, Weights, Blood Glucose

Mice were initially fed a standard chow of NIH-31 modified open formula (Teklad 7013, Madison, WI, USA) containing 18% calories from protein, 6.2% from fat and 45% from carbohydrates. Mice were then transferred to a feed of open source uniform-base diets, for respective studies, containing either 10% calories from fat (low-fat diet [LFD]; D12450B, Research Diets, New Brunswick, NJ, USA) or 60% calories from fat (HFD; D12492, Research Diets, New Brunswick, NJ, USA). Both diets provided 20% calories from protein. Mouse weight was recorded for the respective weeks using an Adventurer Pro digital scale (Ohaus, Parsippany, NJ, USA). Blood glucose

testing results were recorded for the respective weeks by fasting mice for 12 h during their light cycle and sampling tail blood. For glyburide studies, blood glucose testing was conducted immediately post-behavior testing with *ad libitum* access to food. Glucose was quantified by using an AlphaTRAK blood glucose monitoring system (Abbott Laboratories, North Chicago, IL, USA).

Food Intake

As previously described (York et al., 2012), mouse cohorts used to determine food intake were individually housed for up to 6 weeks. Food intake was calculated daily as the difference in weight of food in the feed bowl before and after removal from the food intake arena.

Plasma Non-Esterified Fatty Acids (NEFAs)

As described previously (Moon et al., 2014), mice were euthanized and blood collected via cardiac puncture using BD Microtainer Tubes with Lithium Heparin (BD Diagnostics, Franklin Lakes, NJ, USA). Blood was centrifuged at 8000× g for 10 min at 4°C. Supernatant was collected and analyzed. The resultant plasma non-esterified fatty acids (NEFA) were measured on an AU 680 Chemistry System (Beckman Coulter, Brea, CA, USA) using an enzymatic colorimetric NEFA test kit (Wako, Richmond, VA, USA). This kit is designed to measure total NEFA levels excluding short-chain fatty acids.

Injectables

Glyburide (6.6 mg/kg/mouse; Sigma-Aldrich, St. Louis, MO, USA) was administered IP as described (Chiu et al., 2014) immediately prior to novel object training for mice at 1, 3 and 6 weeks on diet. Mice were injected 4 h prior to elevated zero maze (EZM) measured in mice on diet for 3 weeks only.

Novel Object Recognition (NOR)

Testing was performed as described in the studies by Chiu et al. (2012) and York et al. (2012). In brief, group housed mice were transferred to a shoebox-style training arena (26 cm × 48 cm × 21 cm) containing two identical objects (LEGO toys in distinct configurations) on one side of the arena. Mice were allowed to investigate the objects for 24 h with food provided *ad libitum*. After training, mice were returned to their home cage for 1 h. After the 1 h refractory period, subject mice were transferred to individual testing arenas, without food but with bedding, where they were presented with one familiar object and one novel object in a spatial location comparable to training. Mouse exploration was video recorded for 5 min and evaluated by using EthoVision XT 7 video tracking software (Noldus Information Technology, Leesburg, VA, USA). A discrimination index was used to determine cognition and calculated as the amount of time spent examining the novel object divided by the total time spent investigating both objects.

Object Location Recognition (OLR)

Testing was performed as described (York et al., 2012) and was similar to novel object recognition (NOR) except that upon testing the subject mouse was re-exposed to two familiar objects (LEGO toys) where one was placed at the opposite end from training. Spatial clues were placed on the outside of the cage to assist spatial determination. As above, mouse exploration was video recorded for 5 min and evaluated by using EthoVision XT 7 video tracking software. A discrimination index was used to determine cognition and calculated as the amount of time spent examining the object in a novel location divided by the total time spent investigating both objects.

Elevated Zero-Maze

Testing was performed as described in the study by York et al. (2012). In brief, group-housed mice were individually housed for 24 h. For testing, subject mice were individually placed within the high walls of an EZM (57.15 cm outer diameter, 6 cm track, 72 cm from the floor). The maze was composed of four quadrants with two areas having high walls (14 cm tall) and two areas without walls. Mouse exploration was video recorded for 5 min and evaluated by using EthoVision XT 7 video tracking software. Time spent in the open quadrants was defined as at least 50% of the body being outside of the high-walled areas.

Open Field Test

As above, group-housed mice were individual housed for 24 h. Mice were individually tested by placing subject mice in a lit novel open field arena (66 cm × 45.7 cm × 22.9 cm) generating a 9 cm shadow from respective side walls (York et al., 2012). Mouse exploration was video recorded for 5 min and evaluated by using EthoVision XT 7 video tracking software. Time spent in the open area was equated to time spent in the non-shadowed areas.

Glutathione Assay

As previously described, PBS perfused brain regions were frozen in liquid nitrogen then freeze fractured (Kaczmarczyk et al., 2013) in reaction buffer containing 50 mM NaCl (Fisher Scientific, Fair Lawn, NJ, USA), 1 mM EDTA, 50 mM HEPES, pH 7.0 (USB Corporation, Cleveland, OH, USA) using the TissueLyser II (Qiagen, Valencia, CA, USA) at a rotational frequency of 30 s^{-1} for 2 min. Lysates were centrifuged at 10,000× g for 15 min at 4°C and the supernatant recovered. The supernatant was deproteinated with an equal volume of metaphosphoric acid (Sigma-Aldrich, St. Louis, MO, USA) and vortexing. Samples were re-centrifuged at 8000× g for 5 min. Supernatant and pellets were saved. Glutathione, both reduced and oxidized, was determined using the Glutathione Assay Kit (Cayman Chemical, Ann Arbor, Michigan) following the manufacturer's instructions. Glutathione (GSH) and glutathione disulfide (GSSG) were quantified using an ELx800 Absorbance Microplate Reader (BioTek Instrument, Winooski, VT, USA) at 405 nm in 5 min intervals for 30 min. Protein precipitates were eluted with

reaction buffer and quantified using the DC Protein Assay (Bio-Rad, Hercules, CA, USA).

Quantitative PCR (qPCR)

Brain regions were dissected from PBS perfused whole brains and RNA isolated (York et al., 2012). RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (PN 4368813; Applied Biosystems, Foster City, CA, USA). The TaqMan Gene Expression primers used were: IL1R2 (Mm00439622_m1), BDNF (MM01334042_m1), Arc (Mm01204954_g1), iNOS (Mm00440502_m1), eNOS (Mm00435217_m1), casp1 (Mm00438023_m1), TXNIP (Mm01265659_g1), and superoxide dismutase (SOD1; Mm01344233_g1). Quantitative PCR (qPCR) was performed on a 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). To compare gene expression, a parallel amplification of endogenous RPS3 (Mm00656272_m1) was performed. Reactions with no reverse transcription and no template were included as negative controls. Relative quantitative evaluation of target gene to RPS3 was performed by comparing the values of ΔCts, where Ct is the threshold concentration.

Statistical Analysis

Data analysis was conducted using Sigma Plot11.2 (Systat Software, Chicago, IL, USA). Body weight, fasting blood glucose (FBG), plasma NEFA, food intake, NOR and object location recognition (OLR) total investigation, EZM performance, open field testing (OFT), and PCR analysis used one-way analysis of variance (ANOVA) to define the main effects followed by Tukey adjustment. All experiments with NOR and OLR discrimination index analyzed using a one-sample *t*-test comparing novel object preference to chance level of 0.5. The Kruskal-Wallis one-way ANOVA on ranks was used for GSH:GSSG ratio for analyzing the main effect of diet and treatment in glyburide experiment, due to variance within groups. Glyburide experiments for blood glucose were analyzed by two-way ANOVA and time spent in open of EZM and closed arm entries by one-way ANOVA test to determine the main effects of diet and treatment followed by a Tukey adjustment. Statistical significance was assumed at $p < 0.05$ and all data are presented as means ± SEM.

RESULTS

HFD Feeding for 3 Weeks Increases Body Weight and Blood Glucose Without Impacting Plasma NEFA Concentrations, Food Ingestion, and Inflammatory Gene Expression

To delineate the physiologic impact of the diets administered, the aforementioned biometrics were examined after 1, 3 and 6 weeks of feeding. When HFD-mice were compared to LFD-mice at 3 and 6 weeks post feeding, there was a 30% and 42% increase, respectively, in FBG levels. These findings correlated with a 13.4% and 28.4% rise in body weight, respectively (see **Table 1**). Interestingly, food intake was comparable in HFD-mice and LFD-mice, but this resulted in a 37%, 28%, and 14% increase in calories ingested in HFD-mice after 1, 3 and 6 weeks of feeding, respectively (see **Table 1**). Since HFD-associated brain-based inflammation is implicated in cognitive impairment, biomarkers of pro-inflammation were examined (**Table 2**). Interestingly, only hippocampal iNOS gene transcripts in HFD-mice showed an upregulation (99% vs. LFD-mice).

HFD-Mice Develop Object Memory Impairment and Transient Anxiety-Like Behaviors

HFD-mice and LFD-mice were examined after 1, 3 and 6 weeks of diet using a NOR task (**Figure 1A**). NOR is an effective measure of hippocampal-independent memory (Wan et al., 1999; Brown and Aggleton, 2001; McGaugh, 2004). At 1 and 3 weeks of diet, HFD-mice showed no preference for a novel object while LFD-mice showed preference for novel objects over that of the familiar ones (one-sample *t*-test: 1 week diet (LFD)- $P < 0.001$, 3 week diet (LFD)- $P < 0.022$; **Figure 1A**). After 6 weeks of diet, however, HFD-mice developed novel object preference which was similar to that of LFD-mice (one-sample *t*-test: 6 week diet (LFD)- $P < 0.001$, 6 week diet (HFD)- $P = 0.005$; **Figure 1A**). As an additional control, LFD-mice and chow-fed mice were compared. After 1 week of feeding, NOR performance in chow-fed and LFD-mice were comparable (one-sample *t*-test: SC- $P = 0.002$, LFD- $P = 0.001$; **Figure 1B**). HFD-mice and LFD-mice demonstrated similar total object exploration times

TABLE 1 | Body weight (g), blood glucose (mg/dL), plasma NEFA (mEq/L), food intake (g/d), and energy intake (kcal/g/d) of mice fed with LFD or HFD.

	Time on diet					
	1 week		3 week		6 week	
Diet	**LFD**	**HFD**	**LFD**	**HFD**	**LFD**	**HFD**
Body weight	17.9 ± 0.4^a	19.3 ± 0.9^b	20.9 ± 0.4^a	23.7 ± 0.5^b	22.5 ± 0.5^a	28.9 ± 0.5^b
FBG	129.9 ± 8.2	164.1 ± 17.8	144.0 ± 10.1^a	187.1 ± 6.9^b	146.3 ± 16.2^a	208.0 ± 16.0^b
Plasma NEFA	0.784 ± 0.03	0.662 ± 0.23	0.633 ± 0.06	0.742 ± 0.04	0.638 ± 0.87	0.839 ± 0.10
Food intake	3.20 ± 0.6	3.23 ± 0.08	3.17 ± 0.17	2.98 ± 0.15	3.42 ± 0.7	2.88 ± 0.12
Energy intake	12.31 ± 0.18^a	16.92 ± 0.41^b	12.21 ± 0.18^a	15.59 ± 0.41^b	13.2 ± 0.18^a	15.1 ± 0.41^b

Results are expressed as mean ± SEM, n = 4–30 per group. One-way ANOVA revealed main effect of diet (P < 0.05). Letters within rows indicate significant differences.

(one-way ANOVA: 1 week diet- $F_{(1,22)} = 0.08$; $P = 0.785$, 3 week diet- $F_{(1,22)} = 1.22$; $P = 0.281$, 6 week diet- $F_{(1,22)} = 0.317$; $P = 0.579$; **Figure 1D**).

When hippocampal-memory was examined using OLR (Kesner et al., 1993; Broadbent et al., 2004; Jablonski et al., 2013) a persistent memory impairment was identified in HFD-mice compared to that of LFD-mice (**Figure 1B**). After 1 week of diet OLR was not impacted in HFD-mice (one-sample *t*-test: 1 week diet (LFD)- $P < 0.001$, 1 week diet (HFD)- $P = 0.018$; **Figure 1C**). However, after a period of 3 and 6 weeks of diet, HFD-mice lacked preference for the novel object whereas LFD-mice were able to distinguish between novel and familiar in the OLR task (one-sample *t*-test: 3 week diet (LFD)- $P = 0.048$, 6 week diet (LFD)- $P < 0.001$; **Figure 1C**). As above, diet did not impact combined object exploration (one-way ANOVA: 1 week diet- $F_{(1,12)} = 1.870$; $P = 0.197$, 3 week diet- $F_{(1,14)} = 1.281$; $P = 0.277$, 6 week diet- $F_{(1,22)} = 0.565$; $P = 0.460$; **Figure 1E**).

To explore the impact of a HFD on anxiety-like behavior mice were tested in an EZM. After 3 weeks of diet, time spent in the center of the EZM was reduced in HFD-mice compared to the time spent by LFD-mice (one-way ANOVA: $F_{(1,30)} = 10.333$; $P = 0.003$; **Figure 1F**). After 1 or 6 weeks of diet, no difference between LFD-mice and HFD-mice was observed. To further explore if a HFD engendered trait as opposed to state anxiety (Moon et al., 2015), the OFT was utilized (Prut and Belzung, 2003). HFD-mice spent a decreased amount of time in the open area of the OFT after 3 weeks of diet when compared to LFD-mice (one-way ANOVA: $F_{(1,23)} = 4.446$; $P = 0.047$; **Figure 1G**) and a similar amount of time in open after 1 or 6 weeks of diet.

The GSH:GSSG Ratio in the Amygdala and Hippocampus is Reduced by an HFD

Since antioxidant capacity can impact memory (Alzoubi et al., 2013; Xu et al., 2014) brain GSH and GSSG was examined. After 3 weeks of diet, there was a 54% and 43% increase in the GSH:GSSG ratios in the amygdala and hippocampus, respectively, when LFD-mice were compared to HFD-mice (Kruskal-Wallis one-way ANOVA: amygdala- $Q = 2.935$; $P = 0.038$; **Figure 2A**, hippocampus- $Q = 2.449$; $P = 0.014$; **Figure 2B**). GSH:GSSG ratios were not different in LFD-mice and HFD-mice after 1 and 6 weeks of diet. Glyburide, which is known to increase antioxidant capacity (Patel et al., 1987; Chugh et al., 2001), raised the GSH:GSSG ratio 32.3% in the

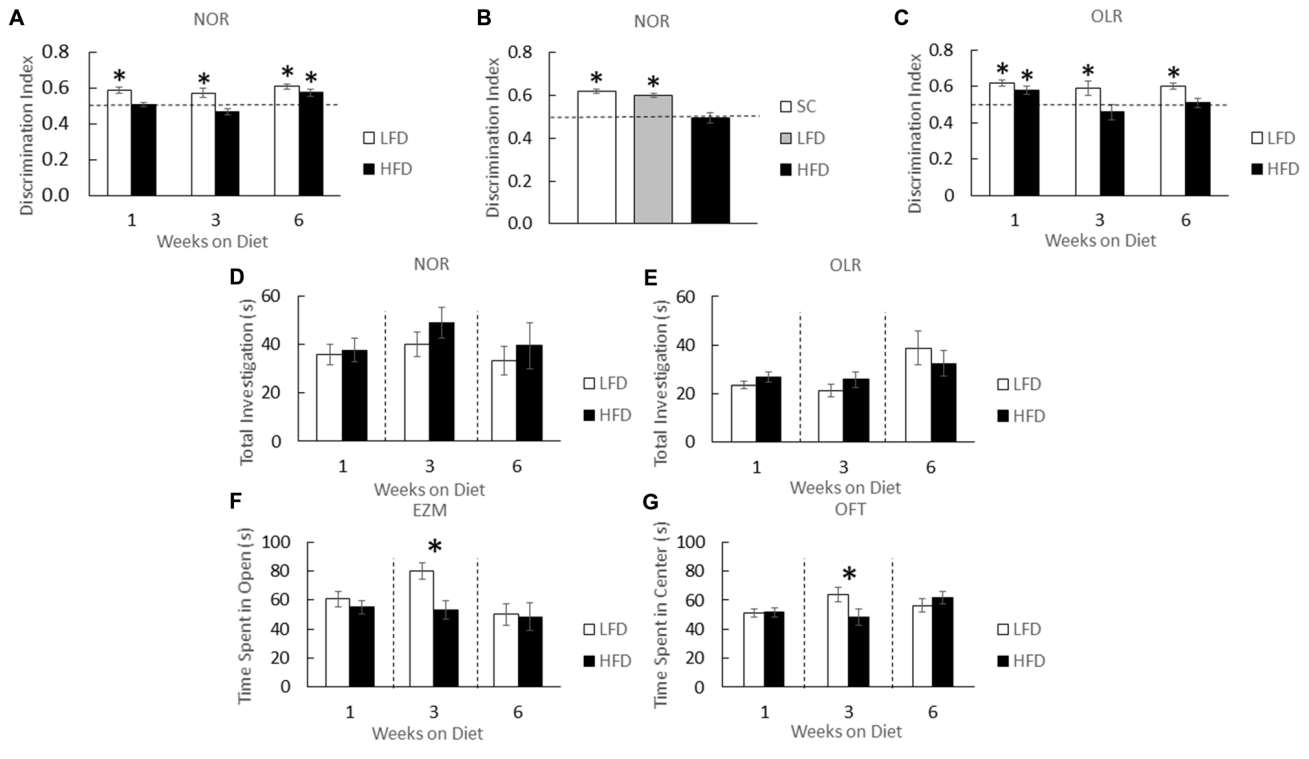

FIGURE 1 | High-fat diet (HFD) mice develop object memory impairment and transient anxiety-like behaviors. HFD-mice (HFD), low-fat diet (LFD)-mice, and/or standard chow-mice (SC) underwent novel object recognition (NOR) at the times indicated (NOR) **(A)**, and at 1 week after diet **(B)**. HFD-mice (HFD) and LFD-mice (LFD) underwent object location recognition (OLR) testing at the times indicated **(C)**. Total time spent investigating both novel and familiar objects were determined for NOR **(D)** and OLR **(E)**. HFD-mice and LFD-mice were examined using elevated zero maze (EZM) **(F)** and the open field test (OFT) **(G)**. Discrimination index for NOR and OLR was defined as time spent exploring novelty divided by time spent investigating both objects. All results are expressed as means ± SEM; $n = 4$–16, values with an asterisk are significant at $p < 0.05$, using one-sample *t*-test with novel object preference compared with chance level of 0.5 and one-way analysis of variance (ANOVA).

TABLE 2 | Impact of LFD or HFD feeding on gene expression in the hippocampus and amygdala after 3 weeks of feeding.

	Amygdala		Hippocampus	
Gene	**LFD**	**HFD**	**LFD**	**HFD**
Arc3.1	1.000 ± 0.140	0.759 ± 0.436	1.000 ± 0.250	0.751 ± 0.216
iNOS	1.000 ± 0.157	0.783 ± 0.147	1.000 ± 0.177[a]	1.993 ± 0.314[b]
eNOS	1.000 ± 0.087	0.965 ± 0.144	1.000 ± 0.119	1.277 ± 0.088
IL1-R2	1.000 ± 0.124	0.879 ± 0.316	1.000 ± 0.103	0.981 ± 0.201
Casp1	1.000 ± 0.035	0.856 ± 0.152	1.000 ± 0.152	0.936 ± 0.068
TXNIP	1.000 ± 0.108	1.075 ± 0.034	1.000 ± 0.196	0.996 ± 0.158
SOD1	1.000 ± 0.121	0.811 ± 0.044	1.000 ± 0.126	1.114 ± 0.080
BDNF	1.000 ± 0.112	1.333 ± 0.130	1.000 ± 0.168	1.092 ± 0.246

Results are expressed as relative fold change in mRNA expression (mRNA), means ± SEM; n = 4. Results within individual rows without a common superscript letter are significantly different. Letters within rows indicate significant main effect of diet.

amygdala, in HFD-mice after 3 weeks of diet (Kruskal-Wallis one-way ANOVA: Amygdala- $Q = 2.748$; $P = 0.050$; **Figure 2C**).

Glyburide Reduces Anxiety-Like Behaviors Associated with a HFD After 3 Weeks of Diet

After glyburide treatment, HFD-mice and LFD-mice spent equivalent times exploring the open and closed arms of the EZM (one-way ANOVA: Gly- $F_{(1,35)} = 0.277$; $P = 0.602$; **Figure 3A**; $F_{(1,35)} = 0.166$; $P = 0.686$; **Figure 3B**). Importantly, injection of saline did not prevent anxiety-like behaviors associated with a HFD (one-way ANOVA: Sal- $F_{(1,33)} = 4.460$; $P = 0.043$; **Figure 3A**; $F_{(1,33)} = 6.264$; $P = 0.018$; **Figure 3B**). HFD-mice and LFD-mice did not differ in distance moved in the EZM (see **Figure 3C**). As expected, *post hoc* analysis showed that glyburide resulted in a 16.5% reduction in non-FBG levels (two-way ANOVA: HFD (Sal vs. Gly)- $Q = 4.506$; $P = 0.003$; **Figure 3D**) and was associated with an overall drug effect (two-way ANOVA: Sal vs. Gly- $F_{(1,41)} = 9.418$; $P = 0.004$; **Figure 3D**).

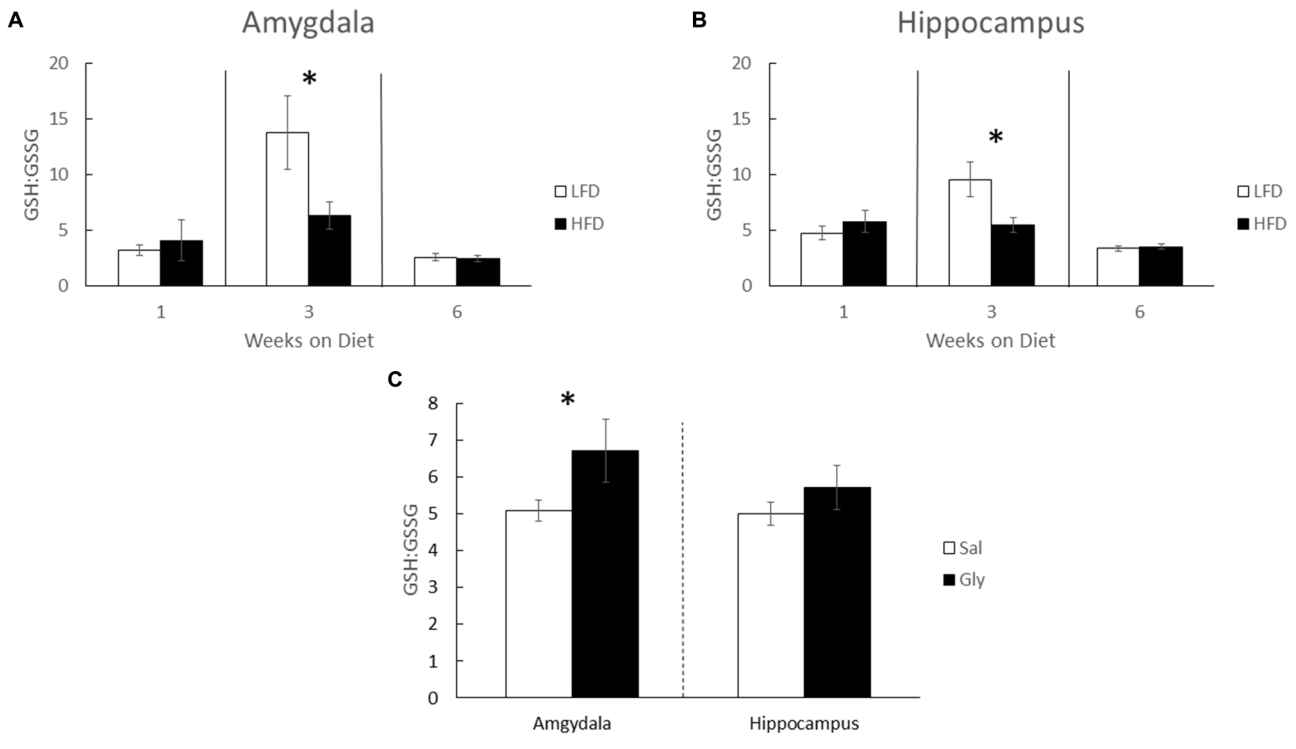

FIGURE 2 | The GSH:GSSG ratio in the amygdala and hippocampus is reduced by a HFD. Glutathione (GSH) and glutathione disulfide (GSSG) were measured in HFD and LFD-mice and presented as GSH:GSSG in both the amygdala **(A)** and hippocampus **(B)**. GSH:GSSG ratios in amygdala and hippocampus of HFD-mice injected with glyburide **(C)**. Results are expressed as means ± SEM, $n = 5$–10, values with an asterisk are significant, $p < 0.05$ using Kruskal-Wallis one-way ANOVA on ranks.

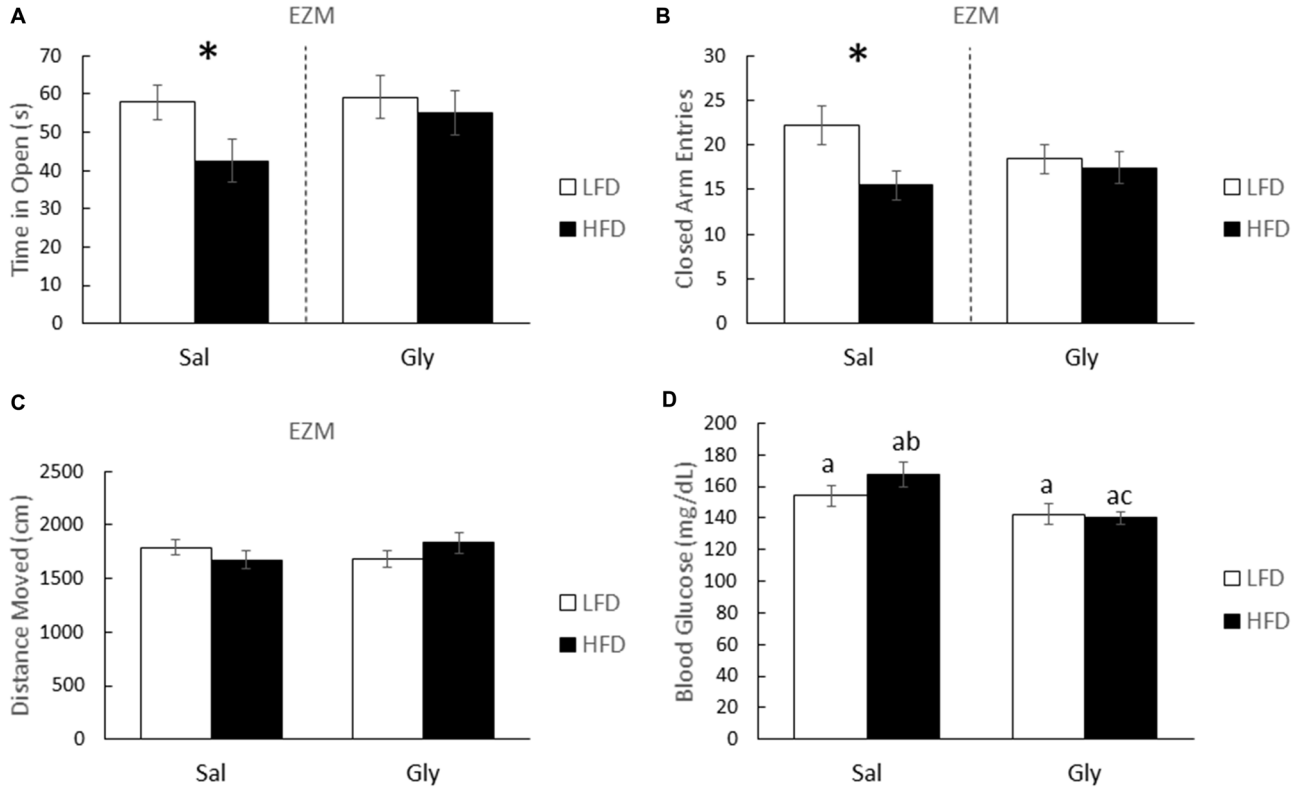

FIGURE 3 | Glyburide reduces anxiety-like behaviors associated with a HFD after 3 weeks of diet. HFD and LFD-mice were administered either saline or glyburide and examined after 4 h in an EZM. Time in the open **(A)** and closed arms **(B)** are presented. Similarly treated HFD- and LFD-mice were examined for locomotion **(C)**, and post-behavior blood glucose **(D)**. All results are expressed as mean ± SEM; $n = 10$–18, values without a common subscript or asterisk are different (a vs. b, $p < 0.05$) with **(A,B)** analyzed using one-way ANOVA and **(C,D)** using two-way ANOVA.

Memory is Improved by Glyburide in HFD-Mice After 3 Weeks of Diet

At 1, 3 and 6 weeks of diet, HFD-mice were unable to discriminate novelty in the NOR and OLR tasks (one-sample *t*-test: 1 week NOR (LFD-Sal)- $P = 0.013$, 1 week (HFD-sal)- $P = 0.249$; **Figure 4A**; 3 week NOR (LFD-Sal)- $P = 0.008$, 3 week (HFD-Sal)- $P = 0.089$; **Figure 4B**; 3 week OLR (LFD-Sal)- $P < 0.001$, 3 week (HFD-Sal)- $P = 0.145$; **Figure 4C**; 6 week OLR (LFD-Sal)- $P = 0.005$, 6 week (HFD-Sal)- $P = 0.644$; **Figure 4D**). Interestingly, glyburide restored novelty preference in HFD-mice in the NOR task after 3 weeks of diet (one-sample *t*-test: 3 week NOR (LFD-Gly)- $P = 0.025$, (HFD-Gly)- $P < 0.001$; **Figure 4B**), but not at 1 or 6 weeks of diet (one-sample *t*-test: 1 week NOR (LFD-Gly)- $P = 0.018$, 1 week (HFD-Gly)- $P = 0.165$; **Figure 4A**; 6 week OLR (LFD-Gly)- $P = 0.002$, 6 week (HFD-Gly)- $P = 0.915$; **Figure 4D**) and not for OLR (one-sample *t*-test: 3 week OLR (LFD-Gly)- P 0.014, 3 week (HFD-Gly)- $P = 0.351$; **Figure 4C**).

DISCUSSION

Overweight children are predisposed to social and emotional complications tied to overnutrition including depression, low self-esteem and learning problems (Mellbin and Vuille, 1989; Daniels et al., 2005). Origination of these learning problems is associated with inferior social skills (Dietz, 1998) and anxiety (Williams, 2001). In addition, controversy exists as to whether the overweight/obese phenotype is a root cause of childhood social and emotional problems or a sequela of resultant bullying (Eisenberg et al., 2003). In juvenile mice, a short-term (1-week) HFD feeding impairs behavior as we have previously shown (Kaczmarczyk et al., 2013) and as shown here (**Figure 1A**). Consistently, impaired NOR appears associated with short-term overnutrition, as opposed to impaired OLR which takes longer to manifest (3 weeks; **Figure 1B**). Interestingly, this early impairment in NOR is not associated with FBG or plasma NEFAs but does positively correlate with energy intake (**Table 1**). These findings support the contention that learning problems associated with overnutrition are not entirely linked to the overweight/obese body type and its negative perception by industrialized peoples (Murray et al., 2009; Holloway et al., 2011).

Another important finding is that impairment of OLR is longer lasting than impairment of NOR (**Figure 1**). The advantage of using NOR and OLR for memory testing is that they are easy to perform, involve a similar paradigm but test

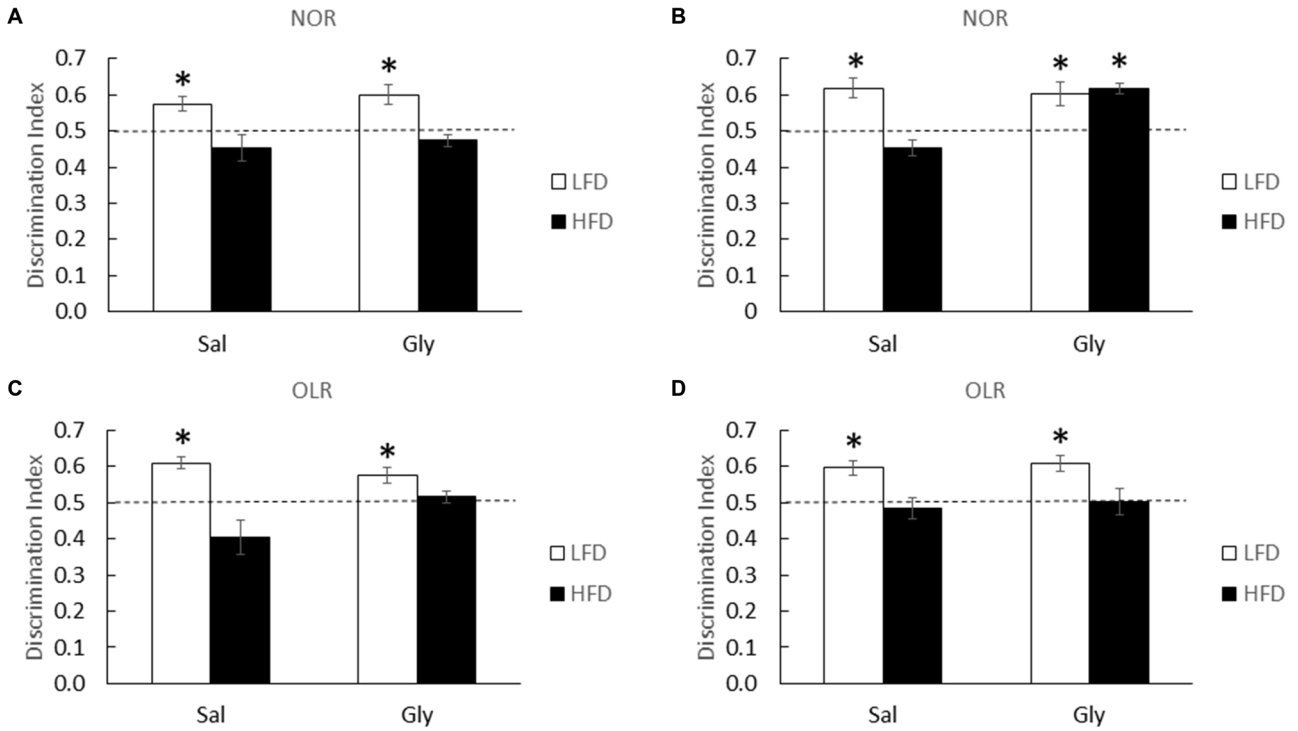

FIGURE 4 | Memory is improved by glyburide in HFD-mice after 3 weeks of diet. HFD and LFD-mice were administered either saline or glyburide after a 1 week of diet and examined using NOR testing **(A)**. HFD- and LFD-mice were administered either saline or glyburide after a 3 weeks of diet and examined using NOR **(B)** and OLR **(C)** testing. HFD and LFD mice were administered either saline or glyburide after a 6 weeks of diet and examined using OLR **(D)** testing. Discrimination index was defined as time spent exploring novelty divided by time spent investigating both objects. Results are expressed as means ± SEM; $n = 4$–6, values with an asterisk are significant at $p < 0.05$, using one-sample *t*-test with novel object preference compared with chance level of 0.5.

different types of memory. NOR is much more hippocampal-independent than is OLR (Wan et al., 1999; Brown and Aggleton, 2001; McGaugh, 2004). In contrast, OLR is more dependent on spatial memory and is, thus, more hippocampal-sensitive (Moses et al., 2005). Our findings are supported by recent work which shows that 23 weeks of HFD feeding in mice impairs OLR but not NOR (Heyward et al., 2013). In addition, we previously found that NOR was intact in HFD-mice on diet for 10–12 weeks (Lavin et al., 2011). Taken together, the clinical significance of these works is their relationship to dementia and type 3 diabetes (T3D; de la Monte and Wands, 2008).

In T3D, lack of brain-based insulin production or the presence of brain-based insulin resistance (Biessels et al., 1998; Lannert and Hoyer, 1998; Kodl and Seaquist, 2008) precipitates memory dysfunction with symptomatology that overlaps that of Alzheimer's disease (Gasparini et al., 2001; Steen et al., 2005; Deng et al., 2009). Insulin is required for new memory creation by facilitating synaptic plasticity (van der Heide et al., 2006) and HFD-induced insulin/IGF-1 resistance (Spielman et al., 2014) which appears important to T3D (Watson and Craft, 2004; Vardy et al., 2007), especially in the hippocampus (McNay et al., 2010; Grillo et al., 2015). Given that HFD-mice have an elevation in FBG (**Table 1**), it is not surprising that impairment in hippocampal-dependent memory would be coincident (**Figure 1**). Furthermore, HFD-induced elevations in FBG are caused by insulin resistance (Hirosumi et al., 2002). What was not anticipated is the rapidity by which a HFD impairs hippocampal memory. Although Beilharz et al. (2014) found a similar phenomenon in their study, they concluded that dietary sugar was the critical factor. Sugar availability (∼6.7% sucrose) was low in the diets used here. Thus, the hippocampal memory impairment observed appears dependent on fat content not on the sugar content.

Previous work by André et al. (2014) showed cognitive impairment and anxiety-like behaviors in mice fed with HFD. In their study, the postulated mechanism was tied to pro-inflammatory cytokines and indoleamine 2, 3-dioxygenase (IDO) activity. In contrast, Kaczmarczyk et al. (2013) demonstrated no brain-based pro-inflammation in HFD-exposed mice and that IDO knockout mice were as susceptible to HFD-induced memory impairment as were the controls. A key difference between the studies of Kaczmarczyk et al. (2013) and André et al. (2014) was the length of diet which was marked shorter in the latter's study. Finally, Del Rio et al. (2016) fed juvenile mice a short-term HFD resulting in cognitive deficits and an anti-depressive phenotype. Anxiety-like behaviors were not observed, but the diet that Del Rio et al. (2016) used contained 45% fat as opposed to the 60% used in this study.

Why a HFD impacts hippocampal-independent functions like NOR and anxiety-like behaviors (**Figures 1A,D,E**) rapidly

and transiently is not clear. Previous work shows that oxidative stress is deleterious to brain function (Shukitt-Hale, 1999; Dröge and Schipper, 2007). Specifically, anxiety-like behaviors manifest in mice when the GSH:GSSG ratio is reduced and cytosolic reactive oxygen species are increased (Llorente-Folch et al., 2013). While **Figure 2** shows a drop in the GSH:GSSG ratio in HFD-mice compared to LFD-mice, this result appears as a consequence of a HFD-induced suppression of a rise in the GSH:GSSG ratio. Interestingly, 7 weeks marks the approximate sexual maturation of male C57BL/6J mice (range 6–8 weeks; Fox and Witham, 1997). Thus, male sexual maturation appears to be accompanied by a spike in the brain GSH:GSSG ratio (**Figure 2**). Mechanistically, this observation may be tied to an increase in testosterone since brain GSH in mice (Atroshi et al., 1990) is augmented by its administration. Additionally, sexual maturation increases the brain-active antioxidant dehydroepiandrosterone (DHEA; Hopper and Yen, 1975; McIntosh and Berdanier, 1991; Aly et al., 2011), and DHEA favorably impacts the hippocampus in neurodegenerative diseases (Charalampopoulos et al., 2008). Glyburide, especially during uncontrolled diabetes, increased reduced glutathione (Chugh et al., 2001). Such findings lend credence to our results demonstrating that glyburide increased the GSH:GSSG ratio in the brain. However, this effect appears brain-region specific, when the amygdala and hippocampus are compared.

As **Figures 3**, **4** illustrate, an acute single dose of glyburide prevents HFD-induced memory impairment and anxiety-like behaviors indicating a role for this sulfonylurea in overnutrition-associated brain dysfunction in juvenile mice. Previously, we demonstrated that glyburide can block the activation of brain caspase-1 triggered by adenosine (Chiu et al., 2014) which is a key biologic in hypoxia-induced anterograde amnesia (Chiu et al., 2012). Since overnutrition is associated with endoplasmic reticulum (ER) stress (Mollica et al., 2011), it is theorized that the overnutrition-associated oxidative stress causes dysregulated purine metabolism (Al-Rubaye and Morad, 2013). Subsequent cellular release of ATP and its precursors rapidly increases the interstitial concentration of adenosine (Chiu and Freund, 2014) which through the A2A adenosine receptor triggers neuronal hyperpolarization in a K_{ATP} channel dependent manner (Popoli et al., 2002). Hence the relevance of adenosine to neurodegeneration and sleep (Portas et al., 1997; Stone, 2005) and the interest in caffeine and its derivatives as neuroprotectants and CNS stimulants (Schwarzschild et al., 2002; Barranco Quintana et al., 2007). Interestingly, a recent study identified the P2X7 receptor as a potential link between anxiety in rats and a HFD (Dutheil et al., 2016). This study, however, demonstrated HFD-induced brain inflammation as the potential mechanism. As noted, brain-based inflammation was not observed here likely due to our use of a short-term HFD feeding. Dutheil et al. (2016) fed rats an HFD for 16 weeks. Thus, different pathways are likely at play during the early (3 week) and late (12–16 week) manifestations of anxiety associated with a HFD.

Glyburide can also boost SOD and catalase activity (Nazaroglu et al., 2009) mitigating oxidative stress in a canonical fashion. However, due to its limited impact on blood glucose (**Figure 3**), this mechanism seems unlikely In HFD-mice fed with fat for at least 6 weeks, memory-impairment may be due to hyperglycemia and the role blood glucose has in elevating extracellular amyloid-β protein in the hippocampus (Macauley et al., 2015).

In summary, short-term HFD-feeding induces both cognitive impairment and anxiety-like behaviors which parallel symptoms seen in childhood obesity. These results coincide with a reduction in antioxidant capacity as exhibited by a suppressed GSH:GSSG ratio. Importantly, we demonstrated that administration of glyburide ameliorated hippocampal-independent brain function in HFD-mice. Unfortunately glyburide did not influence the hippocampal-sensitive memory task tested. Concordantly, glyburide increased the GSH:GSSG ratio in the amygdala but not in the hippocampus. Therefore, glyburide appears to selectively increase antioxidant capacity in the brain resulting in mitigation of hippocampal-independent impairments linked to a short term exposure to a HFD. These results suggest a unique use for glyburide in the prevention of anxiety and hippocampal-independent cognitive impairment. While the exact mechanism of action requires further study, antioxidant capacity appears to be an important glyburide target.

AUTHOR CONTRIBUTIONS

KAK, JKB, MMP, and VLT contirbuted to the acquisition, analysis, and interpretation of the behavioral and biochemical data. SJG contributed to the design, acquisition, analysis, and interpretation of the whole of this manuscript. AET contributed to the design, interpretation, and revision of all aspects of this manuscript. GGF contributed to the design, analysis and interpretation of all aspects of this manuscript. All authors worked on drafting and reviewing this manuscript, and approved the final version for publication.

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frontiers in Behavioral Neuroscience

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Switching Adolescent High-Fat Diet to Adult Control Diet Restores Neurocognitive Alterations

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In addition to metabolic and cardiovascular disorders, obesity is associated with adverse cognitive and emotional outcomes. Its growing prevalence in adolescents is particularly alarming since this is a period of ongoing maturation for brain structures (including the hippocampus and amygdala) and for the hypothalamic-pituitary-adrenal (HPA) stress axis, which is required for cognitive and emotional processing. We recently demonstrated that adolescent, but not adult, high-fat diet (HF) exposure leads to impaired hippocampal function and enhanced amygdala function through HPA axis alteration (Boitard et al., 2012, 2014, 2015). Here, we assessed whether the effects of adolescent HF consumption on brain function are permanent or reversible. After adolescent exposure to HF, switching to a standard control diet restored levels of hippocampal neurogenesis and normalized enhanced HPA axis reactivity, amygdala activity and avoidance memory. Therefore, while the adolescent period is highly vulnerable to the deleterious effects of diet-induced obesity, adult exposure to a standard diet appears sufficient to reverse alterations of brain function.

Keywords: hippocampus, amygdala, obesity, learning, adolescence, neurogenesis, rat

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INTRODUCTION

Overconsumption of energy-dense, palatable foods is a recognized source of weight gain and obesity. Obesity is a serious public health challenge, causing physical disability and premature death as well as cognitive disturbances in adults (Nilsson and Nilsson, 2009; Sellbom and Gunstad, 2012; Francis and Stevenson, 2013) and adolescents (Cserjési et al., 2007; Li et al., 2008; Khan et al., 2015). While the incidence of overweight and obesity is increasing in all age ranges, it is particularly notable in adolescents (Ogden et al., 2012). This is alarming since adolescence is a period of neurobehavioral shaping required for life-long cognitive processing (Spear, 2000).

Adolescence is particularly sensitive to environmental challenges, like diet, and there is now compelling evidence that an energy-dense diet is more harmful when consumed during adolescence than in adulthood. The overconsumption of sugar or fat throughout adolescence, but not adulthood, is associated with changes in reward-related behaviors in rats including deficits in motivation (Vendruscolo et al., 2010) and attenuated conditioned place preference induced via a palatable food reward (Privitera et al., 2011).

We and others have also demonstrated significant deleterious effects on hippocampal function specifically following adolescent consumption of a high-fat diet (HF) and/or high-sugar diet. Rodents that consume a diet supplemented with fat and/or sugar throughout adolescence, but not during adulthood, show impaired performance on a range of hippocampal-dependent behavioral tasks including object location memory (Valladolid-Acebes et al., 2013; Reichelt et al., 2015), spatial memory (Boitard et al., 2014; Hsu et al., 2015; Klein et al., 2016) and relational memory (Boitard et al., 2012). These cognitive deficits are associated with overexpression of hippocampal pro-inflammatory cytokines (Boitard et al., 2014; Hsu et al., 2015) and decreased levels of hippocampal neurogenesis (Boitard et al., 2012). Adolescent HF consumption (from weaning to adulthood) also leads to enhanced aversive memories and emotion-induced neuronal activation of the basolateral complex of the amygdala (BLA) as well as hypothalamo-pituitary-adrenal (HPA) axis deregulations. In contrast, these changes are not observed when the same diet is consumed in adulthood (Boitard et al., 2015). Taken together, these data illustrate that the adolescent period is especially sensitive to the effect of HF and high-sugar diets on memory.

There is now growing interest in determining whether the behavioral and neuronal modifications caused by an energy-dense adolescent diet are permanent. Therefore, in the present study, we investigated whether the neurobehavioral deficits induced by adolescent exposure to a HF could be reversed after removal of the HF. Rats were fed a HF immediately after weaning for 3 months and then were either maintained on the HF or switched to a control diet for an additional 3 months. At adulthood, the rats were tested on both hippocampal- (Morris Water Maze, MWM) and amygdala-dependent (conditioned odor avoidance, COA) memory tasks. A number of metabolic parameters were also measured. Critically, our results demonstrate that shifting to the control diet during adulthood was sufficient to reduce bodyweight and to partially restore all neurobehavioral and endocrine processes that were disrupted following adolescent HF consumption. This indicates that the detrimental neurocognitive effects associated with adolescent HF intake are reversible and can be either restored or maintained depending on the composition of adult diet.

MATERIALS AND METHODS

All experiments were conducted in agreement with the French (Directive 87-148, Ministère de l'Agriculture et de la Pêche) and international legislation (directive 2010-63, September 22, 2010, European Community) and were approved by the local ethical committee (agreement number 5012047-A).

Animals and Diets

Animals were 50 Wistar naïve male rats (Janvier, France) aged 3 weeks old on arrival. They were housed in groups of 2–4 in polycarbonate cages (48 cm × 26 cm × 21 cm) in an air-conditioned (22 ± 1°C) colony room maintained under a 12:12 light/dark cycle. Rats had *ad libitum* access to food and water and were weighed once a week from arrival to sacrifice. On arrival, rats were divided into three groups with similar weights. The first group received only a control diet (C; $n = 17$) offering 2.9 Kcal/g (consisting of 60% carbohydrate, mostly from starch, and 2.5% fat (A04 SAFE, Augy, France); group C), the second group received only a HF ($n = 15$) offering 4.7 Kcal/g (consisting of 24% (45% kcal) fat, mostly saturated fat from lard, and 41% (35% kcal) carbohydrate, with 20% (17.5% kcal) from sucrose (D12451, Research Diets, New Brunswick, NJ, USA); group HF), and the third group received HF from arrival for a duration of 12 weeks, i.e., throughout adolescent development (from weaning to adulthood; see Spear, 2000), directly followed by exposure to the control diet at adulthood (group HF-C; $n = 18$, see **Figure 1A**). One week before the behavioral task, rats were isolated in individual cages (35 cm × 23 cm × 19 cm), to perform behavioral and metabolic assessments under optimal conditions, and habituated to handling by the experimenter. Behavioral experiments were performed on adult rats, starting after 6 months of diet exposure (see **Figure 1A**).

Behavioral Tasks

Morris Water Maze (MWM)

Apparatus

A circular tank (150 cm in diameter and 50 cm high) was filled with opaque water (22 ± 2°C). A platform (10 cm diameter, 30 cm from the edge of the tank) was submerged 5 cm beneath the water surface and was not visible to the rats. Visual cues were provided on the walls of the room to allow spatial navigation. A camera wired to an automated tracking system (SMART v2.5.20, Panlab, Barcelona, Spain) recorded the rat's pathway and behavior.

Learning schedule

The MWM protocol began after 24 weeks (6 months) of diet intervention (**Figure 1A**). As previously described (Boitard et al., 2014), rats were trained to locate the platform for five consecutive days. Before the first trial, rats were placed on the submerged platform for 30 s. Rats were given six trials per day, with different starting locations on each trial, following a pseudo-random sequence. Then, each trial consisted in a swim followed by a 30 s rest on the platform. Rats that did not reach the platform within 90 s were guided to it. The inter-trial interval was 15 s. Latency to reach the platform, distance traveled and swimming speed were recorded.

Memory assessment

Memory was assessed via probe tests occurring 2 h and 4 days (Long-term memory (LTM) assessment) after the final training session. The platform was removed and rats were allowed to navigate in the water maze for 90 s. Latency to reach the target annulus, time spent in the quadrants (each representing $\frac{1}{4}$ of the maze) and the number of each annulus crossings (imaginary circular zones, one in each quadrant, the target annulus being the one where the platform was located during learning) were recorded (Maei et al., 2009). Since our previous results indicate

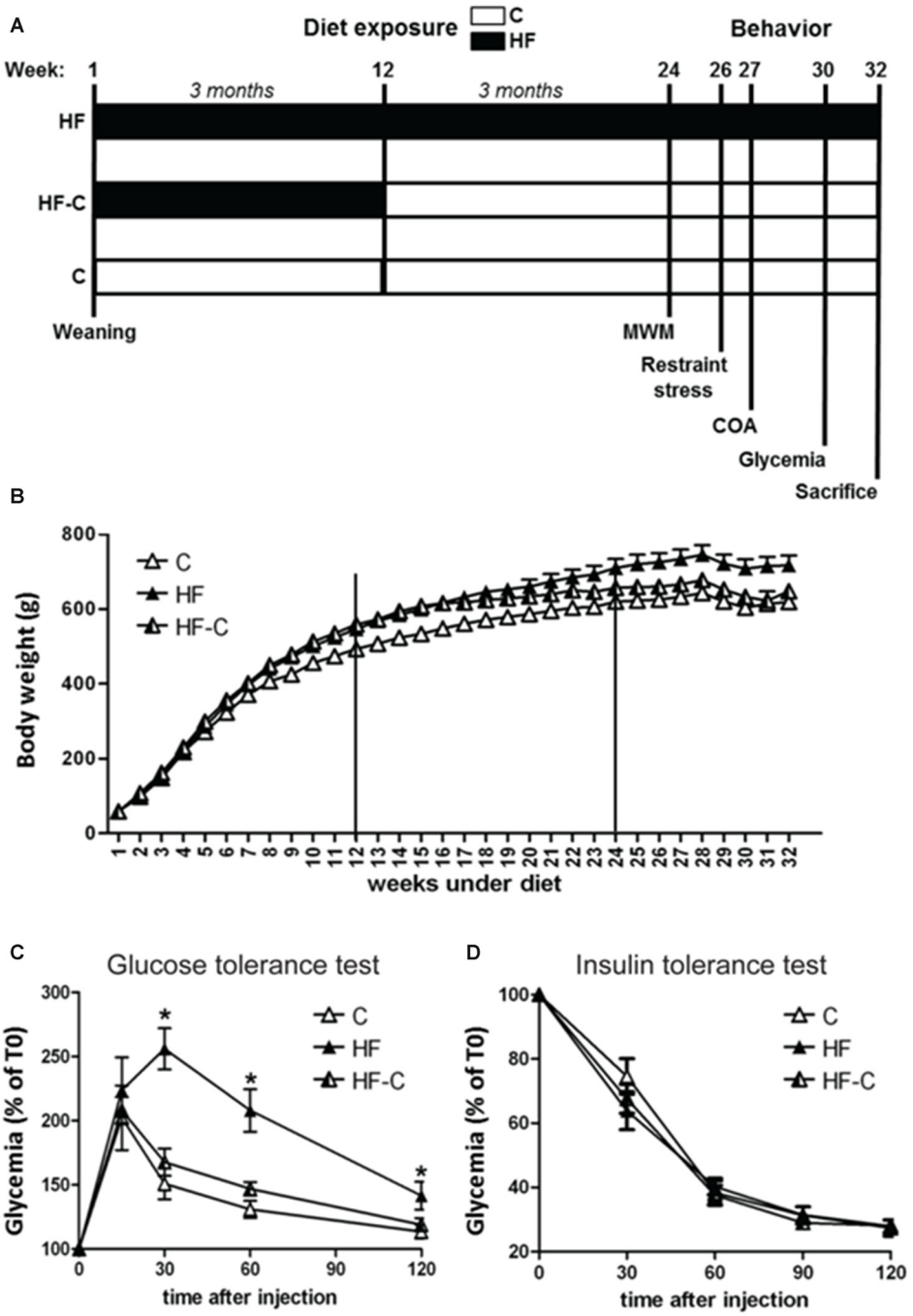

FIGURE 1 | (A) Timeline of the experiments. Diet regimes began at weaning. Rats in group high-fat diet (HF) were continuously exposed to the HF (black bars) from weaning to sacrifice. Rats in group HF-C were given the HF from weaning for 12 weeks (black bars) and were then shifted to the control diet (white bars) until sacrifice. Rats in the control group (C) were given access to the control diet from weaning until sacrifice. Behavioral assessment began after 24 weeks on the diet regime and rats were sacrificed after 32 weeks. **(B)** Bodyweight for each group from weaning to sacrifice. Rats were weighed once per week. **(C)** Glucose metabolism was assessed 30 weeks after starting the diets. Glucose levels were higher in rats maintained on the HF (black triangles) 30–120 min after glucose injection. $^*p < 0.05$ when compared to both C and HF-C groups. **(D)** Intra-peritoneal (i.p.) insulin tolerance test. Injection of insulin induced a similar decrease in blood sugar for all groups.

that HF mainly impacts the number of target annulus crossings (Boitard et al., 2014), we analyzed this parameter by comparing it to chance level (25%) using one sample *t*-tests. Rats failing to learn or accurately remember the location of platform during the test 2 h after training (threshold being less than 30 s to enter the target annulus) were removed from the analyses

(final number of animals per group: C: 9/17, HF: 10/15 and HF-C: 11/18).

Conditioned Odor Avoidance (COA)

The COA protocol began after 27 weeks (6.5 months) of diet intervention for all 50 rats (**Figure 1A**), as previously described (Boitard et al., 2015). Rats were water deprived 24 h before the start of the protocol and acclimated to a water deprivation regimen. Access to tap water was provided in a graded bottle (with 0.5 mL accuracy) for 15 min/day for 3 days between 09:00 and 11:00 in the home cage. On the acquisition day (day 4), rats had 15 min access to almond-scented water, composed of 0.01% benzaldehyde (Sigma Aldrich) diluted in tap water. This almond solution was chosen because previous studies indicated that its processing is mediated by its odor properties, not by its taste properties (Rusiniak et al., 1979; Desgranges et al., 2009). Indeed, anosmic rats were unable to reliably detect 2% almond-scented water (Rusiniak et al., 1979), whereas they performed as well as control for taste detection (Slotnick et al., 1997) indicating that 0.01% almond-scented water is processed by odor, but not taste, cues. The percentage of almond-scented water consumption during acquisition in respect to water consumption the day before was used to measure the strength of neophobic response. After a 30 min delay, rats received an intra-peritoneal (i.p.) injection of the visceral malaise-inducing drug, lithium chloride (LiCl, 0.075 M, 0.75% of bodyweight, 25 mg/kg; Sigma Aldrich), in order to induce the COA. For the next 2 days (day 5 and 6), rats had access to odorless tap water for 15 min/day to re-establish baseline water intake. Finally, COA was assessed on day 7 by providing access to the almond-scented water for 15 min. The percentage of almond-scented water consumption during test in respect to almond-scented water consumption during acquisition was used to measure the strength of the avoidance.

Metabolic Parameters

Glucose metabolism was assessed following the behavioral tasks, i.e., after a total of 30 weeks of diet exposure (**Figure 1A**). Glycemic level was measured in the morning after overnight fasting in all the rats and in response to either insulin or glucose i.p. injection (2UI/kg insulin for half of the rats and 20% glucose solution, 2 g/kg for the remaining rats). A blood drop provided by a tail nick allowed direct reading of the sugar blood level using Accu-Check® devices (Roche diagnostics). Blood sampling from the same wound occurred at the time of injection (T0), and 30, 60, 90 and 120 min after injection for the insulin tolerance tests and T0, 15, 30, 60 and 120 min for the glucose tolerance test. For tolerance tests, data are reported as baseline (% of T0) in order to minimize inter-individual variation and then the total area under the curve (AUC) was calculated.

Triglycerides, cholesterol, leptin and insulin levels were measured in a subset of rats (9–15 rats/group) in plasma samples obtained from blood collected at sacrifice (32 weeks of diet exposure, **Figure 1A**) using specific kits (Cholesterol RTU, Triglycerides enzymatic PAP 150, Biomérieux, France) or milliplex (Rat serum adipokine kit, RADPK-81K, Millipore, Billerica, MA, USA) as previously described (Boitard et al., 2012, 2014).

HPA Axis and Corticosterone Release

Corticosterone release in response to restraint stress was assessed 26 weeks (6.5 months) after the beginning of diet exposure in 7–8 rats/group (**Figure 1A**). Blood from each rat was quickly collected from a tail nick immediately before restraint stress (T0), directly after restraint stress (30 min after T0) and at two additional time points after the rat was returned to the home-cage (90 and 180 min after T0).

Corticosterone levels were also measured in a subset of rats (6–8 rats/group) after a systemic stressor (LiCl, 0.075 M, 25 mg/kg; Sigma Aldrich) used both to induce COA and to assess amygdala activity (see below). Blood sampling occurred 90 min after LiCl injection at the time of sacrifice (32 weeks of diet exposure, see **Figure 1A**).

Total corticosterone level was measured in plasma obtained from blood samples (centrifugation at 4000 rpm, 15 min, RT) by in-house radioimmunoassay (Richard et al., 2010). Briefly, after absolute ethanol steroid extraction from plasma samples, total corticosterone was measured by competition between cold corticosterone and radioactive corticosterone (3H) for a specific anti-corticosterone antibody provided by Dr. H. Vaudry (University of Rouen, France). The sensitivity of this technique is 0.3 μg/dl, with a 10% intra-assay and a 10% inter-assay variability. For the restraint stress-induced corticosterone response, the AUC was calculated in order to detect an overall effect and corticosterone levels.

Amygdala Activation (c-Fos Labeling)

Injection of LiCl, used as the aversive stimulus in the COA, leads to gastric malaise and induces a strong activation of the central nucleus (CeA) and a moderate activation of the basolateral nucleus of the amygdala (BLA; Yamamoto et al., 1997; Koh et al., 2003; Ferreira et al., 2006). We evaluated CeA and BLA activation 90 min following LiCl injection using the neuronal activity marker, c-Fos.

Six to eight rats/group were injected with LiCl (0.075 M, 25 mg/kg; Sigma Aldrich) 90 min before being euthanized with an i.p. injection of a lethal dose of pentobarbital sodium (Ceva Santé Animale, France). Blood was collected transcardially (in order to measure plasma corticosterone, see above) before perfusion with 0.1 M phosphate buffered saline (PBS, pH = 7.4) followed by 4% paraformaldehyde (PFA) in PBS. Brains were quickly removed and stored at 4°C in a 4% PFA for 24 h to allow post-fixation. Plasma obtained from blood samples (centrifugation at 4000 rpm, 4°C for 15 min) was kept at −80°C before assessing metabolic parameters. The next day, brains were submerged in a 30% sucrose solution at 4°C for 48 h to allow cryoprotection before being frozen in isopentane and stored at −80°C. Brains were sectioned coronally at 40 μm using a cryostat (Leica, Paris, France), and stored in a cryoprotective ethylene-glycol solution at −20°C. Coronal sections from 2.5 mm to

3.2 mm posterior to bregma, sampling the whole amygdala (Paxinos and Watson, 2013), were treated. Free-floating sections were rinsed extensively (0.1 M PBS 3× 10 min) and incubated in 3% bovine serum albumin (BSA) and 0.3% Triton (PBS-BSA-T) to block nonspecific binding sites and to facilitate antibody penetration. Sections were first incubated with the primary anti-c-Fos antibody (Santa Cruz Biotechnology, anti-c-Fos rabbit polyclonal antibody, 1:1000 diluted in PBS-BSA-T, 18 h, RT) and incubated with hydrogen peroxide (H_2O_2, 30 min) to eliminate endogenous peroxidase activity. Sections were then incubated with the biotinylated secondary antibody (Vector Laboratories, goat anti-rabbit IgG, diluted 1:1000 in PBS, 2 h, RT) followed by incubation in the avidin–biotin-peroxidase complex solution (ABC solution; Vectastain, Vector laboratories, diluted 1:1000 in PBS, 1 h, RT). Sections were rinsed in sodium acetate (2× 10 min), and the peroxidase complex was visualized after incubation for 30 min in a mix containing diaminobenzidine, sodium acetate, glucose and glucose oxidase leading to a black precipitate. Between each treatment, sections were thoroughly rinsed with 0.1 M PBS (3× 10 min). Finally, sections were mounted on gelatin-coated slides, air-dried, dehydrated in an ascending alcohol series, cleared in xylene and cover slipped.

Labeling was quantified bilaterally on four sections spaced 240 μm apart, chosen to cover the whole amygdala (2.5–3.4 mm posterior to bregma) and representing the same four levels for all rats. Each section of interest was photographed using Nikon-ACT-1® software and labeled cells were counted independently in the CeA and BLA nuclei with ImageJ® software on a surface representing 0.85 mm^2. Results are expressed for a surface of 1 mm^2.

Hippocampal Neurogenesis (DCX Labeling)

Neurogenesis was evaluated by determining the number of immature neurons in the dentate gyrus, characterized by the endogenous marker doublecortin (DCX), a cytoplasmic protein expressed transiently in newborn neurons only (Brown et al., 2003).

In the subset of rats (6–8 rats/group), coronal sections (40 μm) from 2.8 mm to 4.4 mm posterior to bregma, sampling the whole hippocampus (according to Paxinos and Watson, 2013), were treated. Free-floating sections were rinsed (PBS 3× 10 min, 0.1 M) and incubated with methanol/hydrogen peroxide (0.5%) followed by 3% serum normal rabbit and 0.3% Triton (PBS-SNr-T) to block nonspecific binding sites and to facilitate antibody penetration. Sections were incubated with the primary anti-DCX antibody (Santa Cruz Biotechnology, anti-DCX goat polyclonal antibody, 1:1000 diluted in PBS-SNr-T, 72 h, 4°C) followed by biotinylated secondary antibody (Dako, rabbit anti-goat IgG, diluted 1:200 in PBS, 1.5 h, RT) and then incubated in the avidin–biotin-peroxidase complex solution (ABC solution; Vectastain, Vector laboratories, diluted 1:200 in PBS, 1.5 h, RT). Sections were rinsed in sodium acetate (2× 10 min), and the peroxidase complex was visualized after incubation for 30 min in a mix containing diaminobenzidine, sodium acetate, glucose and glucose oxidase. Between each treatment, sections were rinsed with 0.1 M PBS (3× 10 min). Finally, sections were mounted on gelatin-coated slides, air-dried, dehydrated in an ascending alcohol series, cleared in xylene and cover slipped.

Labeling was quantified bilaterally on four sections spaced 400 μm apart, chosen to cover the whole dentate gyrus (2.8–4.4 mm posterior to bregma) and representing the same four levels for all rats. Each section of interest was photographed using Nikon-ACT-1® software and labeled cells were counted with ImageJ® software.

Data Analysis

Data are expressed as the mean ± SEM. All analyses were conducted using Statview software and statistical significance was set at $p < 0.05$. Outliers (individual results significantly differing from the group mean) were calculated with GraphpadQuickCalcs online application, using the extreme studentized deviate method, and were removed from corresponding analyses. The diet effect was assessed using ANOVAs followed by Fisher's *post hoc* tests when ANOVAs were significant. For the MWM, the number of target annulus crossings for each group was compared to chance level (25%) using one sample *t*-tests.

RESULTS

Bodyweight Across Diet Exposure

There was no difference in bodyweight between the groups before starting the diet exposure ($F_{(2,47)} < 1$, $p = 0.90$, see **Table 1**). An analysis restricted to the first 3 months of diet exposure (week 1–12) found a significant effect of diet ($F_{(2,47)} = 11.14$, $p < 0.001$), time ($F_{(11,517)} = 4806.56$, $p < 0.001$) and an interaction between these factors ($F_{(22,517)} = 9.16$, $p < 0.001$, see **Figure 1B**), indicating that the rate of weight gain differed between the groups in the first 3 months. *Post hoc* analyses indicated that the HF fed rats (groups HF and HF-C) were significantly heavier than C fed rats (Fisher's *post hoc*: $p < 0.001$) but no significant difference was found between the two HF fed groups ($p = 0.26$). At the 12th week, rats under HF were significantly heavier than C fed rats ($F_{(2,47)} = 12.3$, $p < 0.001$; *post hoc*: $p < 0.001$; 11–13% overweight compared to group C). At this time point, the bodyweight of HF and HF-C groups did not differ (Fisher's *post hoc*: $p = 0.41$) and rats in group HF-C group were shifted to the control diet.

For week 13–24 (3–6 months), a significant effect was detected for diet ($F_{(2,47)} = 8.16$, $p < 0.001$), time ($F_{(11,517)} = 316.62$, $p < 0.001$) and the diet × time interaction ($F_{(22,517)} = 10.1$, $p < 0.001$). Specifically, rats in group HF and HF-C were significantly heavier than group C (HF vs. C: $p < 0.001$;HF-C vs. C: $p = 0.005$) and groups HF and HF-C did not differ ($p = 0.31$) despite cessation of the HF in group HF-C. At the 24th week, when behavioral testing started, an overall diet effect was detected ($F_{(2,47)} = 7.29$, $p = 0.002$). Rats maintained under HF were significantly heavier than group C (Fisher's *post hoc*: $p < 0.001$, 15% overweight) and group HF-C ($p = 0.03$), which did not differ from each other ($p = 0.11$).

TABLE 1 | Mean body weight and metabolic measures (± SEM).

	C	HF	HF-C
Initial body weight	58.18 (0.48)	58.13 (1.01)	57.94 (0.89)
Body weight after 12 weeks	493.35 (6.6)	546.67 (12.41)*	558.56 (10.64)*
Body weight after 24 weeks	619.00 (9.64)	709.93 (23.57)*°	656.44 (15.27)*
Body weight after 32 weeks	618.67 (13.32)	716.57 (28.27)*°	647.61 (14.08)
After 32 weeks			
Triglycerides (g/l)	124.23 (17.08)	95.226 (17.6)	13212 (11.0)
Cholesterol (g/l)	80.06 (4.82)	96.14 (6.41)*°	70.03 (3.85)
Leptin (ng/ml)	13.57 (2.93)	29.13 (4.62)*	19.02 (4.83)
Insulin (ng/ml)	3.79 (0.65)	4.51 (0.31)°	2.67 (0.53)
Glucose (mg/dl)	96.82 (1.62)	96.67 (1.8)	99.5 (2.26)

*Significantly different from group C; °significantly different from group HF-C.

Finally, from week 25–32 (6–8 months), there was a significant effect of diet ($F_{(2,47)} = 8.81$, $p < 0.001$) and time ($F_{(7,329)} = 18.3$, $p < 0.001$) but no significant interaction between these factors ($F_{(14,329)} = 1.06$, $p = 0.393$), indicating a similar rate of weight gain in all groups. Rats maintained under the HF were overweight compared to both group C (Fisher's *post hoc*: $p < 0.001$) and group HF-C ($p = 0.005$). At the 32th week (the time of sacrifice), and similar to the 24th week (beginning of behavioral assessment), a diet effect was detected ($F_{(2,47)} = 7.70$, $p = 0.001$). *Post hoc* analyses revealed that animals maintained under HF diet were significantly heavier than C rats ($p < 0.001$, 16% overweight) and HF-C rats ($p = 0.009$), and HF-C rats did not differ from C rats ($p = 0.24$).

Metabolic Changes with Diet Exposure

Glucose metabolism was assessed 30 weeks after starting the diets. Glycemic level was not affected by the diets after overnight fasting ($F_{(2,47)} < 1$, $p = 0.504$). However, glucose i.p. injection resulted in a higher and protracted blood sugar level increase in the HF group compared to both C and HF-C groups (diet effect: $F_{(2,17)} = 7.64$, $p < 0.005$, time effect: $F_{(4,68)} = 77.69$, $p < 0.001$, diet × time interaction: $F_{(8,68)} = 5.76$, $p < 0.001$, AUC: $F_{(2,17)} = 7.65$, $p < 0.005$; **Figure 1C**). Blood glucose levels were higher in the HF group compared to both C and HF-C groups 30, 60 and 120 min after injection (Fisher's *post hoc*: all p values < 0.03). Groups HF-C and C did not differ at any time point (all p values > 0.1). Thus, glucose homeostasis was affected following long-term HF exposure, but HF exposure alone was not sufficient to cause enduring changes. In contrast, insulin i.p. injection induced a similar decrease in blood sugar level for all groups (diet effect: $F_{(2,21)} = 0.26$, $p = 0.78$, time effect: $F_{(4,84)} = 417.32$, $p < 0.001$, diet × time interaction: $F_{(8,84)} = 1$, $p = 0.45$, AUC: $F_{(2,21)} = 1.21$, $p < 0.32$; **Figure 1D**), showing HF exposure did not induce insulin resistance under our conditions.

Other metabolic parameters were measured in the plasma at the time of sacrifice (see **Table 1**). Triglyceride levels were not affected by the diet ($F_{(2,33)} = 2$, $p = 0.25$) however, cholesterol levels differed between groups ($F_{(2,37)} = 7.0$, $p < 0.003$). Specifically, HF group had higher cholesterol levels compared to group C ($p = 0.035$) and group HF-C ($p < 0.001$), which did not differ from each other ($p = 0.13$). Leptin levels marginally differed between groups ($F_{(2,31)} = 3.1$, $p = 0.06$). *Post hoc* analyses indicated higher levels in the HF group compared to group C ($p = 0.02$) but no other comparisons were significant (p values < 0.1). Insulin levels were also marginally affected by the diet ($F_{(2,29)} = 3.0$, $p = 0.07$) with lower insulin levels observed in group HF-C compared to group HF (Fisher's *post hoc*: $p = 0.03$).

Spatial Hippocampal-Dependent Memory and Hippocampal Neurogenesis

Spatial learning was assessed in the MWM. All groups learned the location of the hidden platform during the 5 days of training (six trials per day), as evidenced by a decreased latency to reach the platform across days (session effect: $F_{(4,108)} = 33.3$, $p < 0.001$; diet effect and interaction: F values < 2, p values > 0.1; **Figure 2A**). Similar results were obtained for the distance traveled and no group difference was found in swimming speed (data not shown).

A first probe test was performed 2 h after the last training trial in order to evaluate learning performance. Only rats able to accurately remember the platform location during this probe test (i.e., entering the target annulus in less than 30 s) were kept for LTM assessment. As shown in **Figure 2B**, all groups showed a preference for the target annulus compared to the other annuli (comparison to 25% chance level using one sample t-test: $t_{(8)} = 3.0$, $t_{(9)} = 3.0$, and $t_{(10)} = 3.3$, $p < 0.02$ for groups C, HF and HF-C respectively, no diet effect: $F_{(2,27)} < 1$). The number of annuli crossings was similar in all groups (10.1 ± 1.8, 10.8 ± 2.8 and 9.1 ± 2.1 for C, HF and HF-C, respectively; $F_{(2,27)} < 2$, $p > 0.1$).

During the LTM test (4 days after final training session), groups C and HF-C still exhibited significantly more crossings in the target annulus compared to 25% chance level ($t_{(8)} = 2.7$, $p = 0.03$; $t_{(10)} = 2.4$, $p < 0.04$ for C and HF-C, respectively) whereas group HF did not cross the target annulus more than in a random navigation ($t_{(9)} < 1$, $p < 0.1$, **Figure 2C**). However, no diet effect was revealed for target annulus crossings ($F_{(2,27)} < 1$, $p > 0.1$) indicating that the groups did not differ from each other. Moreover, the number of annuli crossings was not different between groups (8.8 ± 2.4, 7.4 ± 3.2 and 7.5 ± 4.5 for groups C, HF and HF-C, respectively; $F_{(2,27)} < 1$, $p > 0.1$).

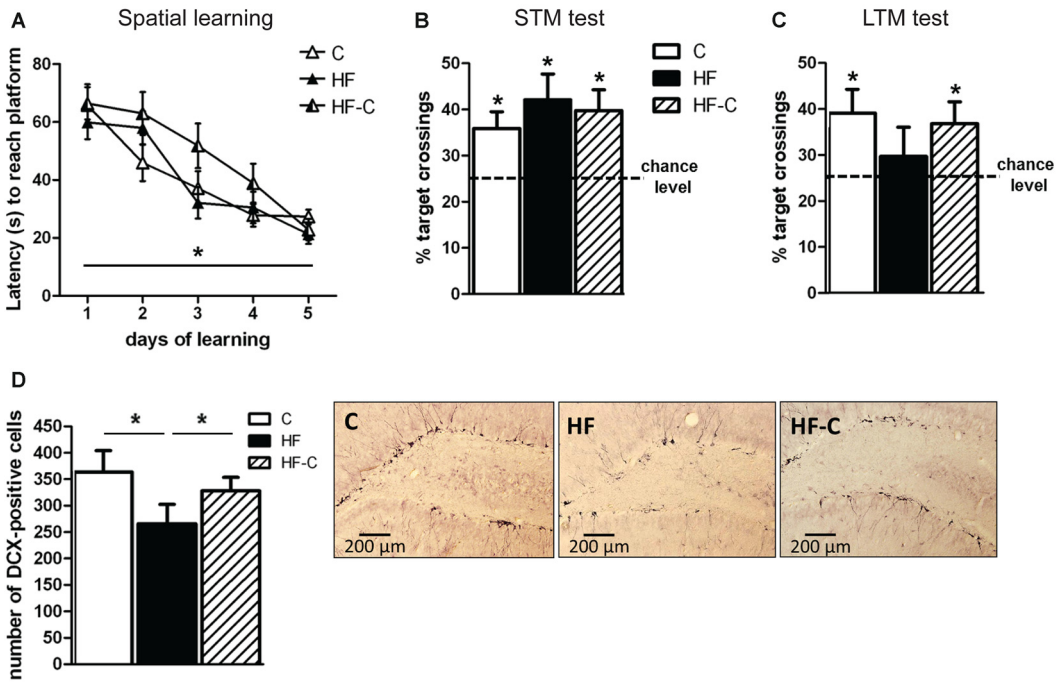

FIGURE 2 | Spatial hippocampal-dependent memory and hippocampal neurogenesis. (A) Performance in the Morris Water Maze (MWM) across training trials. All groups learned the location of the platform and showed a decreased latency to reach the platform across days. *$p < 0.05$ (repeated measure's ANOVA: time effect). **(B)** Short-term memory (STM) was assessed 2 h after the final training trial. The percentage of target annulus crossing was significantly greater than chance level (25%) for all groups. *$p < 0.05$ (one-sample *t*-test). **(C)** Long-term memory (LTM) was assessed 4 days after the final training session. The percentage of target annulus crossings was significantly greater than chance for groups C (white bar) and HF-C (stripped bar) but not for group HF (black bar). *$p < 0.05$ (one-sample *t*-test). **(D)** Less doublecortin (DCX)-positive cells were observed in the dentate gyrus of group HF than in groups C and HF-C. *$p < 0.05$ when compared to both C and HF-C groups (significant one-way ANOVA followed by Fisher's *post hoc*). Representative photomicrographs of DCX-immunoreactivity in the dentate gyrus of groups C, HF and HF-C.

Hippocampal neurogenesis was assessed by counting DCX-positive cells in the dentate gyrus of the hippocampus 7 weeks after the end of MWM to avoid any potential effect of spatial learning on neurogenesis (e.g., Tronel et al., 2010). A diet effect was revealed ($F_{(2,18)} = 4.1$, $p = 0.035$, **Figure 2D**) such that the HF group showed significantly less DCX-positive cells compared to groups C (Fisher's *post hoc*: $p = 0.012$) and HF-C ($p = 0.046$). In addition, DCX-positive cell numbers in C and HF-C groups did not differ from each other ($p = 0.40$).

Avoidance Amygdala-Dependent Memory and Amygdala Activation

Avoidance memory was assessed via COA. To avoid inter-individual differences in liquid consumption, almond-scented odor consumption during acquisition was expressed as a percentage of water consumption the day before. On the acquisition day, all groups showed reduced odorized water intake with respect to water consumption the day before (paired *t*-test: $t_{(16)} = 3.0$, $t_{(14)} = 5.2$ and $t_{(17)} = 3.8$ for groups C, HF and HF-C, respectively, p values < 0.01) indicating a slight yet similar neophobic response for all groups (83%–89% of water consumption, $F_{(2,47)} < 1$; **Figure 3A**). Gastric malaise was induced 30 min after consumption by an i.p. LiCl injection. During the next 2 days, all groups showed similar, normal water consumption (95%–105% of water baseline, $F_{(2,47)} < 1$). On the next day, avoidance memory was assessed and almond-scented odor consumption during test was expressed as a percentage of odorized water consumption during acquisition. All groups showed reduced odorized water intake with respect to acquisition (paired *t*-test: $t_{(16)} = 6.7$, $t_{(14)} = 10.4$ and $t_{(17)} = 9.3$ for groups C, HF and HF-C, respectively, p values < 0.001) indicating an avoidance of the odorized water. However, there was a significant effect of diet on the strength of the avoidance ($F_{(2,47)} = 4.9$, $p = 0.012$; **Figure 3B**). Specifically, the HF group showed a significantly stronger avoidance than group C (Fisher's *post hoc*: $p = 0.003$) and a tendency towards a stronger avoidance than group HF-C ($p = 0.09$). In addition, C and HF-C group did not differ from each other ($p = 0.14$).

Our previous results demonstrate that exposure to HF for 3 months induces higher plasma corticosterone release and c-Fos-positive cells in the BLA 90 min after LiCl injection (Boitard et al., 2015). Here, we assessed if C consumption after exposure to HF for 3 months could restore these endocrine and neurobiological responses. A clear diet effect was revealed on corticosterone levels ($F_{(2,18)} = 6.5$; $p = 0.007$; **Figure 3C**).

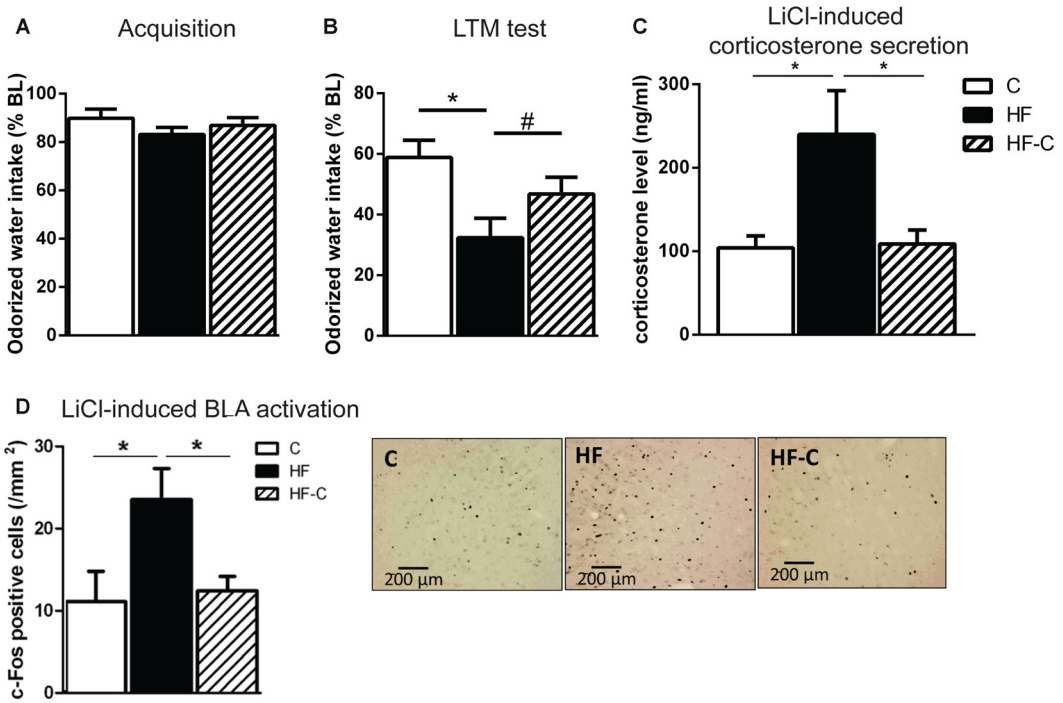

FIGURE 3 | Aversive amygdala-dependent memory and amygdala activation. (A) Odorized water intake did not differ between groups on acquisition day. **(B)** Aversive memory was assessed 3 days after the acquisition trial. All groups showed an avoidance of the odorized water however, the strength of the avoidance was greater in group HF (black bar). $^{*}p < 0.01$, $^{\#}p = 0.09$ (significant one-way ANOVA followed by Fisher's *post hoc*). **(C)** Rats in the group HF showed increase circulating corticosterone levels 90 min after lithium chloride (LiCl) injection compared to groups C (white bar) and HF-C (striped bar). $^{*}p < 0.05$ when compared to both C and HF-C groups (significant one-way ANOVA followed by Fisher's *post hoc*). **(D)** Rats in group HF showed a higher number of c-Fos positive cells in the basolateral amygdala (BLA) 90 min after LiCl injection than groups C and HF-C. $^{*}p < 0.05$ (significant one-way ANOVA followed by Fisher's *post hoc*). Representative photomicrographs of c-Fos immunoreactivity in the BLA for groups C, HF and HF-C.

The HF group differed from groups C ($p = 0.004$) and HF-C ($p = 0.007$) which did not differ from each other ($p = 0.90$). Regarding c-Fos expression in the amygdala, no diet effect was found in the CeA (mean ± SEM number of c-Fos positive cells: C: 42.4 ± 6.2, HF: 58.3 ± 14.4, HF-C: 68.1 ± 5.5; $F_{(2,19)} = 1.9$; $p = 0.17$; data not shown), as previously reported (Boitard et al., 2015). However, a clear diet effect was detected in the BLA ($F_{(2,20)} = 4.0$; $p = 0.034$; **Figure 3D**) revealing increased neuronal c-Fos expression in the HF group compared to both C ($p = 0.028$) and HF-C groups ($p = 0.018$), which did not differ from each other ($p = 0.95$).

Corticosterone Release

To better characterize the effect of HF intake on corticosterone response, we compared the time course of plasma corticosterone levels in the three groups following restraint stress. A repeated-measures ANOVA indicated a significant effect of diet ($F_{(2,20)} = 9.46$, $p = 0.0013$), time ($F_{(3,60)} = 193.31$, $p < 0.0001$) and a diet × time interaction ($F_{(6,60)} = 2.89$, $p = 0.015$; **Figure 4A**). As previously demonstrated (Boitard et al., 2015), no diet effect was revealed on basal corticosterone release ($F_{(2,21)} = 0.95$, $p = 0.40$) or 30 min after stress onset ($F_{(2,21)} = 1.54$, $p = 0.24$). However, a diet effect appeared 90 min after stress onset ($F_{(2,21)} = 8.87$, $p = 0.002$), with the HF group differing from both group C ($p < 0.001$) and group HF-C ($p = 0.006$) and no difference was found between groups HF-C and C ($p = 0.25$). This HF-induced protracted corticosterone release is temporary as, 180 min after stress induction, corticosterone levels did not differ between HF and the two other groups ($F_{(2,21)} = 4.44$, $p = 0.024$; Fisher's *post hoc*: HF vs. C: $p = 0.15$; HF vs. HF-C: $p = 0.26$; HF-C vs. C: $p = 0.007$).

The AUC was significantly higher for the HF group compared to groups C and HF-C ($F_{(2,21)} = 12.6$; $p < 0.001$; Fisher's *post hoc*: HF vs. C: $p < 0.001$; HF vs. HF-C: $p = 0.005$; HF-C vs. C: $p = 0.048$; **Figure 4B**). These data indicate a protracted corticosterone release in response to restraint stress in the HF group that is restored by C exposure after exposure to HF.

DISCUSSION

The adolescent period is particularly vulnerable to the detrimental cognitive effects of a diet laden with sugar or fat (Vendruscolo et al., 2010; Privitera et al., 2011; Boitard et al., 2012, 2014, 2015; Valladolid-Acebes et al., 2013; Hsu et al., 2015; Reichelt et al., 2015; Klein et al., 2016; Naneix et al., 2016). However, the present study, in conjunction with our previous studies, indicates that the neurocognitive alterations associated with adolescent HF intake are partially

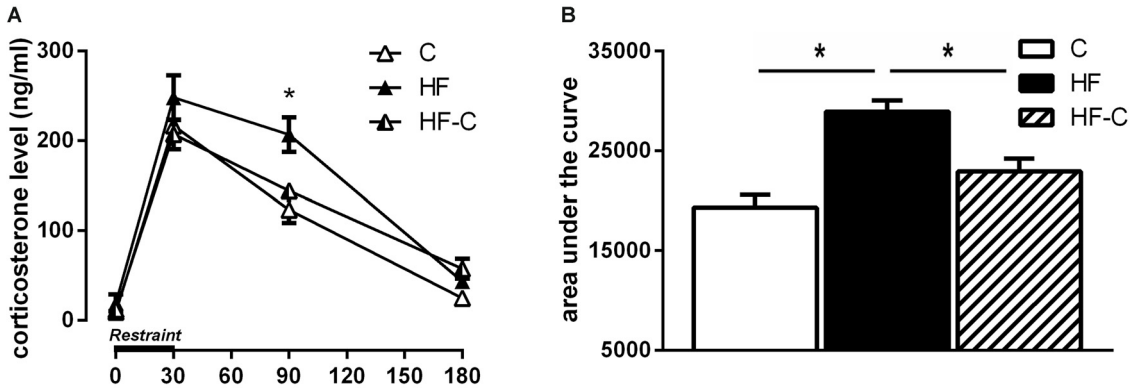

FIGURE 4 | Corticosterone secretion following restraint stress. (A) Higher corticosterone levels were found in the group HF (black bar) 90 min after restraint stress compared to group C (white bar) and group HF-C (striped bar). *$p < 0.05$ when compared to both C and HF-C groups. **(B)** The area under the curve (AUC) was significantly higher for the HF group compared with groups C and HF-C. *$p < 0.05$ (significant one-way ANOVA followed by Fisher's *post hoc*).

reversible. That is, 3 months after removal of the HF, rats with a history of adolescent HF consumption showed no deficits in amygdala-dependent memory and marginal improvements in hippocampal-dependent memory. Moreover, these rats did not show any enduring changes in weight, metabolism (cholesterol, leptin and glucose), corticosterone release, hippocampal neurogenesis or amygdala reactivity.

Contrary to our previous findings (Boitard et al., 2014), we failed to observe group differences in performance during the LTM test of the MWM. This may be due to the large number of rats excluded from all groups due to an inability to learn the task. Nevertheless, when the number of target annulus crossing was compared to chance level for each group, it was revealed that control-fed rats and rats switched from a HF to a control diet spent significantly more time in the target annulus compared to chance whereas rats maintained on a HF did not. Moreover, rats given a HF from weaning to sacrifice showed decreased levels of neurogenesis in the dentate gyrus as we previously reported in mice (Boitard et al., 2012). However, those that received adolescent HF (from weaning for 3 months) and were then switched to a standard control diet showed levels of hippocampal neurogenesis similar to rats with no history of HF consumption.

It was previously reported that adolescent intake of a HF led to long-lasting effects on hippocampal function. Mice consuming a HF starting during adolescence showed impaired object location memory, increased dendritic spine density and desensitization of leptin receptors in hippocampus (Valladolid-Acebes et al., 2013). Critically, these neurobehavioral modifications persisted despite a period (5 weeks) of restricted HF intake. However, not all diet-induced changes were enduring as, similar to the current study, leptin levels normalized following HF restriction (Valladolid-Acebes et al., 2013).

Taken together, these data indicate that reversal of adolescent HF effects may require abstinence from the diet rather than restricted intake only. Indeed, adult rats previously fed with HF then given a short-term (4 weeks) dietary reversal to standard control diet completely recovered hippocampal memory function (Sobesky et al., 2014). Similarly, a recent report indicates that mice maintained on HF for 3 months before being switched to low-fat diet for 2 months showed normalized hippocampal plasticity and hippocampal-dependent memory (Hao et al., 2016). Moreover, it should also be noted that, in our study, group HF-C did not differ in bodyweight from rats fed a control diet whereas, in the study by Valladolid-Acebes et al. (2013), mice remained significantly heavier than control fed mice at the time of behavioral assessment and sacrifice. It could therefore be argued that we may have observed cognitive deficits in the rats switched to a standard control diet if behavioral testing occurred when these rats were still overweight. However, this seems unlikely given that adult rats previously fed with a HF then switched to standard control diet for 4 weeks still weighed more than control fed animals (and not significantly less than the HF rats) but completely recovered hippocampal memory function (Sobesky et al., 2014).

Consistent with our previous findings obtained after 3 months of HF exposure starting at weaning (Boitard et al., 2015), we observed enhanced avoidance memory and amygdala reactivity as well as protracted corticosterone release after maintained HF exposure for 8 months (starting at weaning). While this data is purely correlative, we have previously shown that blockade of glucocorticoid receptors in the BLA of HF-fed rats normalizes aversive memory indicating a causal link between changes in amygdala modulation by glucocorticoids and an increase in emotional memory (Boitard et al., 2015). In contrast, 3 months of HF exposure starting at weaning was without effect 3 months after removal of the HF. That is, after 3 months of control diet, rats with a history of adolescent HF showed normal avoidance memory and neuronal activity in the basolateral amygdala. Corticosterone responses to both restraint and systemic stressors also did not differ from rats fed a control diet only.

Others have also reported that behavioral changes in non-hippocampal systems, caused by adolescent HF, can be rescued following diet cessation. Indeed, alterations in sucrose preference and dopamine system function observed after 12 weeks of adolescent HF were partially reversed 1 month after removal of the diet (Carlin et al., 2016). Similarly,

rats exposed to a HF and high sugar diet for 10 days (postnatal 22–32) showed a reduction in sucrose preference when tested immediately after diet exposure. Rats were then shifted to a standard control diet until adulthood. At adulthood, sucrose preference was restored (Rabasa et al., 2016). However, sucrose preference was also restored at adulthood if rats were maintained on the HF-high sugar diet, thus it was not removal of the high energy diet *per se* that was responsible for the restoration of sucrose preference. In the present study, we showed that rats maintained on HF from weaning until behavioral testing at adulthood showed clear memory, neurobiological and endocrine changes. Switching from a HF to a control diet was therefore critical in reversing these alterations.

While the present results indicate that adolescent HF intake does not produce enduring neurocognitive modifications, it should be noted that animals were only tested during adulthood. It is possible that the effects of adolescent HF may resurface during another vulnerable period, such as aging. Indeed, the effects of HF intake are exacerbated in the aging brain (Ledreux et al., 2016). Aged rats that consumed a HF exhibited reduced hippocampal morphology and greater memory deficits compared to young HF fed rats and age-matched control fed rats (Ledreux et al., 2016). Moreover, a recent article indicates that early-life exposure to HF has long-term deleterious consequences (Wang et al., 2015). Exposure to HF for 4 months (starting at early adulthood) followed by 15 months of normal low-fat diet induces epigenetic modifications and synaptic dysfunction in the hippocampus as well as deficits in hippocampal-dependent memory despite restoration of normal weight and metabolic homeostasis (Wang et al., 2015). Therefore, while we observed that switching to a control diet after adolescent HF intake is sufficient to ameliorate neurocognitive deficits at adulthood, enduring deficits may be observed later in life or during demanding cognitive situations.

We previously demonstrated that 3 months of HF consumption from weaning to adulthood induced a range of neurocognitive alterations including reduced hippocampal neurogenesis (Boitard et al., 2012, 2014) as well as protracted corticosterone release and enhanced amygdala plasticity and aversive memory (Boitard et al., 2015). Here, we have shown that introducing a standard control diet for 3 months after adolescent HF consumption is sufficient to reverse these alterations. That is, the adverse effects of adolescent HF exposure can be overcome by restoring a proper nutritional diet at adulthood. Our results suggest that by introducing diet and weight management in adults, we can improve some of the negative effects of early life obesity.

AUTHOR CONTRIBUTIONS

CB and GF designed research; CB, AC and FT performed research; CB, SLP and GF analyzed data; GF supervised research; CB, SLP and GF wrote the manuscript. All authors edited the manuscript.

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Dietary Intake of Nutrients and Lifestyle Affect the Risk of Mild Cognitive Impairment in the Chinese Elderly Population: A Cross-Sectional Study

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Mild cognitive impairment (MCI) is a pre-clinical stage of Alzheimer's disease afflicting a large number of the elderly throughout the world. However, modifiable risk factors for the onset and progression of MCI remain unclear. A cross-sectional study was performed to explore whether and how daily dietary nutrients intake and lifestyle impacted the risk of MCI in the Chinese elderly. We examined 2,892 elderly subjects, including 768 MCI patients and 2,124 subjects with normal cognition in three different Provinces of China. Dietary intake of nutrients were collected by using a 33-item food frequency questionnaire and calculated based on the Chinese Food Composition database. The MCI patients were first screened by Montreal Cognitive Assessment and then diagnosed by medical neurologists. Multivariate logistic regression and exploratory factor analyses were applied to identify and rank the risk factors. Three dietary nutrient intake combination patterns were identified as the major protective factors of MCI, with eigenvalues of 14.11, 2.26, and 1.51 and adjusted odds ratios (OR) of 0.77, 0.81, and 0.83 ($P < 0.05$), respectively. The most protective combination was featured with eight vitamins and six minerals, and OR for the third and fourth quartiles of these nutrients intake ranged from 0.48 to 0.74 ($P < 0.05$). Carotenoids, vitamin C, and vitamin B_6 exhibited the highest protective factor loadings of 0.97, 0.95, and 0.92 ($P < 0.05$), respectively. Education, computer use, reading, and drinking represented the most protective lifestyle factors (OR = 0.25 to 0.85, $P < 0.05$), whereas smoking and peripheral vascular diseases were associated with higher (OR = 1.40 and 1.76, $P < 0.05$) risk of MCI. Adequate dietary intake of monounsaturated fatty acids and cholesterol were significantly associated with decreased risk of MCI. In conclusion, adequate or enhanced intake of micronutrients seemed to lower the risk of MCI in the Chinese elderly. In addition, improving education and lifestyle such as reading, computer use and moderate drinking might also help to decrease the risk of MCI.

Keywords: dietary nutrients, vitamins, minerals, lipids, lifestyle, mild cognitive impairment, aging

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INTRODUCTION

Alzheimer's disease (AD) has become a serious public health problem, resulting in tremendous economic loss and social burden. There will be an estimated 66 million people with AD or other dementias worldwide by the year 2030, and the number may reach 115 million by 2050 with an aging world population (Wortmann, 2012). Because mild cognitive impairment (MCI) is a precursory stage of AD (Janoutova et al., 2015), the MCI patients constitute a high-risk group that develop AD at a rate of 10–15% per year compared with the general population at a rate of 1–2% per year (Geda et al., 2013). Therefore, there has been a growing interest in preventive strategies to decrease the risk of MCI.

Epidemiological evidence has suggested lifestyle modification as a possible means of protecting against cognitive decline (Hugo and Ganguli, 2014). Likewise, dietary patterns may be of great importance in the prevention of cognitive decline in later life (Smith and Blumenthal, 2016). Several studies have indicated the actual benefits of early control of diets and lifestyle in lowering the rate of MCI (Eshkoor et al., 2015; Roberts et al., 2015; Lara et al., 2016). Meanwhile, prospective studies have shown that individuals with a "Mediterranean-like diets" were less likely to suffer from dementia or cognitive decline (Cao et al., 2015; Gardener et al., 2015). Similarly, two earlier studies by our laboratory have demonstrated that diet rich in marine products, fruit, vegetables, and vegetable juice could prevent cognitive decline in the elderly (Zhao et al., 2015; Dong et al., 2016). However, those studies examined only the association of daily intake of different foods, without considering the influence of individual nutrients on the risk of MCI. Furthermore, conclusions derived from those studies were inconsistent in illustrating the association between lifestyle (such as smoking, drinking or living status) and the risk of MCI (Durazzo et al., 2014; Roerecke and Rehm, 2014). While many factors might have contributed to such inconsistency, one of the limitations in those studies was the relatively small sample size from a single geographic area (Beijing). Another was that the collected data could have been analyzed using more appropriate programs such as exploratory factor analyses (Zhao et al., 2015; Dong et al., 2016). Notably, an important change in lifestyle of the elderly in China, like in other parts of the world, that could influence their cognitive function is the wide availability to personal computers. Therefore, we conducted the present cross-sectional analysis with 2,892 elderly subjects from three different areas in China and explored: (1) the association and importance of specific dietary nutrients intake with the risk of MCI; (2) the association of lifestyle including computer use with the risk of MCI; (3) the combined effects and relative importance of dietary nutrients and lifestyle on the risk of MCI.

MATERIALS AND METHODS

Subjects

The subjects were recruited from February 2014 to March 2016 and selected from Chaoyang District, Beijing; Linyi, Shandong Province; and Jincheng, Shanxi Province. All the subjects were selected for the following inclusion criteria: 50–70 years old, capable of self-managing daily life, without self-recognized cognition dysfunction, and willing to participate in the study. Exclusion criteria for the subjects were as follows: individuals with serious illnesses (e.g., cancer, severe psychiatric disorders such as depression and schizophrenia, recent history of heart or respiratory failure, chronic liver or renal failure); individuals with condition known to affect cognitive function (e.g., a recent history of alcohol abuse, cerebral apoplexy and infarction); individuals with AD, Parkinson's disease (PD) or long-term frequent intake of antidepressants and other medications for neurological diseases. The study protocol was approved by the Ethics Committee of Capital Medical University, Beijing (No.2013SY35). All participants were fully informed of the study, signed a written consent and allowed to terminate their participation at any time.

Data Collection

Data on demographic and clinical characteristics, lifestyle and current use of medications were collected through face-to-face interviews by professionally trained nurses and researchers. The demographic characteristics included age, gender, body weight, height, household income, and education background. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). The lifestyle covered smoking, alcohol consumption, working, reading and computer use. Smoking status were coded as non-smokers and smokers. Alcohol consumption was also dichotomized as current and non-drinkers. Work time was categorized into four groups (<40, 41–50, 51–60, and >60 h/week). History of chronic non-communicable diseases, including hypertension, diabetes and hyperlipidemia, was obtained using a self-guided questionnaire and ascertained on the basis of clinical diagnosis by physicians. Current medications use was recorded by self-reporting at the interview. Educational level was divided into low (illiterate, elementary school), middle (junior high school, senior high school, technical secondary school), and high (college and graduate school) according to average years of formal schooling (Sharp and Gatz, 2011).

Dietary Assessment

A validated semi-quantitative food frequency questionnaire (FFQ) was used to estimate the consumption of a total of 33 items (whole grain, red meat, pork, beef, mutton, chicken, fruit and vegetables, legume and legume product, milk, fish, eggs, nuts, cooking oil, tea, etc.) (Block et al., 1992). All participants filled out the FFQ under the help of trained dietary interviewers and the information including the quantity of foods consumed as well as the consumption frequency (daily, weekly, monthly, yearly, or never). The quantity of food consumed was estimated using food models such as special charts and measuring rulers or cups to help in quantifying the consumed food. The dietary nutrients intake were calculated based on the quantity of total food intake and the China Food Composition Database (Yang et al., 2009).

Assessment of Cognitive Function

Cognitive function was evaluated by Montreal Cognitive Assessment (MoCA) according to standard protocols. The total score of MoCA is 30 and the cut-off score commonly used for screening MCI in developed countries is 25/26 (Bischkopf et al., 2002). However, an increasing number of studies has demonstrated that the MoCA is strongly influenced by educational background and varying cut-offs stratified by educational level are recommended for the purpose of improving the effectiveness of the screening. Consequently, the cutoff score for MCI applied to the present study was 14 for illiterate individuals, 19 for individuals with 1–6 years of education, and 24 for individuals with 7 or more years of education. The same cutoff score proved to be appropriate for screening MCI in the Chinese elderly by a large population-based study (Lu et al., 2011). If the subjects met the above MCI criteria, they were arranged to visit the physicians for final diagnoses (Chertkow et al., 2008).

Statistical Analyses

Data are presented as mean and standard deviation (SD), median [interquartile range (IQR)], or frequency (percentage). Characteristics were compared between MCI and normal cognition groups using factorial design ANOVA analyses for continuous variables and Cochran-Mantel-Haenszel χ^2 test for categorical variables adjusted education levels. Multivariate logistic regression was used to estimate gender-specific associations between different nutrients intake or lifestyle and the risk of MCI. Exploratory factor analysis was used to derive food nutrients from the FFQ for all participants in the study. These factors were orthogonally rotated to generate uncorrelated factors. The retained number of factors was determined on the basis of several criteria including eigenvalue >1.0, hence clearly identifying major dietary nutrients. Factor scores were created for each subject as the linear combination of the dietary variables weighted by an equivalent of the factor loadings. All tests were performed using SAS software version 9.1.3 (SAS Institute, Inc., USA). Graphics were produced using the SAS and Excel (Microsoft, Inc., USA). All the statistical analyses were performed at the conventional two-tailed alpha level of 0.05.

RESULTS

Subjects

A total of 768 MCI patients and 2,124 subjects with normal cognition were enrolled in the study. Sociodemographic characteristics and lifestyle of participants are presented in **Table 1**. Compared with normal group, MCI group had lower educational level ($P = 0.0001$), BMI ($P = 0.0079$) and frequencies of drinking ($P = 0.0068$), reading ($P = 0.0001$), and computer use ($P = 0.0001$). Besides, MCI group had longer working time ($P = 0.0042$) and higher morbidity of peripheral vascular diseases (PVDs) ($P = 0.0019$). The differences of dietary nutrients intake between the two groups are presented in **Table 2**. Except for polyunsaturated fatty acids (PUFAs) and vitamin E, the intake of other nutrients were significantly lower ($P < 0.05$) in MCI group than the control.

Association between Lifestyle and Risk of MCI

In the adjusted multivariate logistic regression models of the overall sample analysis, drinking (OR = 0.85; 95% CI = 0.75, 0.97), reading (OR = 0.72; 95% CI = 0.60, 0.90), and computer use (OR = 0.57; 95% CI = 0.46, 0.70) were less likely to have MCI (**Table 3**). Furthermore, compared with low level of education, middle and high levels of education (OR = 0.69, 95% CI: 0.56, 0.85; OR = 0.25, 95% CI: 0.19, 0.34) were associated with less odds for MCI. Meanwhile, smoking and PVD were related with 1.40 (95% CI = 1.09, 1.80) and 1.76 (95% CI = 1.19, 2.59) times higher odds for MCI, respectively.

Association between Intake of Dietary Nutrients and the Risk of MCI

Dietary intake of various nutrients were divided into four quartiles (Q1–Q4) as categorical variables (**Table 4**). When we applied logistic regression analysis to adjust for potential confounders and took the quartile of nutrient approximate to dietary reference intakes (DRIs, 2013 edition) as the reference, participants with Q3 and Q4 of cholesterol intake (OR = 0.63, 95% CI: 0.49, 0.82; OR = 0.54, 95% CI: 0.42, 0.70) and Q4 of MUFA intake (OR = 0.66, 95% CI: 0.50, 0.89) showed lower risks of MCI whereas Q2 of MUFA intake (OR = 1.33, 95% CI: 1.02, 1.74) had an increased risk. Other quartiles of nutrients intake significantly associated with MCI in the multivariate analysis were: Q1 of five vitamins (A, B_6, B_{12}, C, carotenoids) and Q4 of iodine with increased OR (1.29–1.91, $P < 0.05$); Q3 of vitamin B_3 and three minerals (Mg, Zn, and Cu) with decreased OR (0.71–0.74, $P < 0.05$); Q4 of nine vitamins (A, B_1, B_2, B_3, B_6, B_{12}, C, folic acid, and carotenoids) and six minerals (Zn, Mg, Fe, Se, Cu, and Mn) with lower OR (0.48–0.71, $P < 0.05$).

Gender-Specific MCI Risk

Compared with the pooled gender analysis, the males and females shared similar OR values for dietary intake of all assayed nutrients except for folic acid which was detected as a protective factor only in the females. Besides, vitamin B_1 and magnesium seemed to be stronger protective factors in the females (**Figure 1**). The effects of education were consistent in both genders (**Figure 2**) while smoking, drinking and PVD were significant only in the males, with two as the risk factors and one as the protective factor.

Ranking of Major Risk Factors

Three major factor patterns were identified by the exploratory factor analysis, with eigenvalues of 14.11, 2.26, and 1.51 and adjusted OR of 0.77, 0.81, and 0.83, respectively (**Table 5**). The first pattern was characterized as "vitamin and mineral pattern" that included eight vitamins and six minerals. The three highest factor loadings were carotenoids, vitamin C, and vitamin B_6 as 0.97, 0.95, and 0.92, respectively. Vitamin B_1 had the lowest

TABLE 1 | Comparison of general characteristics between MCI patients and cognitively normal subjects.

Variables	Normal (*N* = 2124)	MCI (*N* = 768)	*P*-value
Gender; *n* (%)			0.1200
Male	1166 (54.9)	355 (46.2)	
Female	958 (45.1)	413 (53.8)	
Age, years	58.2 ± 5.3	58.3 ± 4.4	0.1900
BMI, kg/m^2	25.6 ± 3.8	25.1 ± 3.2	0.0079
Educational level; *n* (%)			0.0001
Low	935 (44.1)	485 (63.1)	
Middle	598 (28.1)	211 (27.5)	
High	591 (27.8)	72 (9.4)	
Live situation; *n* (%)			0.6600
Not alone	2086 (98.2)	751 (97.8)	
Solitude	38 (1.8)	17 (2.2)	
Smoking; *n* (%)			0.0880
Yes	512 (24.1)	203 (26.4)	
No	1612 (75.9)	565 (73.6)	
Drinking; *n* (%)			0.0068
Yes	665 (31.3)	174 (22.7)	
No	1459 (68.7)	594 (77.3)	
Watching TV; *n* (%)			0.8900
Yes	2004 (94.4)	731 (95.2)	
No	120 (5.6)	37 (4.8)	
Reading; *n* (%)			0.0001
Yes	990 (46.6)	256 (33.3)	
No	1134 (53.4)	512 (66.7)	
Computer use; *n* (%)			0.0001
Yes	854 (40.2)	185 (24.1)	
No	1270 (57.8)	583 (75.9)	
Labor intensity; *n* (%)			0.0520
Mild	1903 (89.6)	656 (84.8)	
Moderate	169 (8.0)	81 (10.5)	
Strong	52 (2.4)	36 (4.7)	
Work time; *n* (%)			0.0042
1	1913 (90.1)	662 (86.2)	
2	109 (5.1)	61 (7.9)	
3	39 (1.8)	26 (3.4)	
4	63 (3.0)	19 (2.5)	
Hypertension; *n* (%)	630 (29.7)	242 (31.5)	0.2700
Diabetes; *n* (%)	297 (14.0)	124 (16.2)	0.0710
Hyperlipidemia; *n* (%)	392 (18.5)	147 (19.1)	0.2800
PVD; *n* (%)	78 (3.7)	46 (6.0)	0.0019
CHD; *n* (%)	126 (5.9)	57 (7.4)	0.2500

BMI, body mass index; PVD, peripheral vascular diseases; CHD, coronary heart disease; TV, television.
Educational level: low (illiterate, elementary school), middle (junior high school, senior high school, technical secondary school), and high (college and graduate school).
Work time (h/week): 1, <40; 2, 41–50; 3, 51–60; and 4, >60.
Data are presented as mean ± SD or n (%). Characteristics were compared between the two groups using factorial design ANOVA analysis for continuous variables and Cochran-Mantel-Haenszel χ^2 test for categorical variables adjusted education levels.

loading factor of 0.47. The second pattern was labeled as "fatty acid pattern," in which MUFA and dietary cholesterol had the highest loading factors of 0.86 and 0.53, respectively. The third pattern was marked as "mineral pattern" that was featured by manganese (Mn) and magnesium (Mg) with the two highest loading factors of 0.64 and 0.57, respectively, second to only vitamin B_1 (0.65).

DISCUSSION

One of the most interesting findings of present study is the identification of three major dietary factor patterns with eigenvalues ranging from 14.11 to 1.51 and adjusted OR of 0.77 to 0.83 toward the risk of MCI in the Chinese elderly from three different regions. A panel of eight vitamins and six mineral

TABLE 2 | Comparison of dietary nutrients intake between MCI patients and cognitively normal subjects.

Variables	Normal (*N* = 2,124)	MCI (*N* = 768)	*P*-value
Cholesterol, mg/d	342.3 ± 193.5	278.5 ± 157.8	0.0001
MUFA, g/d	26.6 ± 13.9	22.2 ± 11.6	0.0001
PUFA, g/d	22.6 ± 12.0	22.6 ± 12.4	0.9900
Folic acid, μg/d	392.1 ± 115.5	351.4 ± 104.1	0.0001
Vitamin B_1, mg/d	1.48 ± 0.58	1.30 ± 0.48	0.0001
Vitamin B_2, mg/d	1.46 ± 0.70	1.21 ± 0.60	0.0001
Vitamin B_3, mg/d	21.1 ± 10.0	17.2 ± 8.2	0.0001
Vitamin B_6, mg/d	1.33 ± 0.80	1.02 ± 0.68	0.0001
Vitamin B_{12}, μg/d	2.02 ± 1.80	1.53 ± 1.48	0.0001
Vitamin C, mg/d	133.3 ± 74.5	109.4 ± 68.1	0.0001
Vitamin A, μg RAE/d	922.0 ± 522.6	723.1 ± 475.4	0.0001
Vitamin E, mg/d	37.2 ± 14.3	37.9 ± 15.6	0.2600
Iron, mg/d	38.4 ± 18.3	31.7 ± 15.6	0.0001
Magnesium, mg/d	452.9 ± 185.6	400.8 ± 163.9	0.0001
Zinc, mg/d	15.9 ± 6.6	13.5 ± 5.5	0.0001
Selenium, μg/d	53.1 ± 24.7	44.2 ± 21.1	0.0001
Copper, mg/d	3.75 ± 1.55	3.25 ± 1.36	0.0001
Manganese, mg/d	8.57 ± 3.29	7.68 ± 2.98	0.0001

Data are presented as mean ± SD. Wilcoxon rank sum test was used to compare two groups.

elements accounted for the vitamin and mineral pattern of factor 1 and showed the strongest association in protecting against the development of MCI. Among the 14 nutrients, five of them including carotenoids, vitamins A, C, and B_6, and folic acids depicted the strongest association. Removing these five nutrients came up the factor pattern three that included six elements (Fe, Cu, Mn, Mg, Zn, and Se) and three B vitamins (B_1, B_2, B_3). This group of nutrients was described as mineral pattern and showed the lowest eigenvalue and the least reduction in OR. Compared with the factor pattern 1, the factor pattern 2 included two unique lipid nutrients (MUFA and cholesterol) and shared only four other nutrients (Zn, Se, vitamins B_1 and B_3). The eigenvalue and OR values of the factor pattern 2 were very similar to those of the factor pattern 3. Overall, four nutrients (Zn, Se, and vitamins B_1 and B_3) appeared in all the three patterns, whereas the two lipid nutrients displayed only in the factor pattern 2. It is very intriguing that four nutrients (PUFA, vitamins E and B_{12}, and iodine) were excluded from any of these three patterns.

Dietary nutrients serve as controllable or modifiable environmental factors possibly contributing to the development of MCI. It has been reported that dietary patterns and body nutritional status might affect cognitive function (Smith and Blumenthal, 2016). Previous studies have provided evidence that higher adherence to a Mediterranean diet was associated with slower cognitive decline or reduced risk of MCI (Feart et al., 2015). The identified 'cognition-protective' nutrients combination was manifested with higher intake of fresh fruit and vegetables, fish, and whole grains, and lower intake of sweets, high-fat dairies, and processed butter and meat (Berti et al., 2015). Our previous study also observed the protective effects of diets rich in fish and vegetables on the cognitive function in the elderly (Zhao et al., 2015; Dong et al., 2016). In fact, the revealing of factor pattern 1 in the present study, containing the complete panel of 14 micronutrients as the strongest protective matrix,

TABLE 3 | Odds ratios (95% CI) for lifestyle related risk factors of MCI with different levels of adjustments.

	β*	*P*-value*	Odds ratio (95% CI)*	Adjusted odds ratio (95% CI)#
Education				
Low			1.00	1.00
Middle	−0.3808	0.0001	0.68 (0.56–0.83)	0.69 (0.56–0.85)
High	−1.4279	0.0001	0.24 (0.18–0.32)	0.25 (0.19–0.34)
Live situation				
Not alone			1.00	1.00
Solitude	0.1210	0.4200	1.13 (0.84–1.51)	1.25 (0.85–1.85)
Smoking	0.4305	0.0001	1.54 (1.23–1.92)	1.40 (1.09–1.80)
Drinking	−0.1163	0.0410	0.89 (0.80–1.00)	0.85 (0.75–0.97)
Reading	−0.5120	0.0001	0.60 (0.50–0.71)	0.72 (0.60–0.90)
Computer use	−0.7140	0.0001	0.49 (0.41–0.59)	0.57 (0.46–0.70)
PVD	0.5370	0.0050	1.71 (1.18–2.49)	1.76 (1.19–2.59)
Labor intensity	0.3058	0.0001	1.42 (1.19–1.70)	1.20 (0.97–1.50)
Work time				
1			1.00	1.00
2	0.5538	0.0010	1.74 (1.25–2.42)	1.43 (0.98–2.09)
3	0.6728	0.0092	1.96 (1.18–3.25)	1.53 (0.86–2.72)
4	−0.1106	0.6781	0.90 (0.53–1.51)	0.69 (0.38–1.23)

PVD, peripheral vascular diseases; CI, confidence interval; OR, odds ratio.
Worktime (h/week): 1, <40; 2, 41–50; 3, 51–60; and 4, >60.
**Models adjusted for age and gender.*
#*Model adjusted for age, gender, BMI, education, live situation, smoking, drinking, reading, computer use, labor intensity, working time, hypertension, diabetes, hyperlipidemia, PVD, and CHD.*

TABLE 4 | Odds ratios (95% CI) for dietary nutrients intake related risk factors of MCI with different levels of adjustment.

	β*	*P*-value*	Odds ratio (95% CI)*	Adjusted odds ratio (95% CI)#
Cholesterol				
Q1	0.096	0.363	1.10 (0.90–1.35)	1.07 (0.87–1.33)
Q2			1.00	1.00
Q3	−0.514	0.000	0.60 (0.46–0.77)	0.63 (0.49–0.82)
Q4	−0.826	0.000	0.44 (0.34–0.56)	0.54 (0.42–0.70)
MUFA				
Q1	0.281	0.028	1.33 (1.03–1.70)	1.16 (0.90–1.50)
Q2	0.458	0.001	1.58 (1.22–2.05)	1.33 (1.02–1.74)
Q3			1.00	1.00
Q4	−0.472	0.001	0.62 (0.47–0.83)	0.66 (0.50–0.89)
PUFA				
Q1	−0.111	0.387	0.90 (0.70–1.15)	0.79 (0.61–1.02)
Q2	0.186	0.160	1.21 (0.93–1.56)	1.06 (0.81–1.39)
Q3			1.00	1.00
Q4	0.044	0.743	1.05 (0.80–1.36)	0.95 (0.72–1.24)
Folic acid				
Q1	1.183	0.083	1.20 (0.98–1.48)	1.12 (0.64–1.97)
Q2			1.00	1.00
Q3	−0.207	0.101	0.81 (0.63–1.04)	0.78 (0.48–1.26)
Q4	−0.869	0.000	0.42 (0.33–0.54)	0.51 (0.33–0.79)
Vitamin B_6				
Q1	0.286	0.006	1.33 (1.08–1.64)	1.91 (1.02–3.58)
Q2			1.00	1.00
Q3	−0.374	0.004	0.69 (0.53–0.89)	0.63 (0.39–1.05)
Q4	−0.901	0.000	0.41 (0.32–0.52)	0.54 (0.35–0.89)
Vitamin B_{12}				
Q1	0.359	0.001	1.43 (1.16–1.76)	1.37 (1.11–1.70)
Q2			1.00	1.00
Q3	−0.086	0.502	0.92 (0.72–1.18)	0.98 (0.76–1.26)
Q4	−0.526	0.000	0.59 (0.47–0.75)	0.70 (0.54–0.90)
Vitamin A				
Q1	0.257	0.015	1.29 (1.05–1.59)	1.30 (1.05–1.61)
Q2			1.00	1.00
Q3	−0.194	0.126	0.82 (0.64–1.06)	0.94 (0.72–1.21)
Q4	−0.764	0.000	0.47 (0.37–0.60)	0.58 (0.45–0.74)
Carotenoids				
Q1	0.257	0.015	1.29 (1.05–1.59)	1.31 (1.06–1.62)
Q2			1.00	1.00
Q3	−0.297	0.022	0.74 (0.58–0.96)	0.81 (0.63–1.05)
Q4	−0.684	0.000	0.51 (0.40–0.64)	0.62 (0.48–0.79)
Vitamin E				
Q1			1.00	1.00
Q2	−0.165	0.140	0.85 (0.68–1.06)	0.88 (0.70–1.72)
Q3	−0.295	0.032	0.74 (0.59–0.98)	0.82 (0.61–1.09)
Q4	0.102	0.384	1.11 (0.88–1.39)	1.19 (0.93–1.52)
Vitamin C				
Q1	0.392	0.000	1.48 (1.20–1.82)	1.44 (1.17–1.78)
Q2			1.00	1.00
Q3	−0.116	0.362	0.89 (0.69–1.14)	0.97 (0.75–1.25)
Q4	−0.637	0.000	0.53 (0.42–0.67)	0.65 (0.50–0.84)
Vitamin B_1				
Q1	0.118	0.266	1.13 (0.91–1.39)	1.09 (0.88–1.36)
Q2			1.00	1.00
Q3	−0.076	0.538	0.93 (0.73–1.18)	1.03 (0.80–1.32)
Q4	−0.836	0.000	0.43 (0.34–0.56)	0.54 (0.42–0.70)
Vitamin B_2				
Q1	0.102	0.331	1.11 (0.90–1.36)	1.09 (0.88–1.35)
Q2			1.00	1.00
Q3	−0.330	0.009	0.72 (0.56–0.92)	0.80 (0.62–1.03)
Q4	−0.907	0.000	0.40 (0.32–0.52)	0.50 (0.38–0.64)
Vitamin B_3				
Q1	0.154	0.142	1.17 (0.95–1.43)	1.16 (0.94–1.44)
Q2			1.00	1.00
Q3	−0.455	0.000	0.63 (0.49–0.82)	0.71 (0.55–0.92)
Q4	−0.954	0.000	0.39 (0.30–0.49)	0.48 (0.37–0.63)
Iron				
Q1	0.193	0.066	1.21 (0.99–1.49)	1.18 (0.96–1.46)
Q2			1.00	1.00
Q3	−0.293	0.021	0.75 (0.58–0.96)	0.80 (0.62–1.03)
Q4	−0.936	0.000	0.39 (0.31–0.50)	0.48 (0.37–0.63)
Magnesium				
Q1	0.134	0.203	1.14 (0.93–1.41)	1.12 (0.90–1.38)
Q2			1.00	1.00
Q3	−0.403	0.002	0.67 (0.52–0.86)	0.74 (0.57–0.97)
Q4	−0.709	0.000	0.49 (0.39–0.62)	0.60 (0.47–0.77)
Zinc				
Q1	0.107	0.306	1.11 (0.91–1.37)	1.12 (0.90–1.38)
Q2			1.00	1.00
Q3	−0.448	0.001	0.64 (0.50–0.82)	0.73 (0.56–0.95)
Q4	−0.931	0.000	0.39 (0.31–0.51)	0.50 (0.38–0.64)
Selenium				
Q1	0.336	0.009	1.40 (1.09–1.80)	1.24 (0.96–1.60)
Q2	0.517	0.000	1.68 (1.29–2.98)	1.48 (1.13–1.94)
Q3			1.00	1.00
Q4	−0.491	0.001	0.61 (0.46–0.82)	0.71 (0.53–0.96)
Copper				
Q1			1.00	1.00
Q2	−0.082	0.437	0.92 (0.75–1.13)	0.98 (0.79–1.23)
Q3	−0.548	0.001	0.58 (0.44–0.76)	0.71 (0.53–0.95)
Q4	−0.848	0.001	0.43 (0.33–0.55)	0.60 (0.46–0.80)
Manganese				
Q1			1.00	1.00
Q2	−0.072	0.500	0.93 (0.76–1.15)	1.00 (0.80–1.25)
Q3	−0.371	0.005	0.69 (0.53–0.90)	0.84 (0.64–1.12)
Q4	−0.804	0.001	0.45 (0.35–0.58)	0.64 (0.49–0.85)
Iodine				
Q1	0.389	0.000	1.48 (1.19–1.83)	0.82 (0.46–1.44)
Q2			1.00	1.00
Q3	−0.362	0.011	0.70 (0.53–0.92)	0.85 (0.64–1.13)
Q4	0.379	0.001	1.46 (1.18–1.81)	1.29 (1.03–1.62)

The intake of all dietary nutrients were divided into four quartiles as categorical variables, with the quartiles closest to dietary reference intakes (DRIs) as the respective referent group.

**Models adjusted for age and gender.*

#Model adjusted for age, gender, BMI, education, live situation, smoking, drinking, Reading, Computer use, labor intensity, work time, hypertension, diabetes, hyperlipidemia, PVD, and CHD.

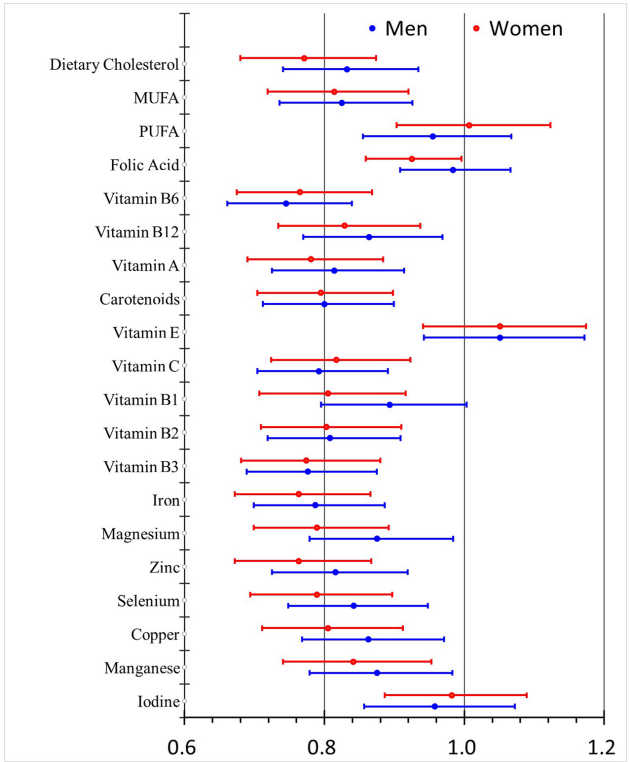

FIGURE 1 | Odds ratios (95% CI) for dietary nutrients intake-related risk factors of MCI with different adjustment levels in men and women.

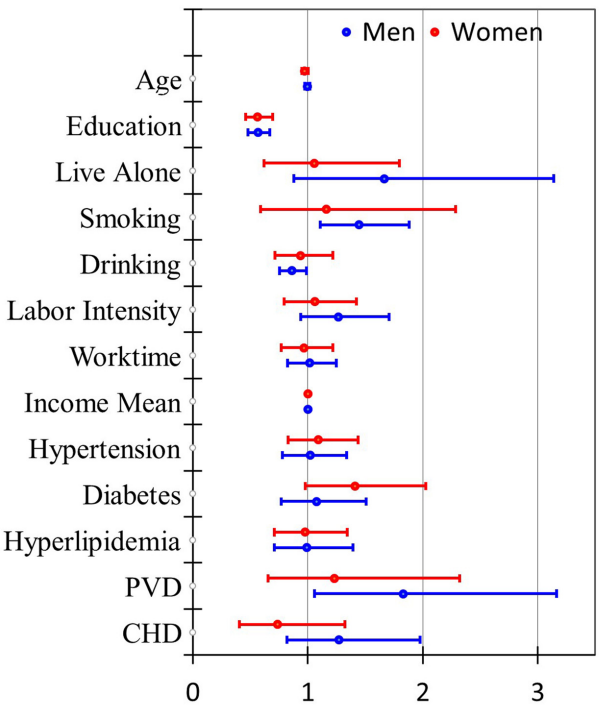

FIGURE 2 | Odds ratios (95% CI) for lifestyle-related risk factors of MCI with different adjustment levels in men and women.

was consistent with our and others' previous observations regarding the cognition-protective nutrient combination and dietary pattern. These results indicated that diets or foods rich in vitamins and minerals, in particular the five top protective nutrients (carotenoids, vitamins C, B_6, and A, and folic acid) could benefit the cognition preservation in the elderly. The presence of vitamins B_1 and B_3 in all the three protective nutrient combination patterns implied an important role of them in that regard. Recently, intake of total B vitamins have been shown to be associated with better cognitive function in the cognitively impaired elderly, especially in AD patients (Kim et al., 2014; Li et al., 2014). Partial mechanisms underlying this association have been proposed including antioxidant defense and lower occurrence of methylation reactions in central nervous system (Haan et al., 2007; Araujo et al., 2015).

In the current study, we have illustrated that dietary intake of certain nutrients were associated with dose (quartile)-dependent changes in OR values of MCI compared with the respective reference quartiles. The significantly elevated OR for Q1 of vitamin A, B_6, B_{12}, C, and carotenoids intake, compared with Q2, suggested that inadequate dietary intake of these nutrients that were lower than DRIs could increase the risk of developing MCI in the elderly. It is also worth noting that the estimated dietary intake of selenium (Se) in the study population in the present study was fairly low (44–53 μg/day). This led to two outcomes: (1) the reference quartile was actually Q3 so that 50% of the subjects did not consume adequate Se; (2) subjects with Q2 of the Se intake had increased risk of MCI while those with Q4 of the Se intake had decreased risk.

The relationship between poor nutritional status and cognitive impairments was previously described. Individuals with low Dietary ingestion or blood concentration of vitamin C and B_{12} performed poorly on the cognitive function test (O'Leary et al., 2012). Similar results were reported in a study of old subjects living in Manhattan (Luchsinger et al., 2007). These results were partly in accordance with our findings which imply significant associations of inadequate dietary intake of certain nutrients with increased risks of MCI in the Chinese elderly. Since oxidative stress has been implicated as one of the primary causes of cognitive impairments (Farr et al., 2014), antioxidant nutrients such as vitamin C are efficient in alleviating oxidative stress at the initial stage of cognitive impairments (Dror et al., 2014). Indeed, several studies have reported protective effects of vitamins C on the development of AD (Arlt et al., 2012; Mohajeri et al., 2015). This supports our finding that adequate vitamin C intake was one of the strongest protective micronutrients in the pattern analysis.

Interestingly, we have revealed the unique combination of two lipid nutrients (MUFA and cholesterol) with micronutrients (Zn and vitamins B_1 and B_3) as the protective nutrient matrix pattern 2. The effects of dietary intake of fatty acids and

TABLE 5 | Factor loadings for rotated factor patterns of daily intake of dietary nutrients.

Nutrients	Pattern 1	Pattern 2	Pattern 3
Carotenoids	0.97	...	...
Vitamin C	0.95	...	...
Vitamin B_6	0.92	...	...
Folic acid	0.87	...	...
Vitamin A	0.84	...	...
Iron	0.82	...	0.44
Vitamin B_2	0.81	...	0.33
Copper	0.71	...	0.54
Manganese	0.67	...	0.64
Magnesium	0.66	...	0.57
Zinc	0.64	0.42	0.52
Vitamin B_3	0.62	0.47	0.47
Selenium	0.52	0.44	0.45
Vitamin B_1	0.47	0.51	0.65
MUFA	...	0.86	...
Cholesterol	...	0.53	...
PUFA	...	...	...
Vitamin E	...	...	...
Vitamin B_{12}	...	...	...
Iodine	...	...	...
Eigenvalue	14.11	2.26	1.51
Odds ratio*	0.72 (0.67–0.77)	0.76 (0.71–0.82)	0.75 (0.70–0.81)
Adjusted OR#	0.77 (0.71–0.83)	0.81 (0.75–0.87)	0.83 (0.76–0.89)

Factor pattern 1: vitamin and mineral pattern; Factor pattern 2: fatty acid pattern; Factor pattern 3: mineral pattern. Factor loadings less than 0.40 are not shown. OR, odds ratio.

**Models adjusted for age and gender.*

#Model adjusted for age, gender, BMI, education, live situation, smoking, drinking, Reading, Computer use, labor intensity, work time, hypertension, diabetes, hyperlipidemia, PVD, and CHD.

cholesterol on cognitive impairments have received particular attention, but the results are inconsistent (Grant, 2003; Del Parigi et al., 2006). Among 6,183 older participants in the Women's Health Study by using serial cognitive tests found that a higher MUFA intake was related to a better global cognition and verbal memory (Okereke et al., 2012). A recent animal experiment has examined the underlying mechanism of the protective effects of cholesterol and indicated that a high-cholesterol diet enriched with polyphenols could improve cognitive function by down-regulating brain cholesterol levels and neurodegenerative-related protein expression (Kuo et al., 2015). Since our study was a cross-sectional analysis, the derived significant association between present consumptions of cholesterol or MUFA and MCI might not actually reflect the impact of past cholesterol and MUFA intake on the current risk or status of MCI. Therefore, the potential mechanism of these dietary lipids in preserving cognitive function in the elderly need to be further tested in randomized controlled study or longitudinal studies.

Our study has also analyzed a number of lifestyle-related factors to the risk of MCI. The illustrated protective factors consist of education, computer use, reading, and drinking, whereas the risk factors include smoking and PVD. Other studies have reported similar protective roles of education and reading against the development of MCI (Luck et al., 2010; Eshkoor et al., 2015; Zhao et al., 2015; Dong et al., 2016; Li et al., 2016). Silbert et al. (2016) have focused on the association between computer uses and volumetric markers of neurodegeneration on brain MRI. They have found that cognitively intact volunteers with less daily computer use showed decreased gray matter density in the temporal lobes and bilateral hippocampi, which may shed light on the potential mechanism underlying the association. Despite the protective role of drinking in decreasing risk of MCI shown in our present study, systematic reviews have summarized major discrepancies regarding the effects of alcohol consumption on AD, probably due to variations of quantity and/or frequency of drinking (Piazza-Gardner et al., 2013; Roerecke and Rehm, 2014). Controversy also exists on the association of smoking with the risk of MCI (Durazzo et al., 2014; Deochand et al., 2015). In the present study, smoking was a strong risk factor (OR = 1.40, 95% CI: 1.09, 1.80) of MCI. Potentially, smoking may cause detrimental effects on memory via oxidative stress induced by decreasing plasma glutathione and serum superoxide dismutase activity and vitamin C concentration (Kim et al., 2003, 2004). The resultant oxidative injuries promote Aβ deposition and abnormal tau phosphorylation (Giunta et al., 2012), thus contributing to the development of AD. Notably, drinking and smoking associated with the risk of MCI remained significant only in the male subjects in our study. This was probably because fewer females were actually drinking or smoking. Besides, PVD was another strong risk factor only in the male subjects. Since PVD often coexists with aging, smoking, and obesity (Cassar et al., 2010), the gender-specific differences of PVD in MCI warrants further research.

Strengths and Limitations

Strengths of the present study are the relatively large sample size consisted of 2,892 participants recruited from three different regions of China, which was adequate for longitudinal studies. The health examination records, stored in the Physical Examination Centers, could provide an objective, accurate, and relatively large dataset for potential confounders. Furthermore, the statistical analyses adopted in this study enabled us to reveal three unique dietary nutrient intake combination patterns as the major protective factors in lowering the risk of MCI in the elderly.

However, the self-reporting derived FFQ data collected from the present study might suffer from recall bias, although the FFQ was one of the most commonly used methods to assess dietary patterns and to calculate the quantity of nutrient consumption. Despite a relatively large sample size, our study was still a cross-sectional analysis. Therefore, longitudinal studies with several follow-up or randomized controlled trials will be needed to explore and better define the cause-effect relationships between dietary nutrients intake or lifestyle and MCI. Equally important, mechanistic studies should be conducted in animals or *in vitro* to elucidate the molecular pathway and physiological regulation underlying the observed associations.

CONCLUSION

Our study revealed three dietary nutrients intake patterns as the major protective factors against the development of MCI in the Chinese elderly. Adequate or enhanced daily intake of carotenoids, vitamin C, and vitamin B_6 ranked as the most protective factors in preserving the cognition in the old Chinese subjects. Improving lifestyle with more education, computer use, reading, and moderate drinking were also protective against, whereas smoking and PVD were potentiating the risk of MCI. Future research is required to confirm and explain the positive effects of dietary MUFA and cholesterol intake on lowering the risk of MCI.

ETHICS STATEMENT

The study protocol was in accordance with the Declaration of Helsinki and ethically approved by the Ethics Committee of Capital Medical University (2013SY35). The consent contains project title, investigators and other project personnel, introduction to project and invitation to participates, what this project is about and why it is being undertaken, project and researcher interests, what participation will involve, participant rights and interests, who to contact. Outline realistically any potential risks and relative preventive arrangements are in place.

AUTHOR CONTRIBUTIONS

RX conceived and designed the study, YL collected the data, performed the analyses and wrote the manuscript. YA, JG, XZ, HW, and HR helped collect and analyze the data. All authors read and approved the final manuscript.

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Association between Exposure to the Chinese Famine in Different Stages of Early Life and Decline in Cognitive Functioning in Adulthood

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Objective: To investigate whether exposure to the Chinese Famine in different life stages of early life is associated with cognitive functioning decline in adulthood.

Methods: We recruited 1366 adults born between 1950 and 1964 and divided them into fetal-exposed, early childhood-exposed (1–3 years old during the famine), mid childhood-exposed (4–6 years old during the famine), late childhood-exposed (7–9 years old during the famine), and non-exposed groups. A selection of cognitive tests was administered to assess their cognitive performance. Association between malnutrition in different famine exposure periods and adult cognitive performance was estimated by multivariate logistic and multiple linear regression analyses.

Results: There were significant differences in cognitive performance between subjects exposed to famine during different life stages. For the general cognitive tests, fetal-exposed period was associated with decreased scores of the Mini-Mental State Examination (MMSE), and late childhood-exposed with decreased scores of the Montreal Cognitive Assessment (MoCA). We also found exposure to famine during mid and late childhood was associated with worse performance on the Stroop color and word test.

Conclusion: Famine exposure *in utero* and during childhood is associated with overall and specific cognitive decline, affecting selective attention and response inhibition particularly.

Keywords: Chinese famine, malnutrition, different life stages, cognitive functioning, early life

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INTRODUCTION

Cognitive functioning is thought to result from complex interactions between genetic and environmental exposures over the course of the life. Good nutrition and sufficient nutrients availability always links brain functioning and cognitive development (Guesry, 1998; Nyaradi et al., 2013). There is an intriguing hypothesis that specific environmental exposures at sensitive or critical time points can alter brain and cognitive development with life-long consequences (Hoeijmakers et al., 2014). Early undernutrition especially during pregnancy and several years after birth thus may be such an environmental exposure increasingly implicated in irreversible and long-term damage to the brain and behavioral function (Venables and Raine, 2012; Raikkonen et al., 2013; Waber et al., 2014b).

Scholars have showed a growing interest in assessing potential long-term cognitive and behavioral functioning consequences of early life nutritional deprivation and thus famines in human history provide distinct opportunities to undertake such research. Dutch famine at the end of World War II was an intense period of food shortage and had a 6-month well-defined but brief duration (de Rooij et al., 2006). Numerous epidemiological studies about the Dutch famine has focused on the impact of exposure during gestation on increased susceptibility to the development of psychiatric disorders and long-lasting cognitive functioning. Some have suggested that the risk of schizophrenia is increased among individuals conceived during the famine (Hoek et al., 1996) and simultaneously people exposed in early gestation had elevated risk of anti-social personality disorder (Neugebauer et al., 1999). Moreover, the association between prenatal famine exposure and affective psychoses (Brown et al., 1995) and depression (de Rooij et al., 2011) was also observed. Furthermore, the Dutch famine birth cohort study also suggested the cognitive functioning in adulthood may be adversely affected by periconceptional exposure to famine (Roseboom et al., 2011).

Compared with Dutch famine, the Chinese famine, whose most severe period persisted from 1959 to 1961, was of longer duration. Superimposed on widespread undernutrition and food unavailability, Chinese famine was regarded as one of the catastrophes in Chinese history (Mu and Zhang, 2011). Therefore, it was expected that the impact of Chinese famine on behavioral and cognitive development to be more marked than the Dutch famine. Similar study results of increased schizophrenia risk were also found in the Chinese famine (St et al., 2005; Song et al., 2009).

However, findings from studies on Dutch famine are contradictory, and they have mostly focused on prenatal exposure only (Stein, 2014). Besides, undernutrition in childhood has been increasingly implicated in adult physical and mental health, which indicated that the inconsistent results in the literature may be caused by different postnatal life exposures (Huang et al., 2010). Since the influence of undernutrition during pregnancy and/or in early postnatal life on cognitive functioning involving multiple domains in late adulthood has not been examined. The purpose of the current study was to estimate the association between them among the pre- and postnatally exposed Chinese famine survivors.

METHODS

Study Population

The data of this study derived from an ongoing epidemiological survey granted by the State Key Program of National Natural Science Foundation of China, which was aimed at investigating the role of dietary cholesterol on Alzheimer's disease (AD) and conducted in different regions from 2013 to 2018. The subjects were recruited from local large hospitals and screened with a series of cognitive functioning tests. The selection of the subjects was required to be satisfied a number of criteria, which included willingness to participate in the study, born between 1950 and 1964 years and Han Chinese residents. The exclusion criteria included suffering from cognitive decline or impairment caused by a history of cerebrovascular disease, depression, traumatic brain injury, drugs, and currently taking medication or dietary supplement to improve cognitive functioning. The study protocol was in accordance with the Declaration of Helsinki and ethically approved by the Ethics Committee of Capital Medical University (2013SY35). All participants were provided written informed consent at the beginning of the study.

Famine Age Categories

The nationwide famine unexpectedly hit China in the late 1950s and continued to the early 1960s. The most severe period was from 1959 to 1961. We selected subjects born between October 1st, 1950, and September 30th, 1964 as our analytic population based on the study Li et al. (2010). Famine exposure is set up according to birth year and subjects were subsequently divided into five groups: non-exposed group (age = 51–53, birth year = 1962–1964, $n = 237$), fetal-exposed group (age = 54–56, birth year = 1959–1961, $n = 217$), early childhood-exposed group (age = 57–59, birth year = 1956–1958, 1–3 years old during the famine, $n = 314$), mid childhood-exposed group (age = 60–62, birth year = 1953–1955, 4–6 years old during the famine, $n = 320$), and late childhood-exposed group (age = 63–65, birth year = 1950–1952, 7–9 years old during the famine, $n = 278$). Our total sample size was 1366 subjects.

Demographic and Clinical Assessment

The demographic and clinical characteristics of the subjects including age, gender, years of education, lifestyle, family history of dementia, and medical history were collected by face-to-face interviews at the baseline evaluation. Smoking status and alcohol consumption were also ascertained. We coded smoking and alcohol as current and other. Current smoking was defined as having smoked three or more cigarettes a week during the past 6 months before recruitment. Current alcohol consumption was defined as alcohol intake three or more times a week during the past 6 months before recruitment. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

Cognitive Assessment

A selection of well-established and conventional cognitive functioning tests on the basis of earlier research was used to assess respondents' cognitive performance, which took about 40 min to complete. All the interviews were done face to face in local hospitals by nurses or researcher who had attended unified training several times before. All the tests were carried out according to provided guidelines and procedures. The assessment contained the following cognitive functioning tests.

THE MINI-MENTAL STATE EXAMINATION (MMSE)

The Chinese version MMSE was chosen for global cognitive status across multiple domains. As a rapid cognitive screening instrument and a practical method of grading cognitive functioning, it comprises 20 individual tests, totaling 30 points and covers 11 domains. The brevity and the broad coverage of cognitive domains make it the most commonly used cognitive

instrument and diagnostic test of dementia (Mitchell et al., 2014). The cutoff score for dementia applied to the Chinese residents is 19 for illiterate individuals, 22 for individuals with 1–6 years of education and 26 for individuals with 7 or more years of education (Zhang et al., 1999).

THE MONTREAL COGNITIVE ASSESSMENT (MoCA)

The Beijing version MoCA is also brief 30-point assessment of global cognitive screening instrument intended to detect mild cognitive impairment (MCI). It also provides a comprehensive assessment including a broad array of cognitive domains but incorporates expanded executive function and visuospatial items, which offers sensitivity and specificity to detect MCI patients and other cognitively impaired subjects with a normal range score on the MMSE (Gluhm et al., 2013; Lam et al., 2013). The cutoff score for MCI applied to the Chinese residents is 14 for illiterate individuals, 19 for individuals with 1–6 years of education, and 24 for individuals with 7 or more years of education (Lu et al., 2011).

LOGICAL MEMORY TEST (LMT)

The test from the Wechsler Memory Scale-Revised, Chinese version (WMS-RC) edited by Gong et al. (1989) was adopted to evaluate memory functions in this study. Logical memory test (LMT) provided measures of verbal memory function and capacity to recall and acquire information over brief time periods. Participants tested by LMT were required to recall two story paragraphs told by investigators immediately. Gist and verbatim scoring systems were used to evaluate the verbal recall of the story paragraphs.

THE STROOP COLOR AND WORD TEST (SCWT)

The test consists of three subtests: subtask I composed of names of four colors printed in black font (red, blue, yellow, and green), subtask II with patches in one of these colors and subtask III that consists of color names printed in an incongruous ink color. Each subtest displays 50 stimuli. Subjects were instructed to first read the color names (subtask I), then recognize color of the patches (subtask II), and finally name the ink color of the printed words (subtask III) as quickly as possible. The outcome of this test was completion time (in seconds) and correct number of each subtest. Stroop interference effects (SIE) were used as the analyzed index, which were composed of time for SIE, calculated according to the formula of (subtask III time- subtask II time), and correct number for SIE, calculated according to the formula of (subtask II correct number- subtask III correct number). The test assesses selective attention and processing speed as well as response inhibition, an index of executive function. Lower scores represent superior cognitive performance (Guo et al., 2005).

Statistical Analyses

The analysis was performed using IBM SPSS software (Version 19.0). All the analyses were two-sided and the statistically significant level was set at 0.05. Continuous variables were expressed as the mean ± standard deviation (SD) when normally distributed or medians (interquartile ranges, IQR) when non-normally distributed. And categorical values were shown in the form of frequencies (percentage,%). Univariate statistical analysis used the following tests: Pearson's chi-square test for categorical variables, analysis of variance (ANOVA), or the Mann–Whitney *U*-test for continuous variables, as appropriate. *Post-hoc* comparisons were evaluated using the Dunnett *t*-tests and Hochberg modification of the Bonferroni correction tests. To assess associations among life stages exposed to famine and cognitive functioning, multivariate logistic regression analyses, and multiple linear regression analyses were used. Non-exposed group was used as reference group. The model took into account potential confounders such as age, gender, years of education, lifestyle, and medical history and model parameters were estimated.

RESULTS

Table 1 shows the results of demographic and clinical characteristics by different life stages exposed to famine. Compared with non-exposed group, childhood-exposed subjects had significantly lower years of education.

Table 2 presents the results of cognitive tests for each of the five groups. Subjects exposed to famine during any stage of childhood had significantly lower scores for MMSE and MoCA. Mid and late childhood exposed subjects had significantly longer time for SIE and only late childhood exposed subjects had lower correct for SIE than those with exposure in other periods. No substantive differences were observed among the groups on LMT.

Table 3 shows no significant association of famine exposure with dementia screened by MMSE and MCI screened by MoCA in multivariate logistic analyses.

Table 4 provides the association of famine exposure with cognitive performance in multiple linear analyses. Exposure to famine during fetal period was associated with a 0.638 point decrease in the scores of MMSE and late childhood exposure was associated with a 0.680 point decrease in the scores of MoCA. Early childhood and late childhood exposure positively affected the correct for SIE. Meanwhile, mid childhood and late childhood exposure showed positive association with time for SIE. However, no association between worse performance on LMT and famine exposure was observed.

DISCUSSION

We investigated whether early-life malnutrition exposure *in utero* and during childhood to the Chinese famine would predict cognitive functioning in later life among Chinese born from 1950 to 1964. Although no association between exposure and general cognitive impairment including dementia screened by MMSE

TABLE 1 | Demographic and clinical characteristics of 1366 individuals exposed to the Chinese famine of 1959–1961.

	Childhood-exposed			Fetal-exposed	Non-exposed
	Late childhood	Mid childhood	Early childhood		
N	278	320	314	217	237
Birth date					
(From October 1,year)	1950	1953	1956	1959	1962
(To September 30,year)	1952	1955	1958	1961	1964
Age in 2015	63–65	60–62	57–59	54–56	51–53
Women (%)	132 (47.5)	158 (49.4)	159 (50.6)	113 (52.1)	108 (45.6)
BMI (kg/m^2)	25.36 ± 3.46	25.27 ± 3.23	25.00 ± 3.21	25.24 ± 3.70	25.72 ± 3.81
Years of education	9 (9, 12)*	9 (9, 12)*	12 (9, 12)*	12 (9, 12)	12 (9, 16)
Family history (%)	22 (7.9)	26 (8.1)	32 (10.2)	22 (10.1)	16 (6.8)
Current smoking (%)	78 (28.1)	79 (24.7)	82 (26.1)	61 (28.1)	76 (32.1)
Alcohol use (%)	76 (27.3)	85 (26.6)	100 (31.8)	71 (32.7)	80 (33.8)
Hypertension (%)	98 (35.3)	110 (34.4)	90 (28.7)	61 (28.1)	66 (27.8)
Hyperlipidemia (%)	56 (20.1)	67 (20.9)	65 (20.7)	46 (21.2)	35 (14.8)
Diabetes (%)	42 (15.1)	46 (14.4)	40 (12.7)	25 (11.5)	32 (13.5)
Coronary heart disease (%)	17 (6.1)	15 (4.7)	21 (6.7)	15 (6.9)	16 (6.8)

BMI, body mass index. Data are presented means ± SD, median (interquartile range), or n (%).
Data shown as median (interquartile range) were compared between 5 groups using the Mann–Whitney U-test.
Data shown as mean ± standard deviation were compared between 5 groups using the analysis of variance.
Data shown as n (%) were compared between 5 groups using the Pearson's chi-square test.
**Compare with non-exposed group using the Hochberg modification of the Bonferroni correction: the adjusted p for statistical significance was calculated as p' = p /[k (k − 1)/2] = 0.05/[5*(5 − 1)/2] = 0.005, p' < 0.005.*

TABLE 2 | Performance on tests of cognitive functioning among 1366 individuals.

	Childhood-exposed			Fetal-exposed	Non-exposed
	Late childhood	Mid childhood	Early childhood		
MMSE	28 (27, 29.5)*	29 (27, 30)*	29 (27, 30)*	29 (27, 30)	29 (28, 30)
MoCA	25 (22, 27)*	25 (23, 27)*	25 (22, 27)*	26 (23, 27)	26 (24, 28)
Correct for SIE	1 (0, 4)*	0 (0, 3)	1 (0, 3)	1 (0, 3)	0 (0, 2)
Time for SIE	38 (29, 50)*	37 (28, 51)*	36 (27, 47)	35.5 (26, 45)	31 (25, 43)
LMT	10.70 ± 5.27	10.42 ± 5.11	10.37 ± 5.28	10.79 ± 5.48	11.03 ± 5.13

MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; SIE. Stroop Interference Effects; LMT, Logical Memory Test.
Data are presented as means ± SD and median (interquartile range). Data shown as median (interquartile range) were compared between 5 groups using the Mann–Whitney U-test.
Data shown as mean ± standard deviation were compared between 5 groups using the analysis of variance.
Time for SIE was calculated according to the formula of (Stroop C time-Stroop B time).
Correct for SIE was calculated according to the formula of (Stroop B correct number-Stroop C correct number).
**Compare with non-exposed group using the Hochberg modification of the Bonferroni correction: the adjusted p for statistical significance was calculated as p' = p /[k (k − 1)/2] = 0.05/[5*(5 − 1)/2] = 0.005, p' < 0.005.*

and MCI screened by MoCA was observed, exposure to famine *in utero* and during childhood is associated with cognitive decline in selective attention and response inhibition, as suggested by lower performance on Stroop color and word test. This study suggested intrauterine periods and a few years of postnatal life were critical for brain functioning and cognitive development.

The current study provides new and further evidence to the accumulating body of literature which have demonstrated early-life nutritional deprivation has a profound and long-term effect on cognitive development (Levitsky and Strupp, 1995; Galler et al., 2005, 2012; Lumey et al., 2011; Ampaabeng and Tan, 2013). The severe period of Chinese famine lasted 3 years, which allows an investigation of cognitive consequences of famine exposure in pregnancy and the first several years of life. We found no effects of pre- and postnatal famine exposure on cognitive impairment that meets screening criteria such as dementia and MCI, suggesting the effects were not severe enough to be linked with dementia and MCI. Despite that, we cannot exclude that exposure to famine is associated with slight changes and decline in cognitive functioning. As we can see from the results of multiple liner regression, exposure to famine during fetal period and late childhood was respectively associated with slightly decreased scores of MMSE and MoCA, indicating slight but significant impact of early life malnutrition on the cognitive development will persist into adulthood and result in a little poorer performance on general cognitive tests. Subsequently, subjects exposed during mid-childhood and late childhood performed significantly worse in time for SIE than non-exposed ones,

TABLE 3 | Association of exposure to the Chinese Famine in different life stages of early life with dementia screened by MMSE and MCI screened by MoCA from logistic regression analyses.

	Childhood-exposed			Fetal-exposed	Non-exposed
	Late childhood	Mid childhood	Early childhood		
DEMENTIA					
N	3	6	12	9	4
OR (95%CI)*	3.23 (0.74–14.01)	1.68 (0.48–5.88)	2.01 (0.72–5.58)	1.96 (0.91–4.26)	Ref.
p	0.12	0.42	0.18	0.88	Ref.
MCI					
N	91	102	106	68	68
OR (95%CI)*	1.27 (0.43–3.76)	0.93 (0.37–2.34)	1.17 (0.55–2.48)	1.14 (0.64–2.03)	Ref.
p	0.66	0.88	0.68	0.66	Ref.

MCI, mild cognitive impairment; OR, odds ratio; Ref, reference group.
**Adjusted for demographic and clinical characteristics.*

TABLE 4 | Association of exposure to the Chinese Famine in different life stages of early life with performance of cognitive functioning tests from multiple linear regression analyses.

	Childhood-exposed			Fetal-exposed	Non-exposed
	Late childhood	Mid childhood	Early childhood		
MMSE					
Median (IQR)	28 (27, 30)	29 (27, 30)	29 (27, 30)	29 (27, 30)	29 (28, 30)
B	–0.336	–0.247	–0.358	–0.638	Ref.
p	0.112	0.227	0.078	0.004*	Ref.
MoCA					
Median (IQR)	25 (22, 27)	25 (23, 27)	25 (22, 27)	26 (23, 27)	26 (24, 28)
B	–0.680	–0.258	–0.542	–0.590	Ref.
p	0.037*	0.411	0.083	0.081	Ref.
CORRECT FOR SIE					
Median (IQR)	1 (0, 4)	0 (0, 3)	1 (0, 3)	1 (0, 3)	0 (0, 2.25)
B	1.292	0.061	0.749	0.422	Ref.
p	0.001*	0.872	0.048*	0.306	Ref.
TIME FOR SIE					
Median (IQR)	38 (29, 50)	37 (28, 51)	36 (27, 47)	36 (26, 45)	31 (25, 43)
B	5.942	7.261	4.249	2.625	Ref.
p	0.011*	0.002*	0.062	0.286	Ref.
LMT					
Mean ± SD	10.70 ± 5.27	10.42 ± 5.11	10.37 ± 5.28	10.79 ± 5.48	11.03 ± 5.13
B	0.778	0.397	0.017	–0.081	Ref.
p	0.119	0.410	0.971	0.875	Ref.

MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; SIE, Stroop Interference Effects; LMT, Logical Memory Test; Ref, reference group.
Data are presented as means ± SD and median (interquartile range).
**Compare with non-exposed group, $p < 0.05$.*

providing evidence of the impairment of selective attention and response inhibition, an index of executive function. Stroop-like tests require selective attention and response inhibition (Van der Elst et al., 2006; Douris et al., 2015), which arises when subjects are completing the third conflicting part of the test between reading the words or naming the color of the words. Spending more time on choosing non-automatic response and inhibiting automatic response means poorer selective attention and response inhibition performance. Carter et al. (1997) have found the lower scores of color-incongruent Stroop task may be linked with less anterior cingulate gyrus activation among patients with schizophrenia. Moreover, age-related diffuse lesions of the white matter also have been reported to be associated with reduced Stroop tests and selective attention performance (van Swieten et al., 1996). Compared with Dutch famine studies, a Stroop color-word interference test found no impact of exposure to famine in the pregnancy on cognitive functioning (de Groot et al., 2011) whereas a Stroop-like task was

associated with famine exposure *in utero* (de Rooij et al., 2010). The conflicting results of these studies demonstrate different association between maternal malnutrition during fetal life and performance of Stroop-like tasks. However, our results showed significant association with mid childhood and late childhood exposure. The inconsistent results may be due to various version and differently operationalized Stroop tests and different timing and duration of exposure.

Several cellular and molecular mechanisms might explain the different associations that fetal-exposed group has deficits in MMSE, late childhood-exposed group in MoCA and mid/late childhood-exposed group in Stroop color and word test. It should be noted that the prenatal period involves most of the process of neurogenesis. Perez-Garcia et al has demonstrated prenatal protein malnutrition in rats led to impaired encoding and consolidation of memory. This learning deficit was associated with reduced hippocampal neurogenesis (Perez-Garcia et al., 2016). However, in the postnatal period synaptogenesis takes place (Alamy and Bengelloun, 2012). Malnutrition during different phases can thus produce permanent alterations in different neurotransmitters systems, which may be responsible for different cognitive performance exhibited in MMSE, MoCA, and Stroop color and word test. It is also noteworthy that long-term intellectual compromise is associated with stunting in the first few years of life (Waber et al., 2014a). Nevertheless, a relevant study in Peru has showed that children who recovered from early stunting did not differ in cognitive functioning in contrast to non-stunted counterparts (Crookston et al., 2011). Since stunted children with subsequent catch-up growth or nutritional recovery had demonstrated normal levels of cognition, weight, and height, body compositions and bone mineral densities (Martins et al., 2011), the persisting effects of chronic postnatal exposure on brain need to be paid more attention and thus help previously malnourished children complete catch-up in mental and physical growth.

The effects of early-life famine exposure on cognitive development depend on a variety of factors (Huang et al., 2013), including timing and duration of exposure and severity of the famine as well as specific instruments and methods of grading cognitive functioning. Some potential threats to validity cannot be neglected. Possible confounders were not included such as birth state, regional disparity, and immigration (Wang et al., 2015). It may not be easy to judge which were really privileges among those confounders and the cognitive decline is not exclusively due to famine exposure, which may contribute to the seemingly small results of comparatively slight decrease in scores of tests.

Since the age at which malnutrition occurs is a critical factor, subjects exposed in different times of life also differs in age. The current study is an observational study with different age groups based on timing and duration of exposure being taken into analysis rather than different groups according to famine and non-famine exposure with comparable age, which mainly because almost all the areas in China were affected by the famine from 1959 to 1961, no valid subjects without famine exposure born at the same time was available. There is no doubt that cognitive functioning changes with normal human aging, and attention and memory are two basic cognitive functions that are most affected by aging. Therefore, the results of both multivariate logistic regression analysis and multiple linear regression analysis took into account potential confounders especially age and significant associations were still observed.

In a word, the study investigates famine exposure not only during gestation but also during infancy and childhood, which could be generalized to the effects of chronic undernutrition. However, it remains uncertain whether the results would be different if the study could establish Chinese Famine Birth Cohort and complete follow-up. Therefore, there are good reasons to plan in-depth study in the future and to evaluate relevant cognitive disorders such as neurodegenerative problems.

CONCLUSION

The findings of the current study suggested that the cognitive decline ensuing from malnutrition during gestation and childhood caused by exposure to the Chinese Famine in different stages of early life is considerable. The impairment of selective attention and response inhibition was especially detected. Given the prevalence of malnutrition during gestation and childhood worldwide, the significant association between early-life malnutrition and long-term cognitive decline in our findings indicate that prevention of malnutrition in early life should remain a major public health goal. Meanwhile, early life interventions should be taken into consideration to help malnourished children to be fully rehabilitated nutritionally and complete catch-up in mental and physical growth, thus mitigating these significant neurodevelopmental insults.

AUTHOR CONTRIBUTIONS

RX designed experiments; CW, YA, HY, YL, HW, LF, and QL carried out experiments; CW and YA analyzed experimental results and wrote the manuscript.

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