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Addiction-like Synaptic Impairments in Diet-Induced Obesity

Robyn Mary Brown, Yonatan Michael Kupchik, Sade Spencer, Constanza Garcia-Keller, David C. Spanswick, Andrew John Lawrence, Stephanie Elise Simonds, Danielle Joy Schwartz, Kelsey Ann Jordan, Thomas Clayton Jhou, and Peter William Kalivas

ABSTRACT

BACKGROUND: There is increasing evidence that the pathological overeating underlying some forms of obesity is compulsive in nature and therefore contains elements of an addictive disorder. However, direct physiological evidence linking obesity to synaptic plasticity akin to that occurring in addiction is lacking. We sought to establish whether the propensity to diet-induced obesity (DIO) is associated with addictive-like behavior, as well as synaptic impairments in the nucleus accumbens core considered hallmarks of addiction.

METHODS: Sprague Dawley rats were allowed free access to a palatable diet for 8 weeks then separated by weight gain into DIO-prone and DIO-resistant subgroups. Access to palatable food was then restricted to daily operant self-administration sessions using fixed ratio 1, 3, and 5 and progressive ratio schedules. Subsequently, nucleus accumbens brain slices were prepared, and we tested for changes in the ratio between α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate currents and the ability to exhibit long-term depression.

RESULTS: We found that propensity to develop DIO is linked to deficits in the ability to induce long-term depression in the nucleus accumbens, as well as increased potentiation at these synapses as measured by AMPA/*N*-methyl-D-aspartate currents. Consistent with these impairments, we observed addictive-like behavior in DIO-prone rats, including 1) heightened motivation for palatable food; 2) excessive intake; and 3) increased food seeking when food was unavailable.

CONCLUSIONS: Our results show overlap between the propensity for DIO and the synaptic changes associated with facets of addictive behavior, supporting partial coincident neurological underpinnings for compulsive overeating and drug addiction.

Keywords: Food addiction, Glutamate, Long-term depression, Nucleus accumbens, Obesity, Synaptic plasticity

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Obesity is rapidly approaching tobacco use as the leading cause of death in the industrialized world (1). While many factors may underlie obesity, the increasing availability of highly palatable, processed foods is a major contributor. Similar to drugs of abuse, highly palatable foods are powerful reinforcers and interact with brain reward circuitry to promote intake (2–7). As with drug addiction, this can lead to pathological overconsumption in susceptible individuals. Thus, it could be argued that in addition to homeostatic feeding mechanisms, excessive intake of palatable food may be explained by dysfunctions in reward circuitry. Indeed, there is emerging evidence in both humans and rodents that supports a hypothesis that the brain's reward circuitry is dysregulated in certain types of obesity, specifically that which results from compulsive overeating (5,7–17). This can manifest in symptoms that parallel those observed in drug addiction such as uncontrolled and excessive consumption, unsuccessful attempts to cut back or reduce intake, and the continuation of overeating despite adverse consequences (18–20).

The transition to drug addiction has been strongly linked to changes in prefrontal cortex regulation of basal ganglia circuitry (21,22). Using animal models of self-administration and relapse, enduring impairments in glutamatergic transmission and synaptic plasticity have been shown on medium spiny neurons in the nucleus accumbens (22). Neuroadaptations in these synapses can be shared between different chemical classes of addictive drugs (22–25). For example, repeated use of drugs such as cocaine and nicotine produces a long-lasting potentiation of these synapses together with a deficit in the ability to induce synaptic plasticity (23,26–28). Critically, an enduring impairment in the ability to induce long-term depression (LTD) in the nucleus accumbens core subcompartment (NAcore) of animals classified as addiction vulnerable to cocaine, but not as addiction resilient, has been implicated in the transition to addiction (27). These data point to potentiated glutamatergic synapses in the accumbens with an impaired ability to undergo LTD as a pathology in psychostimulant addiction. Thus, we sought to

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examine whether rats prone to obesity due to excessive intake of palatable food would exhibit these cardinal synaptic impairments and show similar characteristics toward food that rats classified as addiction vulnerable show toward drugs. We assessed in rats three addiction-like behaviors, used as hallmarks of both drug addiction and pathological overeating (19,25,29,30): 1) a high motivation to obtain the substance; modeled using a progressive ratio schedule, whereby the effort required to obtain food increases progressively within the session; 2) the rapid consumption of significantly larger than normal amounts of the substance; modeled by measuring intake when access to palatable food was limited; and 3) a lack of control to refrain from seeking the substance; modeled by measuring the persistence of lever-pressing during periods that signaled reward unavailability.

METHODS AND MATERIALS

Experimental Subjects

Experimentally naive, outbred male Sprague Dawley rats (Charles River Laboratories, Raleigh, North Carolina) weighing 250 g to 300 g at the start of the experiment were housed individually with nesting/enrichment material made available. A 12-hour light/dark cycle was maintained at all times, with lights turned off at 6:00 AM. Experimental procedures were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee. Rats were given ad libitum access to water and either standard chow (Tekland Global 2018, 18% kcal fat; total density = 3.1 kcal g^{-1}; Harlan Laboratories Inc., Indianapolis, Indiana) or palatable diet (D12451, 45% kcal fat; total density = 4.73 kcal g^{-1}; Research Diets Inc., New Brunswick, New Jersey). Rats were given 7 days to acclimate before experimentation began. A second cohort of Sprague Dawley rats (Monash University, Melbourne, Australia) was housed in similar conditions. Their standard chow diet was obtained from Barastoc (8720610, 9% kcal rat; total density = 3.1 kcal g^{-1}; Barastoc, Melbourne, Australia).

Model of Diet-Induced Obesity

Obesity and drug addiction share the characteristic that when a population is exposed to palatable food or drug, only a subpopulation will develop obesity or addiction. We wanted to model the subpopulation of obese individuals that develop obesity due to excessive overeating of palatable food, as opposed to obesity caused by other factors. We employed a naturalistic diet-induced obesity model that separates outbred rats into obesity-prone (OP) and obesity-resistant (OR) groups based on weight gain in response to a palatable diet (31,32). Diet-induced obese rats in this model exhibit hyperphagia, increased adiposity, and the typical metabolic disturbances found in human obesity (33–36). Rats were placed on a highly palatable diet (D12451, 45% kcal fat; total density = 4.73 kcal g^{-1}; Research Diets Inc.) for a period of 8 weeks. Food intake and body weight were determined twice per week. Rats were then separated into diet-induced OP (top third) and diet-induced OR (bottom third) groups based on weight gain (Figure 1) (31). Weight gain was determined from weeks 3 to 8 of the 8-week period to avoid weight gain due to normal development during the first 2 weeks. A second group of rats was split into two groups and given access to either standard chow or palatable diet (SF04-001, identical formulation to D12451, 45% kcal fat; total density = 4.73 kcal g^{-1}; Specialty Feeds, Glen Forrest, Australia) for a period of at least 8 weeks before being separated by weight gain (also determined from weeks 3 to 8 of the 8-week period) and utilized for electrophysiology experiments.

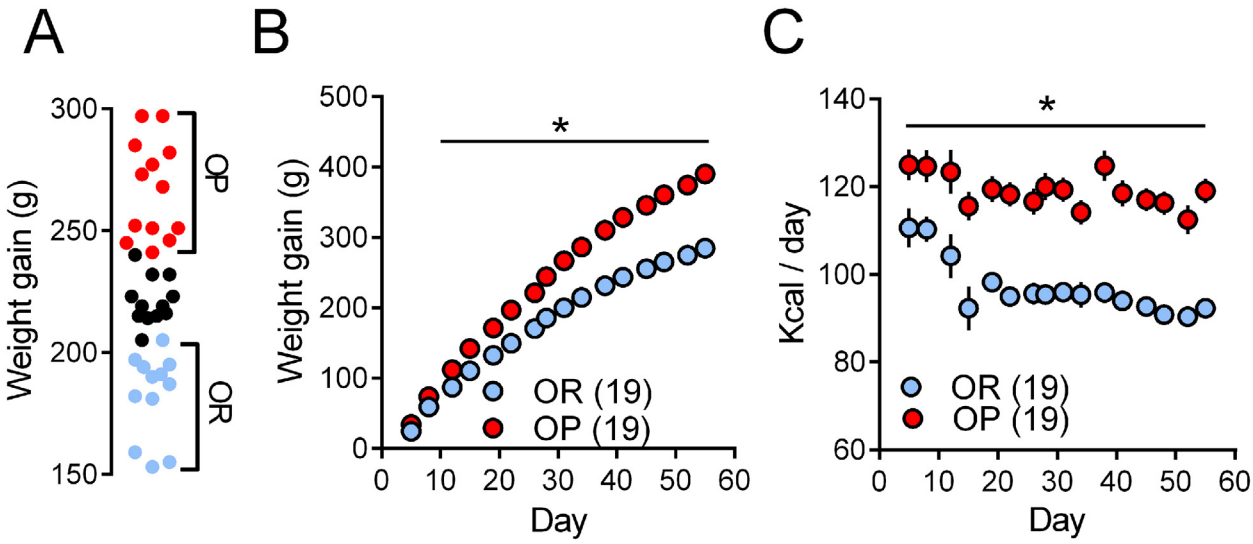

Figure 1. Free access to palatable food diet causes obesity in some rats but not in others. **(A)** Weight gain spread of a representative group of rats following 8 weeks of ad libitum palatable food diet in their home cages. Top third: diet-induced obesity-prone (OP) rats. Bottom third: diet-induced obesity-resistant (OR) rats. **(B)** OP rats gained more weight than OR rats (two-way analysis of variance, $F_{1,36} = 96.64$, $p < .0001$ for weight gain main effect) during the 8-week diet period. **(C)** OP rats consumed more calories than OR rats (two-way analysis of variance, $F_{1,36} = 69.69$ for kcal consumed main effect) during the 8-week diet period. *$p < .05$. Data represent mean ± SEM.

Operant Self-Administration Protocol

After the 8-week diet period, rats were placed on standard chow ad libitum in their home cage, and their access to palatable food was restricted to 45 minutes daily during an operant session. The operant session, a modified version of those previously designed to identify addiction-vulnerable versus addiction-resilient subjects (25,37), consisted of alternating reward-available (designated S+, 15 minutes × 3) and reward-unavailable (designated S−, 5 minutes × 3) periods that were paired with distinct discriminative stimuli. During S+ periods, lever-pressing on the active lever resulted in the dispensing of a 45 mg palatable food pellet (F06162, 45% kcal from fat, total density = 4.6 kcal g^{-1}; Bioserv Inc., Frenchtown, New Jersey). Responding on the active lever during S−, as well as responding on the inactive lever during either S+ or S− periods, resulted in no programmed consequence.

Figure 2 shows the operant protocol whereby rats began on a fixed ratio (FR) of 1 under only S+ conditions for 45 minutes. After 3 days, the S− period was introduced, and sessions were extended to 60 minutes with alternating S+ and S−. Rats experienced FR1 for a further 3 days before the response requirement was increased to FR3 (3 days) and then FR5 (remainder of protocol). The progressive ratio session was conducted in a single session after FR5 responding had been established (typically after 5 days of FR5). The progressive ratio breakpoint was taken as the last step completed before a lapse of 1 hour during which no pellets were earned or the last step completed in 5 hours, whichever occurred first.

Electrophysiology

Slice Preparation. Slices were prepared from animals 24 hours after their final self-administration session at approximately the same time each morning for animals still on the home cage diet. Rats were anesthetized with ketamine hydrochloride (100 mg/kg Ketaset; Fort Dodge Animal Health, Wyeth International, Overland Park, Kansas) and decapitated.

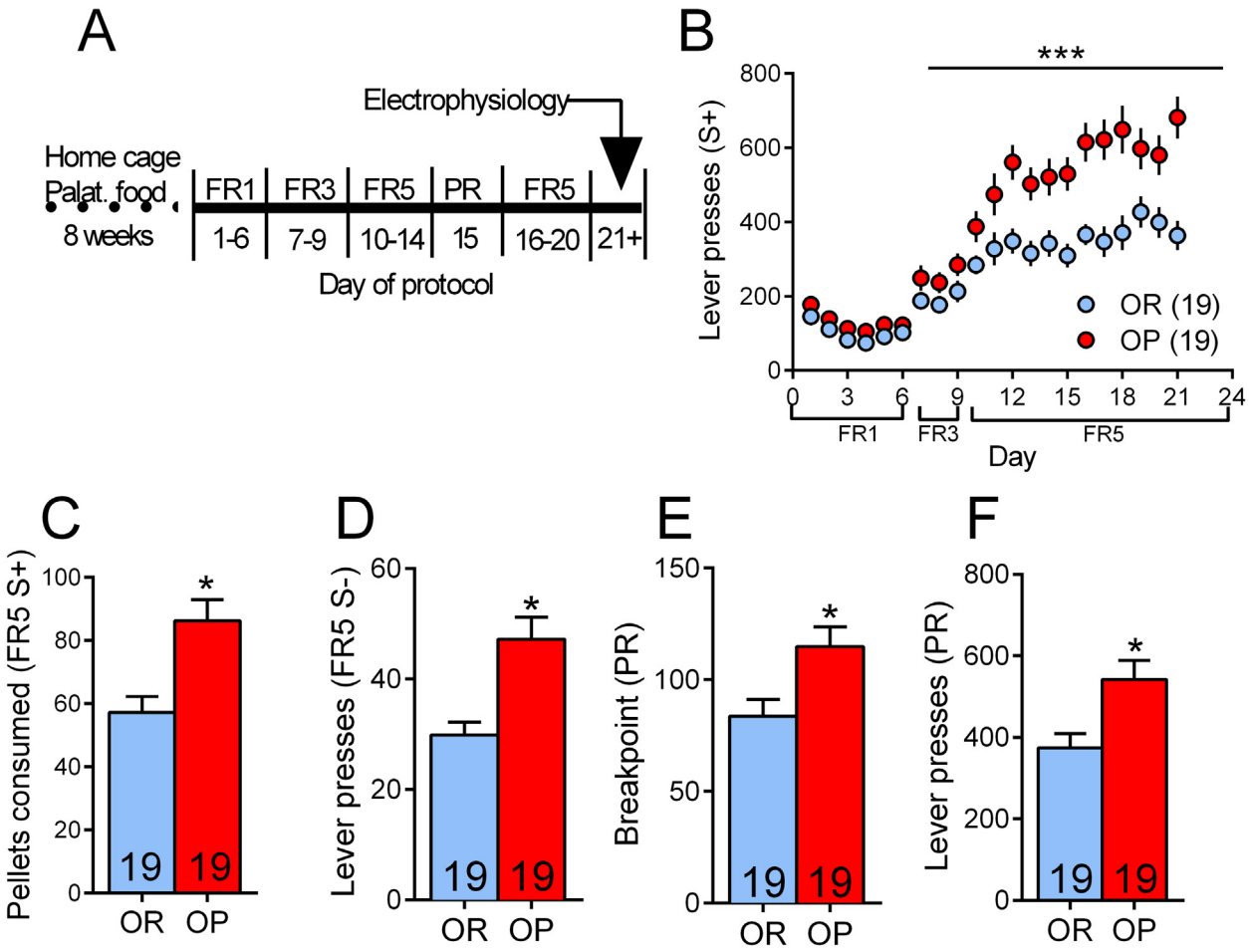

Figure 2. Obesity-prone (OP) rats show increased addictive-like behavior as compared with obesity-resistant (OR) rats. **(A)** Experimental protocol. **(B)** Time course of lever-pressing during the reward-available (S+) periods over the operant protocol schedule. ***p = .0003 (two-way analysis of variance, $F_{1,36}$ = 15.70 for main effect of weight gain). (**C-F**) OP rats showed increased pellet consumption during the fixed ratio (FR)5 S+ period (unpaired t test, t_{36} = 3.49, p =.0013) and higher lever-pressing during the FR5 reward-unavailable (S−) period (unpaired t test, t_{36} = 3.755, p =.0006), higher breakpoint (Mann-Whitney test, U = 90, p = .0063), and lever presses (unpaired t test, t_{36} = 2.87, p = .007) during a progressive ratio (PR) task. Numbers in bars = number of rats. *p < .05. Data represent mean ± SEM.

Brain was removed and coronal accumbens brain slices (220 μm) (VT1200S Leica vibratome; Leica Biosystems Inc., Wetzlar, Germany) were collected into a vial containing artificial cerebrospinal fluid (in mmol/L: 126 sodium chloride, 1.4 monosodium phosphate, 25 sodium bicarbonate, 11 glucose, 1.2 magnesium chloride, 2.4 calcium chloride, 2.5 potassium chloride, 2.0 NaPyruvate, .4 ascorbic acid, bubbled with 95% oxygen and 5% carbon dioxide) and a mixture of 5 mmol/L kynurenic acid and 50 μmol/L D-(-)-2-amino-5-phosphonopentanoic acid. Slices were stored at room temperature until recording.

In Vitro Whole-Cell Recording. All recordings were collected at 32°C (TC-344B; Warner Instrument Corporation, Hamden, Connecticut) in the dorsomedial NAcore, and the stimulating electrode was positioned to optimize activating fibers from prefrontal inputs, although excitatory afferents from other brain regions would also be expected to contribute to the excitatory postsynaptic current (EPSC) (38,39). Medium spiny neurons were visualized with an Olympus BX51WI microscope. Inhibitory synaptic transmission was blocked with picrotoxin (50 μmol/L). Multiclamp 700B (Axon Instruments, Union City, CA) was used to record EPSCs in whole-cell configuration. Glass microelectrodes (1.3 MΩ to 2 MΩ) were filled with cesium-based internal solution (in mmol/L: 124 cesium methanesulfonate, 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid potassium, 1 ethylene glycol tetraacetic acid, 1 magnesium chloride, 10 sodium chloride, 2.0 magnesium adenosine triphosphate, and .3 guanosine 5′-triphosphate sodium salt hydrate, 1 QX-314, pH 7.2–7.3, 275 mOsm). Recordings started no earlier than 10 minutes after the cell membrane was ruptured to allow diffusion of internal solution into the cell. Data were acquired at 10 kHz and filtered at 2 kHz using AxoGraph X software (AxoGraph Scientific, Sydney, Australia). To evoke EPSCs, a bipolar stimulating electrode was placed ~100 to 200 μm dorsomedial of the cell to maximize chances of stimulating prefrontal cortex afferents. The stimulation intensity chosen evoked 30% to 70% of maximal EPSC. Recordings were collected every 20 seconds (for α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid [AMPA]/*N*-methyl-D-aspartate [NMDA] experiments) or 10 seconds (for LTD experiments). Series resistance (Rs) measured with a −2 mV depolarizing step (10 ms) given with each stimulus and holding current were always monitored online. Recordings with unstable Rs or when Rs exceeded 20 MΩ were aborted. AMPA/NMDA ratios and LTD measurements were never obtained from the same slice.

AMPA/NMDA and LTD. AMPA/NMDA was recorded at +40 mV. Currents composed of both AMPA and NMDA were first obtained, followed by bath application of 50 μmol/L D-(-)-2-amino-5-phosphonopentanoic acid, an NMDA receptor antagonist, and recording of AMPA currents alone. NMDA current was obtained by subtraction of AMPA current from total current. Time to which the NMDA current decayed to 37% of its peak was used to estimate NMDA decay. LTD was measured at −80 mV. A baseline of 10 minutes was followed by an LTD protocol (26) after which recording continued for 30 minutes.

LTD Measurements. Baseline EPSCs were first measured for 10 minutes (.1 Hz). After obtaining a stable baseline, we applied the LTD protocol described in Martin *et al.* (26). LTD was induced by applying three 5 Hz trains, each for 3 minutes, with a 5-minute intertrain interval. Trains were paired with depolarization of the cell to −50 mV, while during the intertrain intervals the membrane potential was brought back to −80 mV. After the last train, membrane potential was returned to −80 mV and recording at .1 Hz was resumed for 30 minutes.

Paired-Pulse Ratio Measurement. A pair of electrical pulses was administered with an interpulse interval of 50 ms every 20 seconds. The paired-pulse ratio was calculated as the ratio between the peak amplitude of the second and the first EPSCs.

Spontaneous EPSC Analysis. Spontaneous synaptic activity was measured without stimulation for at least 100 seconds per cell. Events were detected using Axograph X template detection function. Frequency of spontaneous EPSCs (sEPSCs) (Hz) was calculated as the number of spontaneous events per 100 seconds divided by 100. sEPSC amplitude was calculated by first averaging all detected events and then calculating average event peak.

Statistical Analysis. Statistics were performed using Graphpad Prizm 6.0 (GraphPad Software Inc., San Diego, California). Time-course data (weight gain, kcal, lever-pressing) were analyzed by two-way repeated measures analysis of variance (ANOVA) (time and weight gain as factors). LTD data were analyzed by standard two-way ANOVA. Bonferroni post hoc analyses were performed where stated. All other behavioral data, as well as AMPA/NMDA ratio and NMDA decay data, were analyzed by unpaired two-tailed Student *t* test or one-way ANOVA, as they were normally distributed. Breakpoint data were not normally distributed and therefore were analyzed by Mann-Whitney test. For correlations, linear regressions were performed on data and *F*-test determined whether the linear regression slope was different from zero (*p* values and goodness of fit [R^2] values, not *F* values, are displayed on graphs).

RESULTS

Establishing Diet-Induced OP and OR Subpopulations

As depicted in Figure 1A, at the end of the 8-week palatable food diet, rats showed a normal distribution of weight gain (D'Agostino and Pearson omnibus normality test, $K^2 = .789$, $p = .67$), thus allowing their separation into OP (top third) and OR (bottom third) subgroups. OP rats averaged 390 ± 7.1 g of weight gain compared with 284.8 ± 6.2 g for OR rats on the last day of palatable food diet. Importantly, OP rats not only gained significantly more weight over the diet period ($p < .0001$ for main effect of weight gain, two-way ANOVA, $F_{1,36} = 96.64$; Figure 1B) but also ate significantly more (23%), demonstrating a propensity for excessive consumption ($p < .0001$ for weight gain main effect, two-way ANOVA, $F_{1,36} = 69.69$; Figure 1C).

Diet-Induced Obesity Confers Vulnerability to Addictive-like Behavior Toward Palatable Food

After 8 weeks of free access to palatable food, rats were placed on standard chow, and access was restricted to 45 minutes daily during operant sessions. Despite both OP and OR initially responding similarly on FR1 and FR3 schedules, by day 10, when the response requirement increased to FR5, OP rats, escalated to pressing over 50% more than OR rats during S+ periods ($p = .0003$, $F_{1,36} = 15.7$, main effect of group; Figure 2B). This resulted in OP rats' consuming 57% more palatable food when it was available, thus constituting more binge-like consumption in this limited time period in comparison with their OR counterparts ($p = .0013$, $t_{36} = 3.485$; Figure 2C).

A core feature of addiction is loss of control over behavior. The persistence of lever-pressing during periods where the lever cue is not associated with reward delivery models this aspect of addiction-like behavior in rodents (25,27,40). As expected, OP rats pressed more during this period of reward unavailability (S−) than did OR rats ($p = .006$, $t_{36} = 3.755$; Figure 2D), and this occurred throughout the entire protocol (Figure S1 in Supplement 1). Furthermore, OP rats were more highly motivated to obtain a palatable food reward, as they exhibited a higher breakpoint and total number of lever presses on the progressive ratio task ($p = .0063$, U = 90; Figure 2E; and $p = .007$, $t_{36} = 2.87$; Figure 2F).

We then tested whether propensity for diet-induced obesity is a predictor of addictive-like behavior toward palatable food. To that end we correlated the weight gain of each rat with its performance on each of the three behavioral parameters described above. We found that all three parameters were positively correlated with weight gain (Figure 3A–D). Thus, the overall results presented in Figures 2 and 3 strongly indicate that propensity for diet-induced obesity is linked with addictive-like behavior toward palatable food.

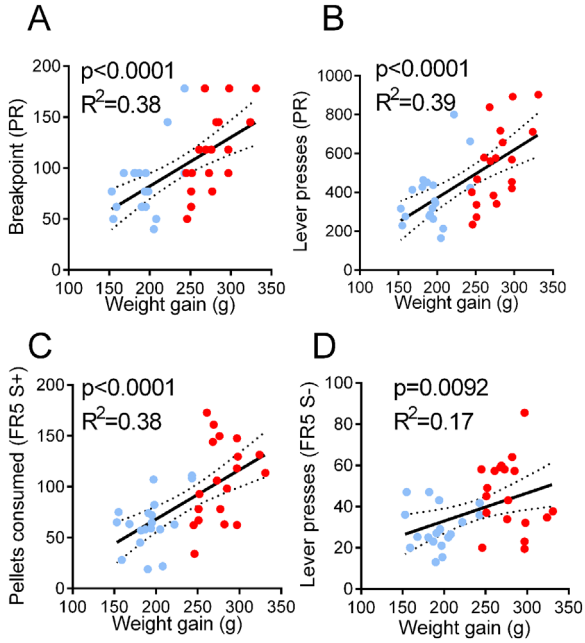

Figure 3. Behavior is positively correlated with previous weight gain. (**A, B**) Breakpoint ($F_{1,36} = 21.77$) and lever presses ($F_{1,36} = 23.18$) in progressive ratio (PR) test. **(C)** Pellets consumed in fixed ratio (FR)5 reward-available (S+) periods ($F_{1,36} = 22.17$). **(D)** Lever presses during FR5 reward-unavailable (S−) periods ($F_{1,36} = 7.587$). Dashed line represents 95% confidence. Blue = obesity-resistant rats. Red = obesity-prone rats.

Diet-Induced Obese Rats Show Synaptic Properties of Addiction in the NAcore

The increased intake, motivation, and persistent palatable food-seeking behavior displayed by OP rats resembles behaviors observed in animals that self-administer drugs of abuse. In the latter, the glutamatergic synapses in the NAcore have been shown to change dramatically. Using animal models of self-administration and relapse, enduring impairments in transmission and synaptic plasticity have been shown at glutamatergic synapses in the NAcore. Three changes, among others, are observed: 1) the glutamatergic synapses are potentiated after a period of drug self-administration (41–43); 2) there is a loss in the ability of these potentiated synapses to undergo LTD (26); and 3) slower NMDA-mediated currents are observed, consistent with a change in subunit composition (23,24). If OP rats display addiction-like behavior toward palatable food, we would expect to observe similar synaptic changes in their NAcore. To test this, we used whole-cell patch-clamp electrophysiology in NAcore brain slices of OP and OR rats (Figure 4A). First, we examined the synaptic strength of the glutamatergic synapses in the NAcore by measuring the ratio between AMPA and NMDA currents (AMPA/NMDA), where a higher ratio indicated potentiated synapses. We found that in the OP rats the glutamatergic synapses in the NAcore resided in an altered state (AMPA/NMDA = 1.34 ± .39) compared with those in the OR rats (.94 ± .23, $p = .0197$, $t_{16} = 2.59$; Figure 4B). This is similar to the long-term potentiation observed in the NAcore of rats that have self-administered drugs such as cocaine (42) and nicotine (23). Also, akin to nicotine (23) and heroin (24) models of addiction, we found (Figure 4C) that the potentiated glutamatergic synapses of the OP rats showed slower decay of the NMDA current (282 ± 29 ms compared with 230 ± 26 ms in OR rats, $p = .0011$, $t_{16} = 3.96$), consistent with a change in subunit composition (44,45). Lastly, using a stimulation protocol that is known to induce LTD in the NAcore (26), we found that the capacity to induce LTD at the glutamatergic synapses in the NAcore is preserved in OR rats but absent in OP rats ($p < .0001$, $F_{1,674} = 159.0$; Figure 4D). This is akin to what is observed in animal models of drug addiction (27,28,42,46).

These synaptic impairments were not observed in control groups that went through the same behavioral protocols but were fed with standard chow instead of palatable diet (Figures S2 and S3 in Supplement 1), demonstrating that exposure to palatable food is required to observe these impairments. To that end, we found that animals that were exposed to the palatable diet alone (no behavior) did not exhibit the potentiation of NAcore synapses observed after the behavioral protocol (one-way ANOVA with Bonferroni post hoc analysis; Figure 5B). Thus, the observed potentiation of nucleus accumbens synapses in OP rats is the result of switching to a more restricted access of the palatable diet when undertaking the operant protocol, the neurophysiological

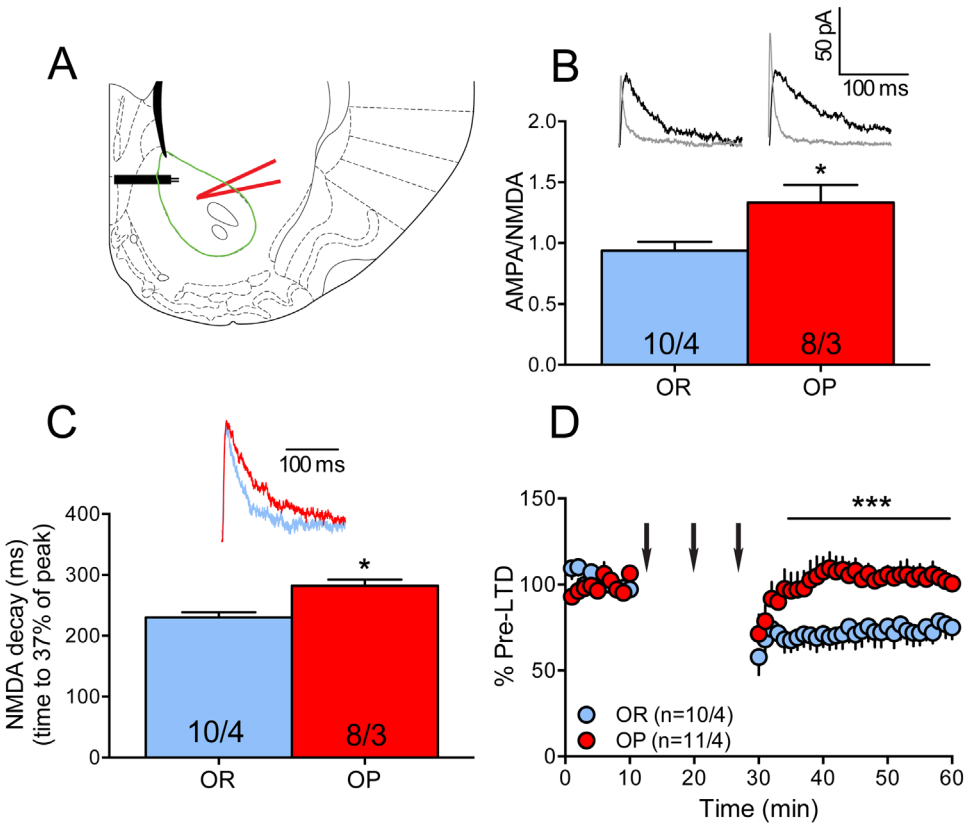

Figure 4. Obesity-prone (OP) rats, but not obesity-resistant (OR) rats, show addiction-like electrophysiological measures. **(A)** Schematic drawing of the recording setup. In a coronal section of the nucleus accumbens core (green line), a medium spiny neuron located dorsomedial to the anterior commissure was patched by a glass pipette (red), and excitatory postsynaptic currents were evoked by a stimulating electrode (black) positioned ~300 μm dorsomedial to the recorded neuron. **(B)** OP rats show higher α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/*N*-methyl-D-aspartate (NMDA) (unpaired *t* test, $t_{16} = 2.59$, $p = .02$) than OR rats. Insets: representative traces (gray = AMPA, black = NMDA). **(C)** OP rats show longer NMDA current decay (unpaired *t* test, $t_{16} = 3.96$, $p = .001$) than OR rats. Inset: representative traces. **(D)** OP rats show lack of long-term depression (LTD) (two-way analysis of variance, $F_{1,674} = 159.0$ for rat type main effect). Arrows indicate LTD protocol—three 3-minute trains of 5 Hz. Numbers in bars and legend represent number of cells/number of rats. $^{*}p < .05$ using unpaired two-tailed Student *t* test. $^{***}p < .0001$ using two-way analysis of variance for main effect of group. [Coronal slice diagram modified with permission from Paxinos and Watson (64)].

manifestation of the development of the addiction-like operant behavior observed, or both. However, induction of LTD was impaired in OP rats compared with OR rats or rats exposed only to standard chow even without the behavioral training ($p < .0001$, $F_{2,833} = 129.8$; Figure 5A). Also, a trend toward slower NMDA decay in OP rats was observed ($p = .0827$, $F_{2,19} = 2.850$; Figure 5C). Thus, an effect of diet alone is apparent (chow vs. palatable diet), as well as an effect of weight gain within rats fed with palatable food (OP vs. OR). The observed differences between OP and OR rats seem to be of postsynaptic origin, as there was no difference in the paired-pulse ratio between the groups (Figure S4 in Supplement 1), consistent with this LTD protocol inducing postsynaptic, NMDA-dependent LTD in the NAcore (47). Although a postsynaptic mechanism was not corroborated by a change in sEPSC amplitude, sEPSC measurements account for all the glutamatergic inputs, while the AMPA/NMDA or LTD measurements arise primarily from medial prefrontal cortex input (recordings were collected in the dorsomedial NAcore, where the prefrontal inputs are most dense) (39,48).

Both Addiction-like Behavior and Propensity for Diet-Induced Obesity Are Positively Correlated With Dysfunction at Excitatory NAcore Synapses

To support that the synaptic changes in the NAcore are linked to the behavior observed in OP rats, we correlated each electrophysiological parameter (i.e., AMPA/NMDA, NMDA current decay, magnitude of LTD) with behaviors shown in Figure 2. For simplification, we generated for each rat an addiction score—the average standardized score of the three behaviors tested (Supplemental Methods in Supplement 1). The three electrophysiological parameters were positively correlated with behavior (Figure 6). Moreover, the electrophysiological parameters were correlated with weight gain (Figure S5 in Supplement 1), thus indicating that the propensity for diet-induced obesity predicts the extent of synaptic dysfunction.

DISCUSSION

Here, we show that obesity resulting from the inability to regulate consumption of palatable food is linked to plasticity at glutamatergic synapses in the NAcore akin to that which is thought to contribute to drug addiction. Specifically, rats that develop obesity when given access to palatable food exhibit features of addictive behavior. Furthermore, they show changes at NAcore excitatory synapses similar to rats that have self-administered psychostimulants, thus supporting the concept of a shared endophenotype underlying both addiction and overeating.

The presence of features of addictive behavior in OP rats speaks to a preexisting vulnerability that interacts with exposure to palatable food to elicit the phenotype observed-excessive consumption, the development of binge-like intake when access is restricted, and increased food-seeking behavior during periods that signal food unavailability. Although the concept of food addiction remains somewhat controversial (49), our results

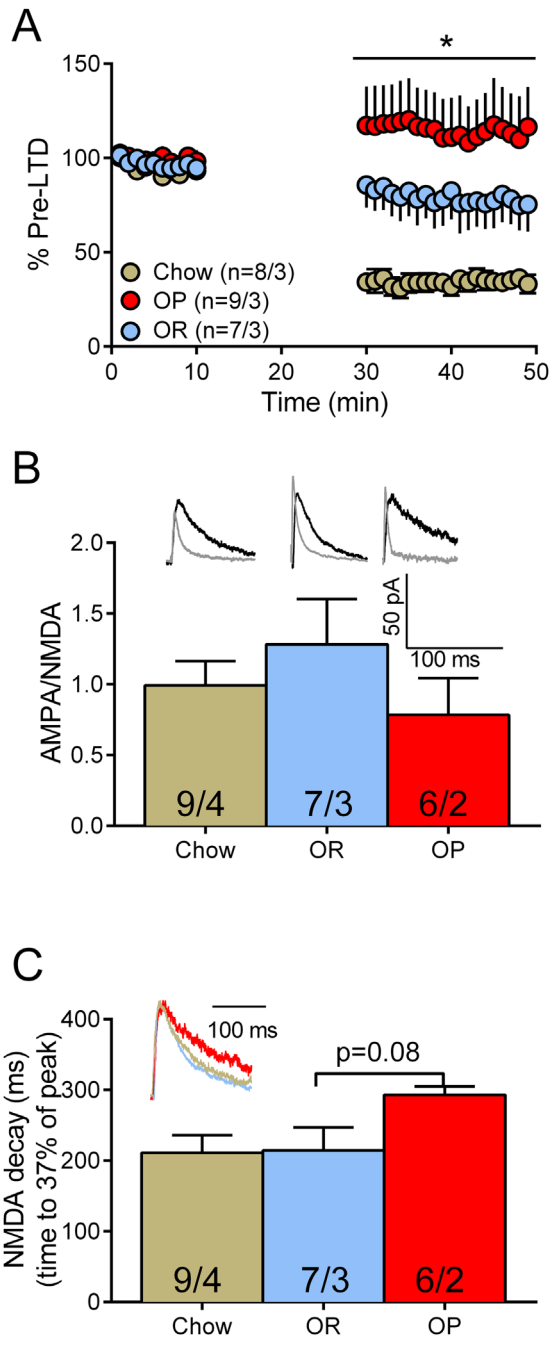

Figure 5. Obesity-prone (OP) rats, but not obesity-resistant (OR) rats or chow rats, show some evidence of addiction-like electrophysiological measures after the home-cage diet alone (no behavior). **(A)** OP rats show lack of long-term depression (LTD), and chow rat LTD is significantly greater than OR rat LTD (two-way analysis of variance [ANOVA], $F_{2,833} = 129.80$ for rat type main effect). Arrows indicate LTD three 3-minute trains of 5 Hz. **(B)** No difference between α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/N-methyl-D-aspartate (NMDA) according to diet or weight gain on diet. Insets: representative traces (gray = AMPA, black = NMDA). **(C)** OP rats show a trend for longer NMDA current decay than OR and chow rats (one-way ANOVA, $F_{2,19} = 2.850$). Inset: representative traces. Numbers in bars and legend represent number of cells/number of rats. $^{***}p < .0001$ using two-way ANOVA for main effect of group.

provide preclinical evidence that a food addiction-like endophenotype exists in vulnerable subpopulations exposed to a highly palatable diet. A recent study showed a similar food addiction-like phenotype in rats that were screened as highly impulsive (30). These results and our own demonstrate that in outbred rat populations, as with humans, only a proportion of those exposed to palatable food will develop pathological consummatory behavior. This similarity to the human situation provides potential translational relevance to our findings in binge eating disorder (29) and in individuals classified as food addicted using the Yale Food Addiction Scale (20).

We show that OP rats display behavior toward palatable food akin to that displayed toward cocaine by rats classified as addiction vulnerable, including increased progressive ratio responding (25,37). Increased responding on a progressive ratio schedule is indicative of heightened motivation, possibly augmented by restricted access to the palatable diet during operant training (50,51). However, increased motivation has been shown to precede the development of diet-induced obesity in this model, suggesting that this is not the case (32). In addition to increased motivation, responses during periods that signal reward unavailability are also elevated in both OP and cocaine addiction–vulnerable rats, consistent with a contingency learning deficit, increased anticipatory and/or impulsive behavior. Perseveration of behavior constitutes one dimension of compulsive behavior (52–54) and thus models the difficulty individuals experience in limiting substance use/overeating. In addition, we found that when access was limited to 3 × 15-minute periods daily, OP rats developed binge-like consumption (57% greater than OR rats) of palatable food pellets in this limited period. This is consistent with previous work demonstrating the development of binge-like behavior in rats given limited access to a palatable food (55) and is akin to a similar addiction-like profile in rats given intermittent access to sucrose in a binge model (56,57).

Though controversy remains regarding the concept of addiction to palatable food, there is no doubt that drugs of abuse and palatable food interact with similar neurobiological substrates and that overlap exists in the circuitry underlying both drug seeking and feeding (19). To date, the presence of synaptic plasticity mechanisms known to underlie the transition to compulsive drug use have not been assessed in animal models of compulsive feeding or obesity. Our work provides the first link between obesity and drug addiction at a cellular level by showing that glutamatergic synapses in the NAcore of obese rats resemble those of rats that have self-administered drugs of abuse. Thus, the increased motivation of OP rats to obtain palatable food may be driven by potentiated glutamatergic inputs to the accumbens and/or impaired synaptic plasticity similar to the potentiated excitatory drive thought to mediate drug seeking (58).

The accumbens serves as a gateway for motivationally relevant information to access motor circuitry (59). It is thought that the transition to compulsive drug seeking arises from an impaired ability of the accumbens to process information about negative environmental contingencies, leading to an inability to inhibit prepotent drug-associated responses (21,22,60). This impairment has been proposed to result from dysregulated glutamatergic signaling at cortico-accumbens synapses. We and others have demonstrated enduring impairments in

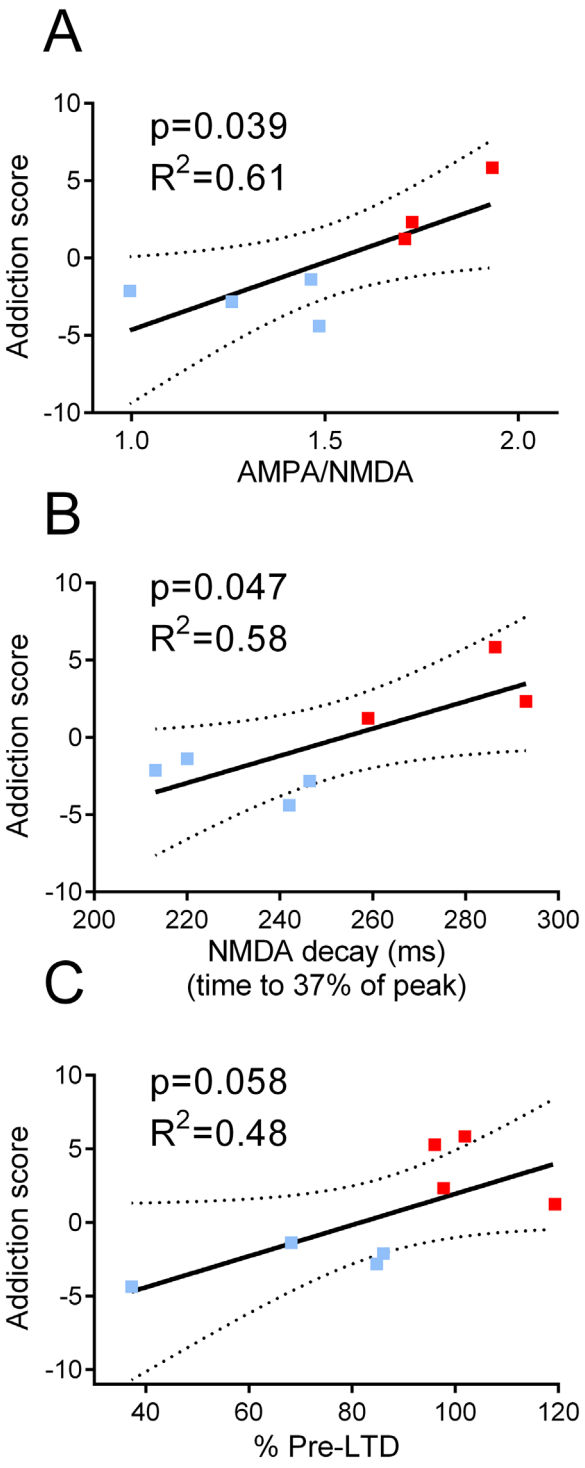

Figure 6. Addiction synaptic hallmarks are positively correlated with addictive-like behavior. **(A–C)** α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/*N*-methyl-D-aspartate (NMDA) ($F_{1,5}$ = 7.772), NMDA decay ($F_{1,5}$ = 6.899), and lack of long-term depression (LTD) ($F_{1,6}$ = 5.486) positively correlate with operant behavior. Operant behavior is presented by an addiction score calculated for each rat (see Supplemental Methods in Supplement 1). Dashed line represents 95% confidence. Blue = obesity-resistant rats. Red = obesity-prone rats.

glutamatergic transmission and synaptic plasticity on medium spiny neurons in the accumbens that underlie relapse vulnerability in animal models of drug addiction (23,28,46,61,62). The presence of similar synaptic dysfunction at NAcore synapses in OP rats suggests similar neurobiological mechanisms lead to overeating beyond energy requirements. In particular, a deficit in the ability of excitatory synapses in the NAcore to undergo LTD speaks to the lack of behavioral flexibility observed in people who cannot refrain from overeating or using drugs (28). As what has been found with models of cocaine addiction (28), it may be that reversing this impairment (pharmacologically or otherwise) restores eating in obese individuals to a normal adaptive behavior.

Many questions remain for future investigation. Our findings provide evidence for addiction-like synaptic impairments in the NAcore of diet-induced obese rats, but whether the mechanisms leading to these impairments are similar to those implicated in drug addiction is not known. For example, it is thought that increased motivation in addicted individuals is translated to uncontrollable action by changes in a common final pathway that includes the accumbens (63). Although we presented three examples of synaptic changes occurring in obese rats and rats trained to self-administer cocaine or nicotine, other changes may not be shared. The persistence of these synaptic changes also remains to be determined. Both palatable food and drugs of abuse are strong reinforcers, but drugs of abuse have specific pharmacological effects whereas food does not. Another question that remains open is whether these synaptic impairments represent a biomarker for an addiction-like endophenotype that can be generalized to other compulsive disorders (e.g., compulsive gambling, internet gaming addictions). Future studies focused on these questions are needed to better understand how addiction-like plasticity may be involved in the development of obesity and more generally, addictive behavior.

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ARTICLE INFORMATION

From the Department of Neurosciences (RMB, YMK, SS, CG-K, DJS, KAJ, TCJ, PWK), Medical University of South Carolina, Charleston, South Carolina; Florey Institute of Neuroscience & Mental Health (RMB, AJL), University of Melbourne, Parkville, Australia; Department of Medical Neurobiology (YMK), The Hebrew University, Jerusalem, Israel; Departamento de Farmacología (CG-K), Universidad Nacional de Córdoba, Córdoba, Argentina; and Department of Physiology (DCS, SES), Monash University, Clayton, Australia.

RMB and YMK contributed equally to this work.

Address correspondence to Robyn M. Brown, Medical University of South Carolina, Department of Neurosciences, Charleston, SC 29425; E-mail: robyn.brown@florey.edu.au.

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