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Addiction-like synaptic impairments in diet-induced obesity

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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 (NAcore) 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 (OP) and resistant (OR) subgroups. Access to palatable food was then restricted to daily operant self-administration sessions using fixed (FR1, 3 and 5) and progressive ratio (PR) schedules. Subsequently, NAcore brain slices were prepared and we tested for changes in the ratio between AMPA and NMDA currents (AMPA/NMDA) and the ability to exhibit long-term depression (LTD).

Results—We found that propensity to develop DIO is linked to deficits in the ability to induce LTD in the NAcore, as well as increased potentiation at these synapses as measured by AMPA/NMDA currents. Consistent with these impairments, we observed addictive-like behavior in OP

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rats, including i) heightened motivation for palatable food (ii) excessive intake and (iii) 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; obesity; nucleus accumbens; synaptic plasticity; glutamate; long term depression

Introduction

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 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 examine whether rats prone to obesity due to excessive intake of palatable food would exhibit these cardinal synaptic impairments and show similar characteristics towards food that rats classified as addiction-vulnerable show towards drugs. We assessed in rats three addiction-like behaviors, used as hallmarks of both drug addiction and pathological overeating (19, 25, 29, 30): (i) A high motivation to obtain the substance; modeled using a progressive ratio (PR) schedule, whereby the effort required to obtain food increases progressively within the session; (ii) The rapid consumption of significantly larger than normal amounts of the substance; modeled by measuring intake when access to

palatable food was limited; (iii) 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) weighing 250-300 g at the start of the experiment were housed individually with nesting/enrichment material made available. A 12-h light/dark cycle was maintained at all times, with lights turned off at 6:00 a.m. Experimental procedures were approved by the MUSC Animal IACUC. 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) or palatable diet (D12451, 45% kcal fat; total density=4.73 kcal g⁻¹; Research Diets Inc, New Brunswick, NJ, USA). Rats were given 7 days to acclimate before experimentation began. A second cohort of Sprague-Dawley rats (Monash University) 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 and resistant groups based on weight gain in response to a palatable diet (31, 32). Diet-induced obese rats in this model exhibit hyperphagia, increased adiposity, as well as 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, New Brunswick, NJ, USA) for a period of 8 weeks. Food intake and body weight were determined twice per week. Rats were then separated into diet-induced obese prone (OP, top third) and diet-induced obese resistant (OR, bottom third) groups based on weight gain (Figure 1) (31). Weight gain was determined from weeks 3-8 of the 8 week period in order 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 3-8 of the 8 week period) and utilized for electrophysiology experiments.

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 restricted to 45min 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+, 15min × 3) and 'reward unavailable' (designated 'S-', 5min × 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, NJ, USA). Responding on the active lever during S-, as well as responding on the inactive lever during either S+ or S- periods also 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 45min. After 3 d the S- period was introduced and sessions were extended to 60min with alternating S+, S-. Rats experienced FR1 for a further 3 d before the response requirement was increased to FR3 (3d) and then FR5 (remainder of protocol). The progressive ratio (PR) session was conducted in a single session after FR5 responding had been established (typically after 5d FR5). The PR breakpoint was taken as the last step completed prior to a lapse of 1h during which no pellets were earned or the last step completed in 5h, whichever occurred first.

Electrophysiology

Slice preparation—Slices were prepared from animals 24h after their final self-administration session approximately the same time each morning for animals still on the homecage diet. Rats were anesthetized with ketamine HCl (1 mg/kg Ketaset, Fort Dodge Animal Health) and decapitated. Brain was removed and coronal accumbens brain slices (220µm) (VT1200S Leica vibratome) were collected into a vial containing aCSF (in mM: 126 NaCl, 1.4 NaH₂PO₄, 25 NaHCO₃, 11 glucose, 1.2 MgCl₂, 2.4 CaCl₂, 2.5 KCl, 2.0 NaPyruvate, 0.4 ascorbic acid, bubbled with 95% O₂ and 5% CO₂) and a mixture of 5mM kynurenic acid and 50µM D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5). 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) in the dorsomedial NAc core, and the stimulating electrode positioned to optimize activating fibers from prefrontal inputs, although excitatory afferents from other brain regions would also be expected to contribute to the EPSC (38, 39). Medium spiny neurons were visualized with an Olympus BX51WI microscope. Inhibitory synaptic transmission was blocked with picrotoxin (50 µM). Multiclamp 700B (Axon Instruments, Union City, CA) was used to record excitatory postsynaptic currents (EPSCs) in whole cell configuration. Glass microelectrodes (1.3-2 MΩ) were filled with cesium-based internal solution (in mM: 124 cesium methanesulfonate, 10 HEPES potassium, 1 EGTA, 1 MgCl₂, 10 NaCl, 2.0 MgATP, and 0.3 NaGTP, 1 QX-314, pH 7.2-7.3, 275mOsm). Recordings started no earlier than 10min after the cell membrane was ruptured, to allow diffusion of internal solution into the cell. Data were acquired at 10kHz, and filtered at 2kHz using AxoGraph X software (AxoGraph Scientific, Sydney). To evoke EPSCs, a bipolar stimulating electrode was placed ~100-200 µm dorsomedial of the cell to maximize chances of stimulating PFC afferents. The stimulation intensity chosen evoked a 30-70% of maximal EPSC. Recordings were collected every 20sec (for AMPA/NMDA experiments) or 10sec (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 R_s , or when R_s exceeded 20 M Ω 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 μ M (2*R*)-amino-5-phosphonovaleric acid (D-AP5), 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 10min was followed by an LTD protocol (26) after which recording continued for 30min.

LTD measurements—Baseline EPSCs were first measured for 10min (0.1Hz). After obtaining a stable baseline, we applied the LTD protocol described in (26). LTD was induced by applying three 5Hz trains, each for 3min, with 5min inter-train interval. Trains were paired with depolarization of the cell to –50 mV while during the inter-train intervals the membrane potential was brought back to –80 mV. After the last train membrane potential was returned to –80 mV and recording at 0.1Hz was resumed for 30min.

Paired-pulse ratio measurement—A pair of electrical pulses was administered with an inter-pulse interval of 50ms every 20sec. The paired-pulse ratio (PPR) was calculated as the ratio between the peak amplitude of the second and the first EPSCs.

sEPSC analysis—Spontaneous synaptic activity was measured without stimulation for at least 100sec per cell. Events were detected using Axograph X template detection function. Frequency of sEPSCs (Hz) was calculated as the number of spontaneous events per 100sec 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 Prism 6.0 (Graphpad Software Inc, San Diego, CA). Time-course data (weight gain, kcal, lever-pressing) were analyzed by 2way RM ANOVA (time and weight gain as factors). LTD data was analyzed by standard 2-way ANOVA. Bonferroni posthoc 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 was not normally distributed and therefore 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 obesity prone and obesity resistant 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 & Pearson omnibus normality test, $K^2=0.789$, $p=0.67$), 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 ($p < 0.0001$ for main effect of weight gain, two-way ANOVA, $F_{(1,36)} = 96.64$; figure 1B), they also ate significantly more (23%), demonstrating a propensity for excessive consumption ($p < 0.0001$ for weight gain main effect, two way ANOVA, $F_{(1,36)} = 69.69$; figure 1C).

Diet-induced obesity confers vulnerability to addictive-like behavior towards palatable food

After 8-weeks of free access to palatable food rats were placed on standard chow and access was restricted to 45min 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 ($p = 0.0003$, $F_{(1,36)} = 15.7$, 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 to their OR counterparts ($p = 0.0013$, $t_{(36)} = 3.485$; 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 ($p = 0.006$, $t_{(36)} = 3.755$; Figure 2D), and this occurred throughout the entire protocol (supplemental figure S1). 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 ($p = 0.0063$, $U = 90$; Figure 2E and $p = 0.007$, $t_{(36)} = 2.87$; Figure 2F).

We then tested whether propensity for diet-induced obesity is a predictor of addictive-like behavior towards 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 2-3 strongly indicate that propensity for diet-induced obesity is linked with addictive-like behavior towards palatable food.

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: i) the glutamatergic synapses are potentiated after a period of drug self-administration (41-43); ii) loss of the ability of these potentiated synapses to undergo long-term depression (LTD) (26); iii) slower NMDA-mediated currents, consistent with a change in subunit composition (23, 24). If OP rats display addiction-like behavior towards palatable food we would expect to observe similar synaptic changes in their NAcore. To test this we used whole-cell patch-clamp

electrophysiology in NAc core brain slices of OP and OR rats (Figure 4A). First, we examined the synaptic strength of the glutamatergic synapses in the NAc core by measuring the ratio between AMPA and NMDA currents (AMPA/NMDA), where a higher ratio indicates potentiated synapses. We found that in the OP rats the glutamatergic synapses in the NAc core reside in an altered state (AMPA/NMDA=1.34±0.39) compared to those in the OR rats (0.94±0.23, $p=0.0197$, $t_{(16)}=2.59$; Figure 4B). This is similar to the long-term potentiation (LTP) observed in the NAc core 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 to 230±26 ms in OR rats, $p=0.0011$, $t_{(16)}=3.96$), consistent with a change in subunit composition (44, 45). Lastly, using a stimulation protocol that is known to induce LTD in the NAc core (26), we found that the capacity to induce LTD at the glutamatergic synapses in the NAc core is preserved in OR rats but absent in OP rats ($p<0.0001$, $F_{(1,674)}=159.0$; 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 fed with standard chow instead of palatable diet (supplemental Figure S2 and 3), demonstrating that exposure to palatable food is required in order 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 NAc core synapses observed after the behavioral protocol (one-way ANOVA with bonferroni posthoc analysis; Figure 5B). Thus, the observed potentiation of NAc synapses in OP rats is either the result of switching to a more restricted access of the palatable diet when undertaking the operant protocol and/or the neurophysiological manifestation of the development of the ‘addiction-like’ operant behavior observed. However, induction of LTD was impaired in OP rats compared to OR rats or rats exposed only to standard chow even without the behavioral training ($p<0.0001$, $F_{(2,833)}=129.8$; Figure 5A). Also, a trend towards slower NMDA decay in OP rats was observed ($p=0.0827$, $F_{(2,19)}=2.850$; Figure 5C). Thus, an effect of diet alone is apparent (chow versus palatable diet), as well as an effect of weight gain within rats fed with palatable food (OP versus OR). The observed differences between OP and OR rats seem to be of postsynaptic origin, as there was no difference in the PPR between the groups (supplemental Figure S4); consistent with this LTD protocol inducing postsynaptic, NMDA-dependent LTD in the NAc core (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 mPFC input (recordings were collected in the dorsomedial NAc core, 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 NAc core synapses

To support that the synaptic changes in the NAc core 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 (see Supplemental Methods). The three electrophysiological parameters were positively correlated with behavior (Figure 6). Moreover, the electrophysiological parameters were correlated with weight gain (Supplemental Figure S5), 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 NAc core 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 NAc core 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 pre-existing 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 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 (BED)(29), and in individuals classified as 'food addicted' using the Yale Food Addiction Scale (20).

We show that OP rats display behavior towards palatable food akin to that displayed towards 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 (54, 55). However, increased motivation has been shown to precede the development of diet-induced obesity in this model, suggesting 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 (56-58), and thus models the difficulty individuals experience in limiting substance use/overeating. In addition, we found that when access was limited to 3×15min 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 (59), and is akin to a similar 'addiction-like' profile in rats given intermittent access to sucrose in a binge model (60, 61).

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 NAc core 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 (62).

The accumbens serves as a gateway through for motivationally relevant information to access motor circuitry (63). 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, 64). This impairment has been proposed to result from dysregulated glutamatergic signalling at cortico-accumbens synapses. We and others have demonstrated enduring impairments in glutamatergic transmission and synaptic plasticity on medium spiny neurons in the accumbens which underlie relapse vulnerability in animal models of drug addiction (23, 28, 46, 65, 66). The presence of similar synaptic dysfunction at NAc core synapses in OP rats suggests similar neurobiological mechanisms lead to overeating beyond energy requirements. In particular a deficit the ability of excitatory synapses in the NAc core 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 NAc core 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 (67). 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 do these synaptic impairments represent a 'biomarker' for an addiction-like endophenotype that 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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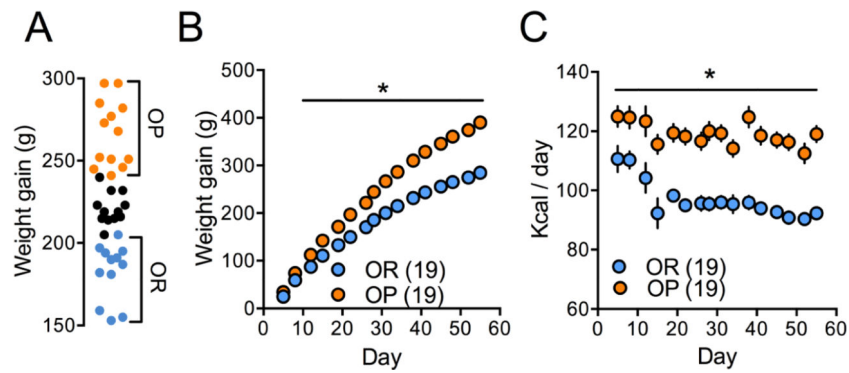


Figure 1.

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 obese (OP) rats. Bottom third – diet-induced obesity resistant (OR) rats. **(B)** OP rats gained more weight than OR rats (two-way ANOVA, $F_{(1,36)}=96.64$, $p<0.0001$ for weight gain main effect) during the 8 week diet period. **(C)** OP rats consumed more calories than OR rats (two-way ANOVA, $F_{(1,36)}=69.69$ for Kcal consumed main effect) during the 8 week diet period. * - $p<0.05$. Data represents mean \pm SEM.

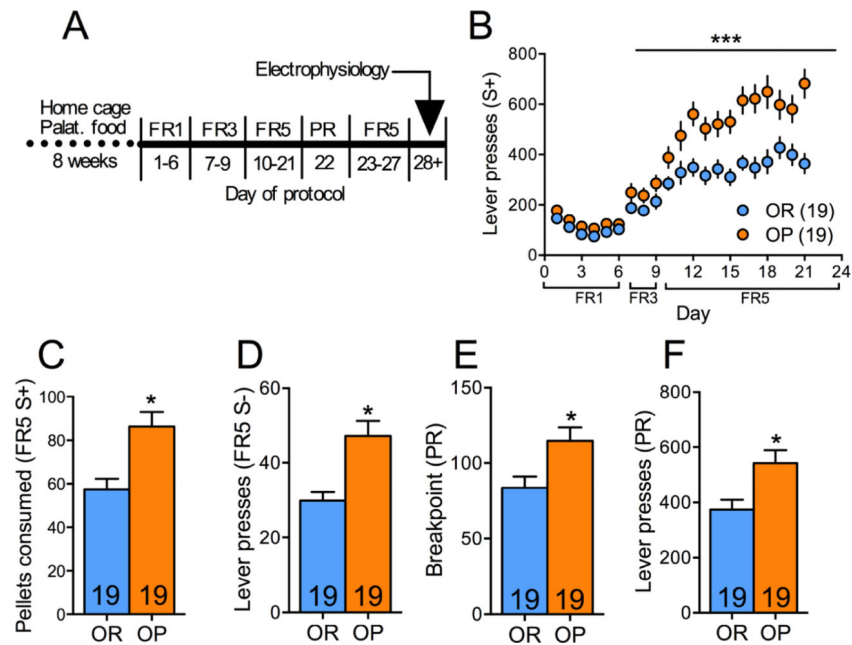


Figure 2. OP rats show increased addictive-like behavior as compared to OR rats. **(A)** Experimental protocol. FR – fixed ratio. **(B)** Timecourse of lever pressing during the S+ periods over the operant protocol schedule. *** - $p=0.0003$ (2-way ANOVA, $F(1,36)=15.70$ for main effect of weight gain). **(C-F)** OP rats showed increased pellet consumption during the FR5 S+ period (unpaired t-test, $t_{(36)}=3.49$, $p=0.0013$) and higher lever pressing during the FR5 S- period (unpaired t-test, $t_{(36)}=3.755$, $p=0.0006$), higher breakpoint (BP, Mann-Whitney test, $U=90$, $p=0.0063$) as well as lever presses (unpaired t-test, $t_{(36)}=2.87$, $p=0.007$) during a progressive ratio (PR) task. Numbers in bars – rats. * - $p<0.05$. Data represents mean \pm SEM.

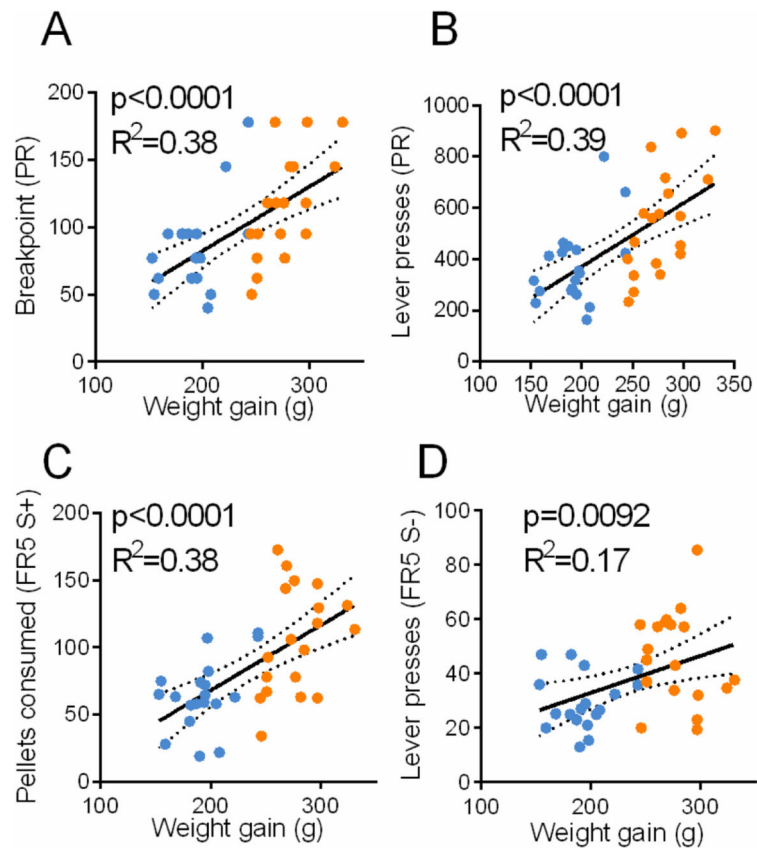


Figure 3. Behavior is positively correlated with previous weight gain. **(A-B)** breakpoint ($F_{(1,36)}=21.77$) and lever presses ($F_{(1,36)}=23.18$) in PR test. **(C-D)** Pellets consumed in FR5 S+ ($F_{(1,36)}=22.17$) and lever presses during FR5 S- ($F_{(1,36)}=7.587$) are positively correlated with weight gain. Dashed line represents 95% confidence. Blue – OR. Orange – OP.

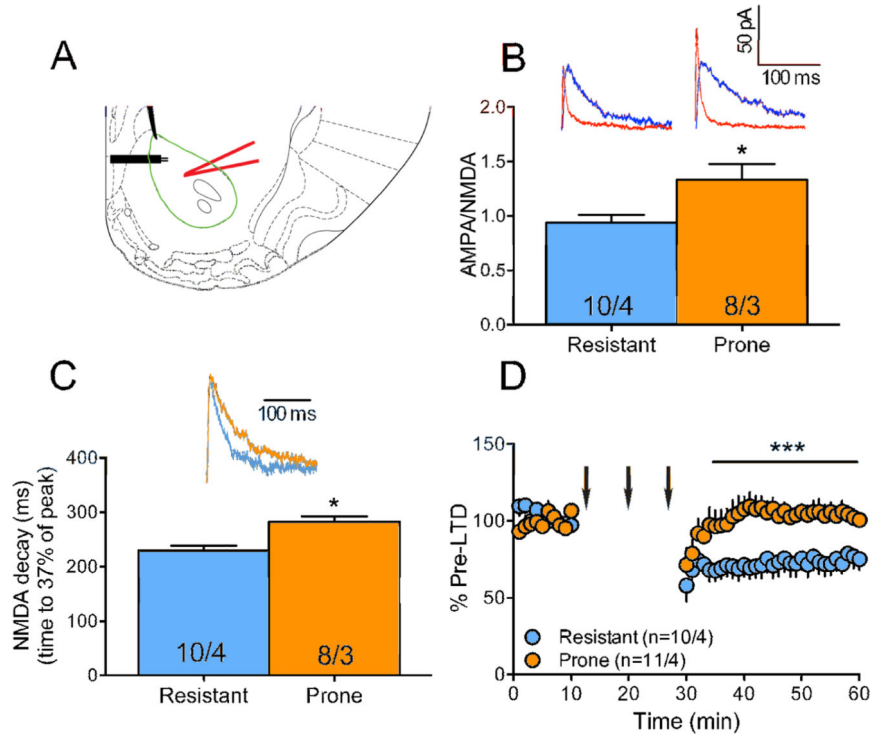


Figure 4. OP rats, but not OR rats, show “addiction-like” electrophysiological measures. **(A)** Schematic drawing of the recording setup. In a coronal section of the NAc core (green line) a medium spiny neuron located dorsomedial to the anterior commissure was patched by a glass pipette (red) and EPSCs were evoked by a stimulating electrode (black) positioned ~300 μm dorsomedial to the recorded neuron. Coronal slice diagram modified from (68). **(B)** OP rats show higher AMPA/NMDA (unpaired t-test, $t_{(16)}=2.59$, $p=0.02$, red trace = AMPA, blue = NMDA) than OR rats. **(C)** OP rats show longer NMDA current decay (unpaired t-test, $t_{(16)}=3.96$, $p=0.001$) than OR rats. Insets: representative traces. **(D)** OP rats show lack of LTD (two-way ANOVA, $F_{(1,674)}=159.0$ for rat type main effect). Arrows indicate LTD protocol – three 3-min trains of 5 Hz. Numbers in bars and legend represent number of cells / number of rats. * $p<0.05$ using unpaired two-tailed student's t-test. *** $p<0.0001$ using Two-way ANOVA for main effect of group.

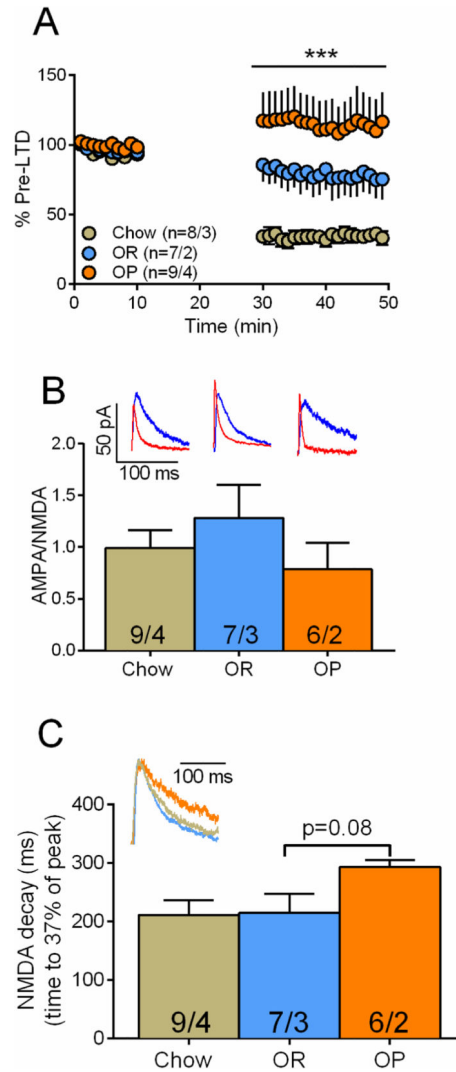


Figure 5.

OP rats, but not OR rats or chow rats show some evidence of “addiction-like” electrophysiological measures after the homecage diet alone (no behavior) (A) Schematic drawing of the recording setup. In a coronal section of the NAcore (green line) a medium spiny neuron located dorsomedial to the anterior commissure was patched by a glass pipette (red) and EPSCs were evoked by a stimulating electrode (black) positioned ~300 μ m dorsomedial to the recorded neuron. Coronal slice diagram modified from (68). (B) OP rats show lack of LTD and chow rat LTD is significantly greater than OR rat LTD (two-way ANOVA, $F_{(2,833)}=129.80$ for rat type main effect). Arrows indicate LTD protocol – three 3-min trains of 5 Hz. (C) No difference between AMPA/NMDA according to diet or weight gain on diet. (D) OP rats show a trend for longer NMDA current decay than OR and chow rats (one-way ANOVA, $F_{(2, 19)}=2.850$). Insets: representative traces. Numbers in bars and legend represent number of cells / number of rats. *** $p < 0.0001$ using Two-way ANOVA for main effect of group.

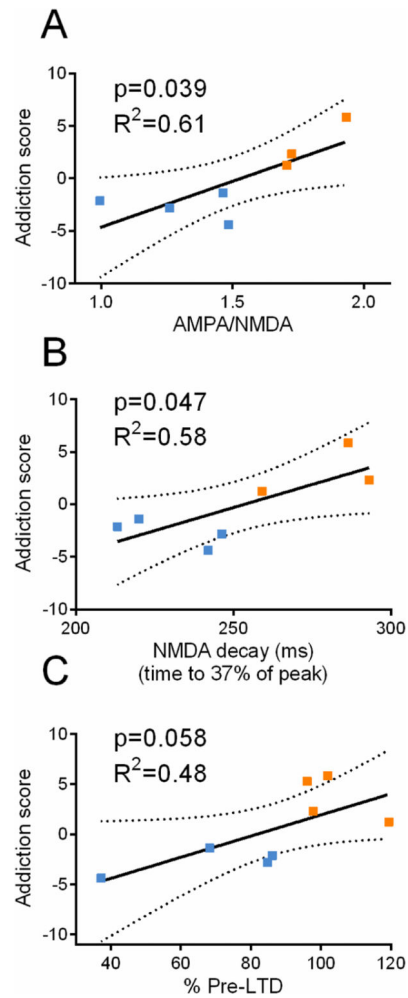


Figure 6. Addiction synaptic hallmarks are positively correlated with addictive-like behavior. (**A-C**) AMPA/NMDA ($F_{(1,5)}=7.772$), NMDA decay ($F_{(1,5)}=6.899$), and lack of LTD ($F_{(1,6)}=5.486$) positively correlate with operant behavior. Operant behavior is presented by an ‘addiction score’ calculated for each rat (see Supplemental Methods). Dashed line represents 95% confidence. Blue – OR. Orange – OP.