

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Sketriene, D;Battista, D;Lalert, L;Kraiwattanapirom, N;Thai, HN;Leeboonngam, T;Knackstedt, LA;Nithianantharajah, J;Sumithran, P;Lawrence, AJ;Brown, RM

Title:

Compulsive-like eating of high-fat high-sugar food is associated with 'addiction-like' glutamatergic dysfunction in obesity prone rats

Date:

2022-09-01

Citation:

Sketriene, D., Battista, D., Lalert, L., Kraiwattanapirom, N., Thai, H. N., Leeboonngam, T., Knackstedt, L. A., Nithianantharajah, J., Sumithran, P., Lawrence, A. J. & Brown, R. M. (2022). Compulsive-like eating of high-fat high-sugar food is associated with 'addiction-like' glutamatergic dysfunction in obesity prone rats. *Addiction Biology*, 27 (5), <https://doi.org/10.1111/adb.13206>.







Persistent Link:

<https://hdl.handle.net/11343/327099>

License:

[CC BY-NC-ND](#)

Compulsive-like eating of high-fat high-sugar food is associated with ‘addiction-like’ glutamatergic dysfunction in obesity prone rats

Diana Skettriene^{1,2}  | Damien Battista¹ | Laddawan Lalert^{1,3}  |
Natcharee Kraiwattanapirom^{1,4} | Han Ngoc Thai¹ | Tanawan Leeboonngam^{1,2} |
Lori A. Knackstedt⁵  | Jess Nithianantharajah¹  | Priya Sumithran^{6,7} |
Andrew J. Lawrence¹  | Robyn M. Brown^{1,2,8} 

¹Mental Health Research Theme, The Florey Institute of Neuroscience and Mental Health, Parkville, Melbourne, Australia

²The Florey Department of Neuroscience and Mental Health, University of Melbourne, Parkville, Melbourne, Australia

³School of Medicine, Walailak University, Nakhon Si Thammarat, Thailand

⁴Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand

⁵Psychology Department, University of Florida, Gainesville, Florida, USA

⁶Department of Medicine (St Vincent's), University of Melbourne, Melbourne, Australia

⁷Department of Endocrinology, Austin Health, Melbourne, Australia

⁸Department of Biochemistry and Pharmacology, University of Melbourne, Parkville, Melbourne, Australia

Correspondence

Robyn M. Brown, Department of Biochemistry and Pharmacology, University of Melbourne, Grattan St, Parkville, Melbourne, Victoria 3010, Australia.

Email: robyn.brown@unimelb.edu.au

Funding information

Australian Research Council, Grant/Award Number: DE190101244; National Health and Medical Research Council, Grant/Award Numbers: 1108092, 1116930, 1178482

Abstract

Chronic overeating is a core feature of diet-induced obesity. There is increasing evidence that in vulnerable individuals, such overeating could become compulsive, resembling an addictive disorder. The transition to compulsive substance use has been linked with changes at glutamatergic synapses in the nucleus accumbens. In this study, we investigated a potential link between such glutamatergic dysregulation and compulsive-like eating using a rat model of diet-induced obesity. A conditioned suppression task demonstrated that diet-induced obese rats display eating despite negative consequences, as their consumption was insensitive to an aversive cue. Moreover, nucleus accumbens expression of GluA1 and xCT proteins was upregulated in diet-induced obese animals. Lastly, both a computed ‘addiction score’ (based on performance across three criteria) and weight gain were positively correlated with changes in GluA1 and xCT expression in the nucleus accumbens. These data demonstrate that the propensity for diet-induced obesity is associated with compulsive-like eating of highly palatable food and is accompanied by ‘addiction-like’ glutamatergic dysregulation in the nucleus accumbens, thus providing neurobiological evidence of addiction-like pathology in this model of obesity.

KEYWORDS

compulsive eating, conditioned suppression, glutamate, nucleus accumbens, Sprague–Dawley

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Addiction Biology* published by John Wiley & Sons Ltd on behalf of Society for the Study of Addiction.

1 | INTRODUCTION

Obesity is a chronic and relapsing condition, typically driven by overconsumption of foods high in fat and sugar (HFHS). People suffering from diet-induced obesity often continue to overeat despite knowledge of its negative health consequences, resembling a hallmark diagnostic feature of addiction—the persistence of behaviour despite adverse consequences. Indeed, increasing evidence suggests that the brain's reward circuitry is dysregulated in diet-induced obesity.^{1–3} To study this phenomenon, a rodent model of diet-induced obesity can be used.⁴ Previous work using this model has demonstrated that diet-induced obese (DIO) rats exhibit addiction-like behaviour towards HFHS food compared to their diet-resistant (DR) counterparts. This is evidenced by (1) heightened motivation for palatable food, (2) binge-like consumption (3) and a lack of control over HFHS food-seeking during periods of signalled food-unavailability.^{5,6} We aimed to investigate whether DIO rats show another important diagnostic feature of addictive behaviour: difficulty to restrain from consumption in the face of negative consequences and a hallmark of both substance use disorder and compulsive overeating.^{7–10} The presence of this specific hallmark has not yet been investigated in an animal model of DIO. To assess this, we used a conditioned suppression paradigm where a mild foot shock was utilized as a negative consequence.¹¹

Previously, Brown et al.⁵ demonstrated that addiction-like behaviour towards HFHS food in DIO rats is associated with glutamatergic plasticity deficits in the nucleus accumbens (NAc). This joined a body of evidence implicating dysregulated glutamate transmission in the nucleus accumbens in the development of addictive behaviours associated with chronic consumption of drugs of abuse and, more recently, of palatable foods.^{7–9} Thus, we decided to assess changes in the expression of some glutamate proteins that may underlie the observed deficits in accumbal glutamatergic plasticity in this DIO model. Our first target was the GluN2B subunit of the N-methyl-D-aspartate (NMDA) receptor as upregulation of this subunit in the NAc is observed in animal models of drug relapse.^{8,10,11} Further, NMDAR antagonism in the NAc shell has been shown to suppress compulsive-like food intake.¹² NMDARs participate in synapse-membrane homeostatic crosstalk with α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors.¹³ Trafficking of GluA1-containing AMPA receptors in the NAc plays an important role in drug-induced neuroplasticity,^{14,15} and increased levels of GluA2-lacking AMPA receptors have been implicated in increased sensitivity of NAc neurons to drug-associated cues, as well as relapse to cocaine-seeking.¹⁶

Another possible mechanism underlying the behaviour observed in DIO animals after HFHS diet is altered glutamate homeostasis. The maintenance of glutamate homeostasis is achieved via transporters and exchangers, such as the cystine-glutamate exchanger and the EAAT2 transporter (rodent GLT1). The cystine-glutamate exchanger exports glutamate to maintain extra-synaptic levels of glutamate,¹⁷ while the GLT1 transporter performs re-uptake.^{18,19} Changes in expression of the cystine-glutamate exchanger catalytic subunit, xCT, as well as GLT1 have been implicated vulnerability to drug relapse and the transition to addiction.^{17,18,20,21} Importantly, changes in expression of these proteins are

linked to synaptic plasticity adaptations in NAc. We also investigated changes in the dorsal striatum, part of the brain that plays an important role in the development of habitual and compulsive behaviours.²²

Our first aim was to extend previous findings of addiction-like behaviour towards palatable food in the DIO model and assess the presence of eating despite negative consequences. We hypothesized that even without food restriction, some DIO rats would display difficulty restraining from HFHS food consumption in the conditioned suppression paradigm where a mild foot shock was utilized as a negative consequence.²³ The second aim was to investigate brain changes potentially associated with the transition to compulsive-eating behaviour in DIO rats. We hypothesized that DIO rats would show altered striatal expression of one or more of the glutamatergic proteins GluN2B, GluA1, GLT1 and xCT and these changes would be associated with compulsive-like behaviour towards HFHS food.

2 | MATERIALS AND METHODS

2.1 | Experimental subjects

Outbred male Sprague–Dawley rats (340 \pm 20 g, 9–10 weeks old, $n = 60$) (ARC, Canning Vale) were housed individually with nesting/enrichment material at a room temperature of 20 \pm 2°C on a reverse 12 h light/dark cycle (lights off at 7:00 AM). Rats were provided ad libitum access to water and either standard rodent diet (Chow, 18% kcal fat; total density = 3.17 kcal g⁻¹, Barastoc, Australia) or a high-fat high-sugar (HFHS) diet, as described below. Rats were allowed to acclimate for 7 days before experimentation began. All procedures performed were in accordance with the Prevention of Cruelty to Animals Act (2004), under the guidelines of the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of animals for Experimental Purposes (2013) and approved by The Florey Institute of Neuroscience and Mental Health Animal Ethics Committee.

2.2 | Model of diet-induced obesity

Sixty rats were placed on an 8-week HFHS diet (SF04-001, 45% kcal from fat; total density = 4.73 kcal g⁻¹, Specialty Feeds, Glen Forrest, Australia). Food intake and body weight were measured twice per week. Rats were then separated into DIO (top third, $n = 20$) and DR (bottom third, $n = 20$) groups based on weight gain from weeks 3 to 8 to avoid weight gain due to normal development during the first 2 weeks.^{4,5} For the subsequent duration of the experiment, rats were placed back on standard chow diet.

2.3 | Conditioned suppression test

Rats underwent a conditioned suppression task 2 days after being placed back on the standard chow diet (Figure 1A). In fear conditioning chambers (MED Associates; St. Albans, VT), rats were given access to HFHS food

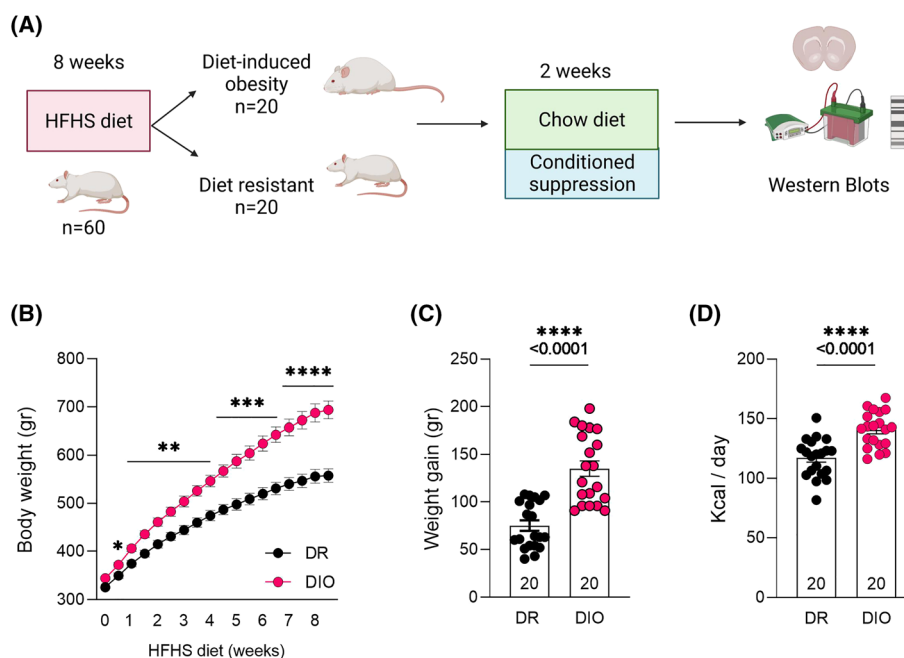
(20 g) for 30 min, made available in a plastic tray affixed to the grid floor. Food was weighed before and after the session to establish baseline HFHS intake; this was repeated daily until the daily food intake no longer varied beyond 10% from total average.²³ Average training time was 13 days. Once the baseline was established, we ran a single shock session. Rats were placed in the same fear chambers, this time without any food. During the first and last 10 min of the 30 min session, a flashing cue light (conditioned stimulus, CS) was accompanied by a 1 s electric shock (0.5 mA, unconditioned stimulus, US) every minute. During minutes 11 to 20, there was no US or CS presentations. The following day, rats were placed back into the chambers with HFHS pellet, and the effect of CS presentation (cue light) on food consumption was measured. We recorded baseline and test day latencies to commence eating, time spent interacting with food (scored manually via NIR video recording) and total amount of food consumed during these sessions (kcal and grammes).

2.4 | Western blots

GluN2B (NMDAR2B, NR2B), GluA1 (GluR1, GRIA1), GLT1 and xCT expression in the dorsal striatum and nucleus accumbens was determined by western blot analysis, according to previously described methods²⁴; the membrane subfraction protocol was adapted from Reissner et al.²⁰ At conclusion of experiment, half of the rats were transferred to another experiment, and half were culled via i.p. injection of pentobarbital (1.00 ml) followed by rapid decapitation (DIO $n = 12$, DR $n = 13$). Snap-frozen brains from DIO, DR and control Chow were cut into 1 mm thick slices at Bregma +2.2 mm,²⁵ and 2 mm diameter patches were collected. A control group, never exposed to the HFHS diet, was included to normalize western blot analysis (Chow, $n = 14$), all protein expression data were normalized

to protein expression levels in this Chow control group. The Chow group was matched by age and weight. Brain punches were homogenized on ice in sucrose buffer containing protease (cOmplete™, Roche, IN) and phosphatase inhibitors (PhosSTOP EASYpack, Roche, IN). Some brain punches were lost due to freezer failure. For nucleus accumbens, we analysed punches from 10 DR, 6 DIO, 9 Chow, for dorsal striatum 4 DR, 4 DIO, 5 Chow. The membrane protein fraction was separated in two steps. First, the 1000g, 4°C for 10 min to separate supernatant from tissue debris and the final membrane fraction was obtained by centrifugation at 12 000g, 4°C for 20 in and resuspending protein in 35 μ l of sucrose buffer. Protein content was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Protein (20 μ g) was separated on Tris/Glycine 4–20% gels and transferred onto a PVDF membrane. Membranes were cut in three parts. Part A was incubated with mouse anti-GluN2B (1:2000; Merck 05-920), mouse anti-GluA1 antibodies (1:1000; Merck SAB6201086), part B with rabbit anti-GLT1 antibody (1:5000; gift from David Pow, RMIT University, Australia) and part C with rabbit anti-xCT (1:1000; Novus NB300-318, Lot M-1 and N) diluted in 1% skimmed milk in Signal Enhancer solution 1 (SignalBoost Immunoreaction Enhancer kit, Merck Millipore, Cat# 407207) and mouse anti- β -actin antibodies (1–10 000; Sigma-Aldrich, USA). Part A was incubated with goat anti-mouse (1:10 000, IRDye®800cw [Green], LI-COR, USA) for NR2B and GluA1. Part B: goat anti-rabbit (1:10 000, IRDye®680cw [Red], LI-COR, USA) was used for GLT1 detection. Part C: First, xCT was detected with goat anti-rabbit (1:10 000, IRDye®800cw [Green], LI-COR, USA) diluted in 5% skimmed milk in Signal Enhancer Solution 2 (SignalBoost Immunoreaction Enhancer kit, Merck Millipore, Cat# 407207) and then goat anti-mouse (1:10 000, IRDye®680cw [Red], LI-COR, USA) for β -actin. Expressions of GluN2B, GLT1, GluA1, xCT and β -actin bands were quantified with ImageJ ver. 1.52p (NIH).

FIGURE 1 Protocol, body weight and food intake. (A) Schematic representation of protocol and conditioned suppression test. (B) Body weight changes through the diet period, grammes, mean \pm SEM. Two-way RM ANOVA, main effect of Group $p < 0.0001$, $F_{(1, 38)} = 24.68$, main effect of Time $p < 0.0001$, $F_{(17, 646)} = 783.4$, significant interactions $p < 0.0001$, $F_{(17, 646)} = 34.10$. Bonferroni's multiple comparisons test, * $p < 0.005$, ** $p < 0.001$, *** $p < 0.0005$, **** $p < 0.0001$. (C) Cumulative weight gain in grammes after the end of 8 weeks of diet. Unpaired two-tailed t test, $p < 0.0001$, $t_{(38)} = 6.096$. (D) Average daily kcal intake during the last week of the diet. Unpaired two-tailed t test, $p < 0.0001$, $t_{(38)} = 4.819$. All data are mean \pm SEM. DR $n = 22$, DIO $n = 20$



2.5 | Addiction score

The 'addiction score' was calculated using an approach previously described^{5,26,27} for three behavioural criteria: (1) overeating palatable food measuring kcal intake during the HFHS diet period, (2) lack of control to refrain from eating despite negative consequences, measuring latency to start eating HFHS on the test day in the presence of an aversive signal (CS previously associated with electrical shock), and (3) binge-like eating by measuring kcal intake of palatable pellets in a 30 min session. Standardization of a score for each behaviour was done based on data obtained from the whole group: subtracting the average from each data point and dividing by the standard deviation. The addiction score for each rat equals the sum of the standardized scores in the 3 behaviours. According to DSM-5,²⁸ substance use disorder is categorized 'mild', 'moderate' or 'severe' according to the number of criteria fulfilled. In line with this, animals in our study that were positive for the maximum number of criteria (3) we will consider *severe*, two criteria *moderate*, one criterion *mild* and zero criteria not addicted.

2.6 | Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.1 (GraphPad Software Inc., San Diego, California). Two-way repeated measures analysis of variance (two-way RM ANOVAs) were used to examine group (DIO vs. DR) differences in weight, latency to food consumption and total kcal intake. For western blotting, target protein expression was first normalized to β -actin expression and next divided by mean expression in the Chow group. The weight gain after 8 weeks of HFHS diet, daily kcal intake on the final week of HFHS diet, time spent eating in the first 5 min of the test day session, the addiction score and western blot results were analysed by unpaired two-tailed Student *t* tests. *Post hoc* Bonferroni's analyses were performed where indicated. Alpha values of <0.05 were considered significant. For correlations, linear regressions were performed, and *F* test determined whether the linear regression slope was different from 0 (*P* values and goodness of fit [R^2] values, not *F* values, are displayed on graphs).

3 | RESULTS

3.1 | Establishing DIO and diet resistant subpopulations

A two-way ANOVA conducted on body weight over the 8 weeks of access to palatable food (Figure 1B) found main effects of Group ($F_{(1, 38)} = 24.68$, $p < 0.0001$), and a significant Time X Group interaction ($F_{(17, 646)} = 34.10$, $p < 0.0001$). Bonferroni post hoc test found that prior to palatable food diet (Week 0), there were no group differences in weight, but by Week 8, DIO rats were heavier than DR rats ($p < 0.0001$). Before the diet, regular chow intake did not differ between groups (week 0) (data not shown; $t_{(38)} = 1.348$, $p = 0.186$).

Total amount of weight gain was greater in the DIO rats (Figure 1C; $t_{(38)} = 6.096$, $p < 0.0001$). DIO rats also consumed more HFHS food ($t_{(38)} = 4.819$, $p < 0.0001$; Figure 1D) (overeating in the home cage was used as the first criterion for the addiction score).

3.2 | DIO rats display pervasive consumption of HFHS food despite aversive stimuli

After 8 weeks of ad libitum access to HFHS diet, animals were placed on standard chow, and access was restricted to 30 min daily during conditioned suppression baseline sessions (see Figure 1A). On the test day, the CS was presented, without the US, and HFHS food was available. A two-way RM-ANOVA computed on food intake (Figure 2A) found a main effect of Group ($F_{(1, 38)} = 12.67$, $p = 0.001$), no main effect of Shock and a significant Group X Shock interaction ($F_{(1, 38)} = 7.568$, $p = 0.009$). Bonferroni's multiple comparisons test showed that while the Baseline consumption was similar between DIO and DR groups ($p = 0.256$), the Test Day consumption was higher in DIO group ($p < 0.0001$), accordingly comparison of consumption during Baseline versus Test Day was different only in DR ($p = 0.005$) and unchanged in DIO ($p > 0.999$) group. DIO rats spent more time eating in the presence of the aversive cue (Figure 2B; unpaired *t* test, $t_{(38)} = 2.355$, $p = 0.024$). Both groups increased their latency to approach food during test (second criterion for the addiction score), but DIO accessed HFHS pellets earlier than DR (Figure 2C; two-way RM-ANOVA: main effect of Group, $F_{(1, 38)} = 7.821$, $p = 0.008$, main effect of Shock, $F_{(1, 38)} = 86.45$, $p < 0.0001$, Group X Shock interactions, $F_{(1, 38)} = 4.452$, $p = 0.042$). Bonferroni's multiple comparisons found no group differences at baseline ($p > 0.999$), but a difference on test day ($p = 0.002$).

3.3 | DIO rats display a higher addiction score compared to DR rats

The final criterion, binge-like eating of HFHS in 30 min, was higher in DIO rats as revealed by unpaired *t* test of the baseline data ($t_{(38)} = 2.182$, $p = 0.035$, data not shown). When computing performance on these three criteria (binge-like eating during baseline consumption, overeating of HFHS in homecage and eating despite negative consequences), it was found that DIO animals had a higher addiction score compared to DR rats (Figure 2D; unpaired two tailed *t* test: $t_{(38)} = 5.687$, $p < 0.0001$).

3.4 | DIO rats display increased expression of GluA1 and xCT protein in nucleus accumbens

Western blots of membrane fraction from NAc revealed that DIO and DR animals did not show any significant differences in GluN2B or GLT1 expression (Figure 3A,B). However, DIO showed increased expression of both GluA1 ($t_{(14)} = 4.230$, $p = 0.0008$; Figure 3C) and

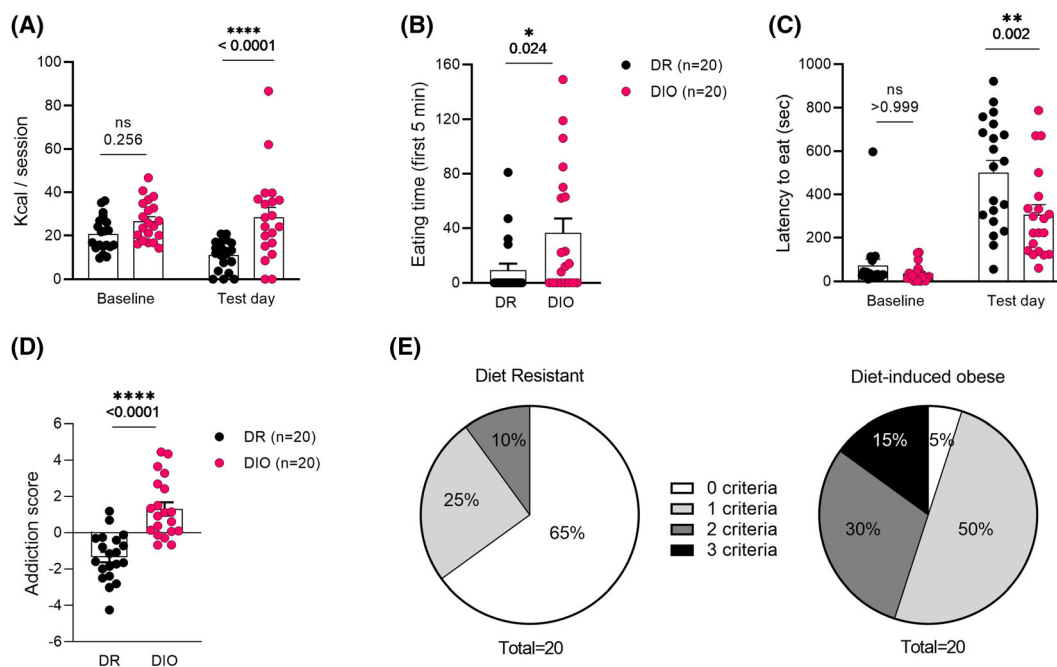


FIGURE 2 Conditioned suppression and addiction score in diet-induced obese (DIO) and diet-resistant (DR) rats. (A) Kcal consumption. Baseline: average for 3 days (less than 10% variation between days), two-way RM ANOVA, main effect of Group $p = 0.001$, $F_{(1, 38)} = 12.67$, no main effect of Time $p = 0.070$, $F_{(1, 38)} = 3.484$, significant interactions $p = 0.009$, $F_{(1, 38)} = 7.568$. Bonferroni's multiple comparisons test, between groups DR versus DIO baseline $p = 0.256$, test day $p < 0.0001$, within the group baseline versus test day, DR $p = 0.005$, DIO $p > 0.999$. (B) Time spent eating in the first 5 min of the session (main difference occur in this period, later animals realize that light cue does not signal food shock anymore), unpaired t test, $t_{(38)} = 2.355$, $p = 0.024$. (C) Latency to start eating in the presence of aversive signal compared to latency before the shock exposure, two-way RM ANOVA, main effect of Group $p = 0.008$, $F_{(1, 38)} = 7.821$, main effect of aversive signal, $p < 0.0001$, $F_{(1, 38)} = 86.45$, interactions Group X Aversive signal, $p = 0.042$, $F_{(1, 38)} = 4.452$, Bonferroni's multiple comparisons DIO versus DR at baseline $p > 0.999$, on the test day $p = 0.002$. (D) Addiction score (binge-like eating in 30 min sessions, chronic overeating in the home-cage, lack of control to refrain from eating despite known negative consequences), unpaired t test, $p < 0.0001$, $t_{(38)} = 5.687$. (E) Percentage of animals with addiction criteria. All data are mean \pm SEM. DR, $n = 20$; DIO, $n = 20$

xCT ($t_{(14)} = 2.382$, $p = 0.032$; Figure 3D). There was no significant difference in the expression of any of the proteins in dorsal striatum (Figure 3E–K).

3.5 | Addiction score is positively correlated with weight gain, GluA1 and xCT expression

Both addiction score and weight gain were positively correlated with GluA1 (Figure 4C; $R^2 = 0.289$, $p = 0.0316$, Figure 4G; $R^2 = 0.257$, $p = 0.045$) and xCT expression in the nucleus accumbens (Figure 4D; $R^2 = 0.377$, $p = 0.011$, Figure 4H; $R^2 = 0.289$, $p = 0.0317$). There were no correlations with GluN2B and GLT1 (Figure 4A,E: GluN2B, $p = 0.345$, $p = 0.745$; Figure 4B,F: GLT1, $p = 0.372$, $p = 0.232$, respectively). Addiction score and propensity for weight gain were positively correlated (Figure 4L; $R^2 = 0.341$, $p < 0.0001$).

4 | DISCUSSION

The goal of this study was to examine whether the propensity for diet-induced obesity was associated with compulsive-like behaviour

towards HFHS food and glutamatergic dysregulation in the striatum. We show that DIO rats exhibit an impaired ability to refrain from eating in the presence of cues that signal an aversive consequence (foot shock): one of the key hallmarks of addiction (consumption of the substance despite known negative consequences).²⁸ Further, addiction-like behaviour towards HFHS food was associated with glutamatergic dysfunction in the NAc of DIO rats, and the extent of this dysfunction predicted the extent of addiction-like behaviour.

Although to date, the theory of food addiction remains somewhat controversial, there is growing evidence that diet-induced obesity is often associated with addiction-like behaviour towards HFHS food in both humans and animal models.^{23,29} We have previously shown that DIO but not DR animals display binge-like eating, loss-of-control over food-seeking behaviour and increased willingness to work for food in an operant self-administration paradigm.^{5,6} Our current findings add another crucial parameter to this list of aberrant eating behaviours, namely, eating despite known negative consequences. This was evidenced by the fact that DIO animals failed to reduce their kcal intake or latency to start eating in the presence of aversive signal.

Taken together, these data demonstrate that the propensity for obesity is associated with compulsive-like behaviour towards HFHS,

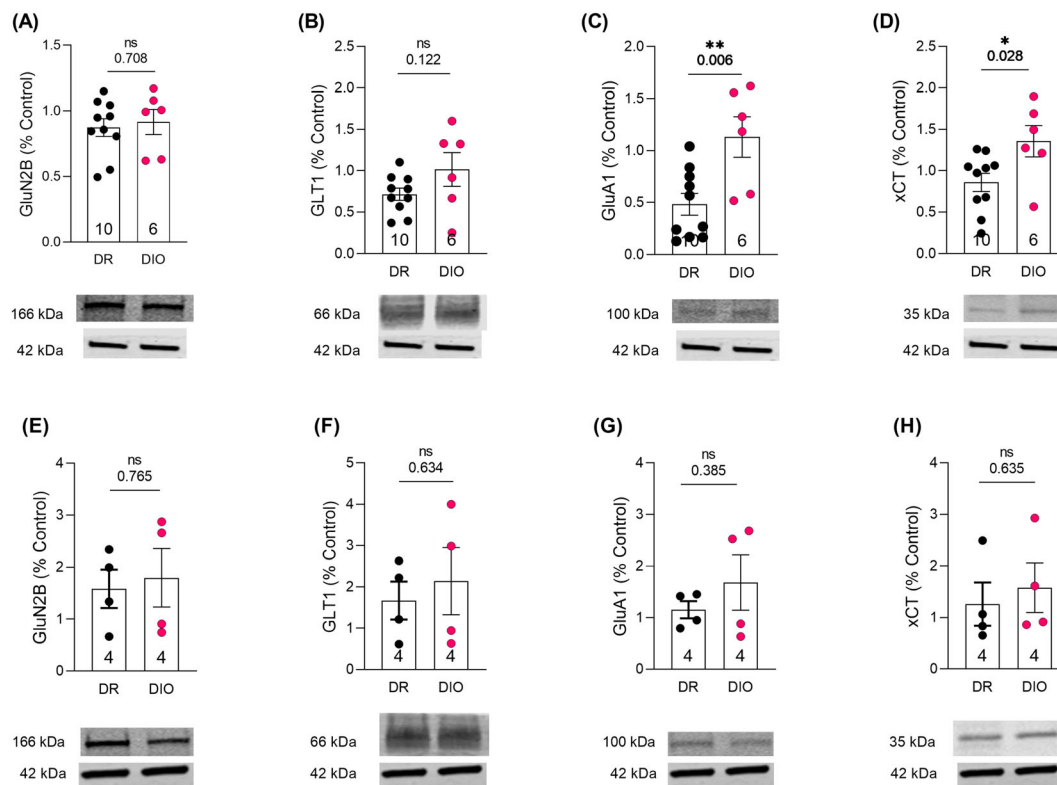


FIGURE 3 Western blots (A–D) membrane fraction from the nucleus accumbens, all gels we normalized to actin. Results expressed as a percentage of control group Chow ($n = 9$). Unpaired two-tailed t tests: GluN2B, $t_{(14)} = 0.383$, $p = 0.708$; GLT-1, $t_{(14)} = 1.645$, $p = 0.122$; GluA1, $t_{(14)} = 3.228$, $p = 0.006$; xCT, $t_{(14)} = 2.443$, $p = 0.028$. (E–H) membrane fraction from the dorsal striatum, all gels we normalized to actin. Results expressed in ratio to the control group, Chow ($n = 5$). All data are mean \pm SEM. Unpaired two-tailed t tests, GluN2B, $t_{(6)} = 0.313$, $p = 0.765$; GLT-1, $t_{(6)} = 0.502$, $p = 0.634$; GluA1, $t_{(6)} = 0.937$, $p = 0.385$; xCT, $t_{(6)} = 0.500$, $p = 0.635$. Black: diet-resistant rats, $n = 4$. Pink: diet-induced obese rats, $n = 4$

thus providing support for a common endophenotype underlying some forms of diet-induced obesity and drug addiction. Moreover, our data provide further evidence of the utility of the Levin outbred DIO model. Robust preclinical models of addictive eating will facilitate the development of effective and comprehensive obesity treatments which should go beyond weight loss to reduce food craving, promoting the maintenance of healthy eating habits.

4.1 | Increased GluA1 expression in DIO rats is similar to cocaine-induced changes in the nucleus accumbens

Accumbal glutamatergic dysfunction has been demonstrated in animal models of cocaine,¹⁴ nicotine,²¹ alcohol³⁰ and heroin³¹ addiction. More recently, accumbal glutamatergic dysfunction (viz., an increase in AMPA/NMDA currents in parallel with a deficit in LTD) was also reported in DIO animals,⁵ suggesting dysregulated accumbal glutamate signalling in DIO. To investigate this further, assessed GluA1 expression in the NAc and dorsal striatum, finding that 8 weeks of HFHS diet were sufficient to increase expression of GluA1-containing AMPA receptors selectively in the NAc of DIO

animals. Not only is this consistent with the increased ratio of AMPA receptor mediated currents as compared to NMDA previously observed⁵ but a body of literature exists demonstrating a role for NAc GluA2-lacking (presumably GluA1-containing) AMPAR in incubation of drug craving and enduring vulnerability to relapse.^{9,16,32} Furthermore, it seems that AMPA receptors lacking the GluA2 subunit (presumably GluA1-AMPA) are responsible for the increased sensitivity of NAc neurons to drug associated cues.^{13,16} For example, a study of rats undergoing prolonged withdrawal from cocaine using whole-cell patch-clamp recording of medium spiny neurons in the NAc core showed an enhanced responsiveness of those neurons to excitatory inputs, largely relying on GluA2-lacking AMPA receptors.¹⁶ Plus, in the same study, they showed that intra-NAc administration of 1-naphthylacetylspermine (Naspm), a selective antagonist of GluA2-lacking AMPA receptors, reduced cue-induced cocaine-seeking.

Our findings are also consistent with work demonstrating exposure to HFHS selectively increases the function of calcium permeable AMPAR in the NAc of DIO rats, but not their DR counterparts.³³ Moreover, enhanced cue triggered approach behaviour (as measured by Pavlovian Instrumental Transfer) in DIO rats is mediated by calcium-permeable AMPAR in NAc core,⁹ providing a possible

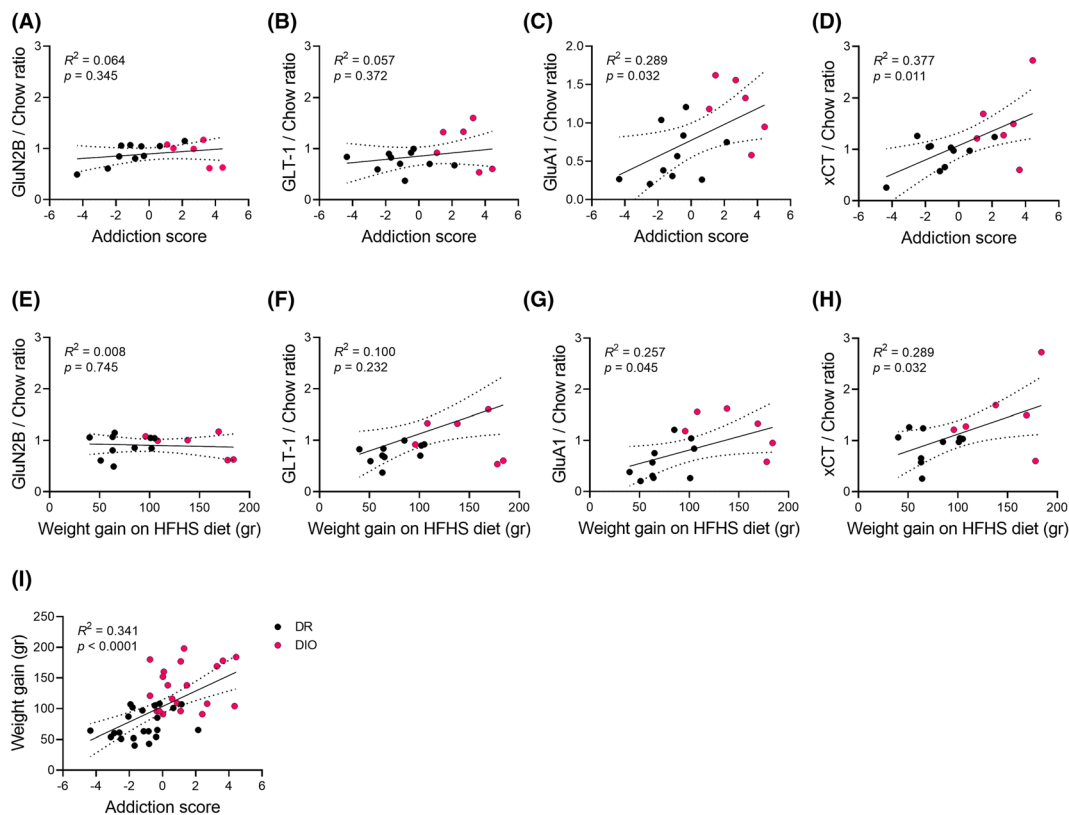


FIGURE 4 Pearson r correlations between western blot measurement of expression of GluN2b, GLT-1, GluA1 and xCT in the nucleus accumbens and addiction score (A–D)/weight gain (E–H); between addiction score and weight gain (I). Dashed line represents 95% confidence. Pearson r correlations R^2 and p values are as presented on graphs. All data are mean \pm SEM. Black: diet-resistant rats, $n = 10$. Pink: diet-induced obese rats, $n = 6$

mechanism by which enhanced cue triggered motivation could drive compulsive-like behaviour towards HFHS (see also Oginsky et al.³³ and Alonso-Caraballo et al.³⁴). Thus, it has previously been established that, as with cocaine, AMPAR plasticity in NAc occurs in response to food rewards. We extend this research by showing a specific association between the extent of GluA1 expression in NAc and the level of compulsive-like behaviour towards HFHS food. This provides a link between glutamatergic dysfunction in NAc and compulsive-like behaviour towards ‘junk food’ in obesity. One possible mechanism by which HFHS food is increasing GluA1 in DIO rats is via the generation of de novo silent synapses,³⁴ as observed following cocaine use.³⁵

4.2 | DIO rats display xCT upregulation in the nucleus accumbens but no change in GLT1

We also observed changes at the level of xCT, a catalytic chain subunit of the cystine/glutamate antiporter (also known as the x_c^- system or Slc7a11). This system transports cystine into neurons in exchange for glutamate at a ratio of 1:1. It plays an important role in glutamate homeostasis in many parts of the brain, and in the NAc, this system is responsible for 60% of extracellular glutamate.³⁶ In drug addiction studies, the x_c^- system is thought to contribute to

pathological glutamate signalling linked to addiction.³⁷ In these studies, downregulation of xCT is typically observed in NAc after a period of withdrawal.^{38–40} The directionality of these findings is in contrast with our data where we observe an *increase* in xCT expression in NAc in the DIO rats displaying addictive-like behaviour towards HFHS. However, importantly, in the current study, the NAc tissue used was obtained from rats that were not subject to withdrawal from HFHS food. Indeed, increased levels of xCT are observed in ethanol-dependent rats, and the opposite is observed once rats are placed into withdrawal.³⁹ xCT upregulation in the NAc is also associated with an increased risk for relapse to alcohol seeking in mice.⁴¹ Thus, it would appear that upregulation of accumbal xCT levels promotes vulnerability to addiction-like behaviour. In contrast to xCT, no changes were observed in NAc expression of GLT1. Simplistically, this suggests that xCT may play a bigger role in glutamate-mediated changes in DIO rats than GLT1. However, it should be noted that one recent study suggests that in NAc membrane GLT1 expression is activity dependent and can undergo rapid transition from upregulation to downregulation.⁴²

In summary, we have observed that the predisposition to obesity on a ‘junk food’ diet is associated with differences in the xCT system in NAc, providing support for the proposal that glutamatergic dysfunction in NAc underlies compulsive eating in obesity. Future studies

could specifically examine changes in behaviour and xCT/GLT1 expression induced by withdrawal from 'junk food'. It would be of interest to observe whether prolonged withdrawal causes incubation of 'craving' in DIO animals and concomitant HFHS food-seeking behaviour which correlates with upregulation of xCT expression in NAc. These data support a common mechanism between substance abuse and aberrant eating behaviour.

4.3 | No significant differences observed between DIO and diet resistant rats with respect to dorsal striatum levels of GluA1, xCT, GluN2B and GLT1

A potentially intriguing finding is the lack of observed differences in the dorsal striatum levels of any of the glutamatergic markers observed. Given the compulsive nature of the behaviour assessed and the known role for dorsal striatum in the transition to compulsive drug use,²² in addiction, we would potentially hypothesize that glutamatergic dysfunction in dorsal, not ventral, striatum would underlie the differences observed. However, this was found not to be the case. Considering the established involvement of the dorsal striatum in the later stages of addiction,²² it may be that our protocol was simply not long/extensive enough to recruit this brain region. Another possibility could be due to food being a natural reward, as opposed to a pharmacological agent, and, as such, not providing as strong a stimulus to induce these changes. However, binge-like consumption of palatable food has been shown to accelerate habitual control of behaviour in a dorsolateral striatum-dependent manner,⁴³ providing direct evidence to the contrary. Thus, given the distinct role for the dorsolateral subregion of the striatum compared to the dorsomedial in the transition from goal-directed to more habitual behaviour, it may be that had we tested protein levels from these distinct striatal subregions that we may have observed an effect. Future studies should explore these possibilities.

4.4 | Weight gain on the HFHS diet and nucleus accumbens GluA1 and xCT expression are positively associated with addiction scores

While compulsive overeating is mostly reported in people suffering from eating disorders and some people with obesity, milder variations of this behaviour are also observed in healthy individuals (e.g., stress-related overeating, emotional eating).⁴⁴ It is unclear what precipitates the shift from occasional overeating of junk food to more maladaptive patterns of consumption. To generate effective treatment and prevention strategies, we need appropriate preclinical models. Eating is essential for life and in that regard is very different from drug use. Yet, as is the case with drug use, most individuals who occasionally overindulge in junk food are generally in control of their behaviour and do not develop compulsive habits. Similarly, in our model, only a small proportion of outbred DIO rats developed 'severe' addiction-like behaviour (15% positive for all 3 addiction

criteria) after chronic exposure to the HFHS diet. Importantly, weight gain on the HFHS diet was predictive of propensity to develop addiction-like behaviour (animals positive for all three addictive criteria were only found in the DIO group, i.e., top gainers). Strikingly, we observed an association between both the development of addiction-like behaviour and changes in brain reward circuits. We hypothesize that a propensity for overeating and thus weight gain when exposed to HFHS food is an early indication of increased risk for developing compulsive eating.

In this regard, compounds acting on glutamate homeostasis may show promise for the treatment of compulsive eating in diet-induced obesity. As we described above, DIO rats with compulsive-like eating display dysregulation of glutamate homeostasis in NAc.⁵ In several psychostimulant studies, dysregulated glutamate homeostasis in NAc and, thus, relapse-like behaviour is restored by N-acetylcysteine, a cysteine pro-drug.^{20,45-48} We recently showed that sub-chronic administration of N-acetylcysteine reduced escalation of operant responding for HFHS pellets and addictive-like behaviour towards junk food in DIO rats.⁶ Moreover, N-acetylcysteine has been shown to reduce binge-like eating⁴⁹ and cue-induced reinstatement of food-seeking in rats.⁴⁶ In light of the present results showing increased xCT expression in this model, it is possible that N-acetylcysteine reduces binge eating/addictive eating due to its well-established effects on increasing basal glutamate, reducing the motivation to overeat to increase xCT expression. Collectively, these data support a common mechanism between compulsive drug taking and compulsive eating behaviour and highlights the therapeutic potential of glutamate homeostasis restoring treatments.

Limitations of this study deserve note. First, the western blot analyses were performed on tissue obtained from animals that were subjected to the conditioned suppression protocol, which could potentially affect results. However, this allowed us to examine the putative molecular basis of behavioural differences observed and perform correlations between protein expression and behavioural endpoints. Second, studies suggest that there are sex-differences in addictive-like behaviours and preclinical evidence suggests that there could be sex-differences in the glutamate system related to drug-exposure.⁵⁰ Moreover, we know that women are at a higher risk for stress-induced HFHS eating, and preclinical studies show sex-differences in binge-like eating.⁴⁴ Therefore, it is important that in future studies addiction-like behaviour and glutamatergic changes are assessed in female rats too. In conclusion, DIO rats exhibit compulsive-like behaviour towards HFHS junk food accompanied with increased GluA1 and xCT expression in the NAc. Further, the extent of glutamatergic dysregulation observed predicted the extent of addiction-like behaviour. These data suggest addiction-like glutamatergic dysfunction underlies compulsive eating of HFHS foods in diet-induced obesity.

ACKNOWLEDGEMENTS

We thank David Pow (RMIT University, Australia) via Tony Hannan (Florey) for the gift of the GLT1 antibody. This research was funded by National Health and Medical Research Council project grant 1108092. RMB is supported by RMB is supported by Australian

Research Council (ARC) DECRA (DE190101244). PS is supported by a National Health and Medical Research Council Investigator Grant (1178482), and AJL is supported by a National Health and Medical Research Council Principal Research Fellowship (1116930). We acknowledge the Victorian State Government Operational Infrastructure Scheme. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

RMB designed the experiments with input from AJL. DB ran behavioural testing under supervision of RMB and AJL. LL, NK, HT and TL optimized and ran western blots under supervision of RMB and JN, with input from LK. DS analysed data and wrote the manuscript with input from PS, RMB and AJL.

DATA AVAILABILITY STATEMENT

The data from this manuscript are available upon request.

ORCID

Diana Skettriene  <https://orcid.org/0000-0002-6510-042X>

Laddawan Lalert  <https://orcid.org/0000-0003-4178-9282>

Lori A. Knackstedt  <https://orcid.org/0000-0003-2524-6739>

Jess Nithianantharajah  <https://orcid.org/0000-0002-3501-1143>

Andrew J. Lawrence  <https://orcid.org/0000-0001-6836-727X>

Robyn M. Brown  <https://orcid.org/0000-0003-4167-3634>

REFERENCES

- Gold MS, Avena NM. Animal models lead the way to further understanding food addiction as well as providing evidence that drugs used successfully in addictions can be successful in treating overeating. *Biol Psychiatry*. 2013;74(7):e11. doi:10.1016/j.biopsych.2013.04.022
- Meule A, Gearhardt AN. Food addiction in the light of DSM-5. *Nutrients*. 2014;6(9):3653-3671. doi:10.3390/nu6093653
- Hauck C, Cook B, Ellrott T. Food addiction, eating addiction and eating disorders. *Proc Nutrition Soc*. 2020;79(1):103-112. doi:10.1017/S0029665119001162
- Levin BE, Dunn-Meynell A, Balkan B, Keesey RE. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *Am J Physiol*. 1997;273:R725-R730.
- Brown RM, Kupchik YM, Spencer S, et al. Addiction-like synaptic impairments in diet-induced obesity. *Biol Psychiatry*. 2017;81(9):797-806. doi:10.1016/j.biopsych.2015.11.019
- Skettriene D, Battista D, Lawrence AJ, Brown RM. N-acetylcysteine reduces addiction-like behaviour towards high-fat high-sugar food in diet-induced obese rats. In: Conference Poster The 11th FENS Forum of Neuroscience (FENS 2018): poster. 2017. doi:10.1111/ejn.15321
- Wei S, Hertle S, Spanagel R, Bilbao A. NMDA receptors in accumbal D1 neurons influence chronic sugar consumption and relapse. *Eneuro*. 2021;8(3):1-9. doi:10.1523/ENEURO.0029-21.2021
- Scofield MD, Heinsbroek JA, Gipson CD, et al. The nucleus accumbens: mechanisms of addiction across drug classes reflect the importance of glutamate homeostasis. *Pharmacol Rev*. 2016;68(3):816-871. doi:10.1124/pr.116.012484
- Derman RC, Ferrario CR. Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPA receptors. *Neuropharmacology*. 2018;131:326-336. doi:10.1016/J.NEUROPHARM.2017.12.039
- Wang J, Ishikawa M, Yang Y, et al. Cascades of homeostatic dysregulation promote incubation of cocaine craving. *J Neurosci*. 2018;38(18):4316-4328. doi:10.1523/JNEUROSCI.3291-17.2018
- Shen H, Moussawi K, Zhou W, Toda S, Kalivas PW. Heroin relapse requires long-term potentiation-like plasticity mediated by NMDA2b-containing receptors. *Proc Natl Acad Sci U S A*. 2011;108(48):19407-19412. doi:10.1073/pnas.1112052108
- Smith KL, Rao RR, Velázquez-Sánchez C, et al. The uncompetitive N-methyl-D-aspartate antagonist memantine reduces binge-like eating, food-seeking behavior, and compulsive eating: role of the nucleus accumbens shell. *Neuropsychopharmacology*. 2015;40:1163-1171. doi:10.1038/NPP.2014.299
- Dong Y, Taylor JR, Wolf ME, Shaham Y. Circuit and synaptic plasticity mechanisms of drug relapse. *J Neurosci*. 2017;37(45):10867-10876. doi:10.1523/JNEUROSCI.1821-17.2017
- Niedzielska-Andres E, Pomierny-Chamioło L, Andres M, et al. Cocaine use disorder: a look at metabotropic glutamate receptors and glutamate transporters. *Pharmacol Ther*. 2021;221:107797. doi:10.1016/j.pharmthera.2020.107797
- Wolf ME, Ferrario CR. AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neurosci Biobehav Rev*. 2010;35(2):185-211. doi:10.1016/j.neubiorev.2010.01.013
- Conrad KL, Tseng KY, Uejima JL, et al. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*. 2008;454(7200):118-121. doi:10.1038/nature06995
- Baker DA, McFarland K, Lake RW, et al. Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nat Neurosci*. 2003;6(7):743-749. doi:10.1038/nm1069
- Kalivas PW. The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci*. 2009;10(8):561-572. doi:10.1038/nrn2515
- Pendyam S, Mohan A, Kalivas PW, Nair SS. Computational model of extracellular glutamate in the nucleus accumbens incorporates neuroadaptations by chronic cocaine. *Neuroscience*. 2009;158(4):1266-1276. doi:10.1016/j.neuroscience.2008.11.014
- Reissner KJ, Gipson CD, Tran PK, Knackstedt LA, Scofield MD, Kalivas PW. Glutamate transporter GLT-1 mediates N-acetylcysteine inhibition of cocaine reinstatement. *Addict Biol*. 2015;20(2):316-323. doi:10.1111/adb.12127
- Knackstedt LA, LaRowe S, Mardikian P, et al. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. *Biol Psychiatry*. 2009;65(10):841-845. doi:10.1016/j.biopsych.2008.10.040
- Everitt BJ, Robbins TW. From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. *Neurosci Biobehav Rev*. 2013;37(9):1946-1954. doi:10.1016/J.NEUBIOREV.2013.02.010
- Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci*. 2010;13(5):635-641. doi:10.1038/nn.2519
- Sheehan RK, Weston RH, Perera ND, D'Amico A, Nutt SL, Turner BJ. Effect of thymic stimulation of CD4+ T cell expansion on disease onset and progression in mutant SOD1 mice. *J Neuroinflammation*. 2015;12(1):1-12. doi:10.1186/s12974-015-0254-3
- Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Vol. 10. 7th ed; 2017.
- Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. *Science*. 2004;305(5686):1014-1017. doi:10.1126/science.1099020
- Velázquez-Sánchez C, Ferragud A, Moore CF, Everitt BJ, Sabino V, Cottone P. High trait impulsivity predicts food addiction-like behavior in the rat. *Neuropsychopharmacology*. 2014;39(10):2463-2472. doi:10.1038/npp.2014.98

28. DSM-5. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. American Psychiatric Publishing; 2013.
29. Burrows T, Hides L, Brown R, Dayas C, Kay-Lambkin F. Differences in dietary preferences, personality and mental health in Australian adults with and without food addiction. *Nutrients*. 2017;9(3):285-298. doi:[10.3390/nu9030285](https://doi.org/10.3390/nu9030285)
30. Avshalumov Y, Mandyam CD. Synaptic plasticity and its modulation by alcohol. *Brain Plasticity*. 2020;6:103-111. doi:[10.3233/BPL-190089](https://doi.org/10.3233/BPL-190089)
31. LaLumiere RT, Kalivas PW. Glutamate release in the nucleus accumbens core is necessary for heroin seeking. *J Neurosci*. 2008;28(12):3170-3177. doi:[10.1523/JNEUROSCI.5129-07.2008](https://doi.org/10.1523/JNEUROSCI.5129-07.2008)
32. Wolf A, Bauer B, Abner EL, Ashkenazy-Frolinger T, Hartz AMS. A comprehensive behavioral test battery to assess learning and memory in 129S6/Tg2576 mice. *PLoS ONE* Published Online. 2016:e0147733. doi:[10.1371/journal.pone.0147733](https://doi.org/10.1371/journal.pone.0147733)
33. Oginsky MF, Goforth PB, Nobile CW, Lopez-Santiago LF, Ferrario CR. Eating 'junk-food' produces rapid and long-lasting increases in NAC CP-AMPA receptors: implications for enhanced cue-induced motivation and food addiction. *Neuropsychopharmacology*. 2016;41(13):2977-2986. doi:[10.1038/npp.2016.111](https://doi.org/10.1038/npp.2016.111)
34. Alonso-Caraballo Y, Fetterly TL, Jorgensen ET, Nieto AM, Brown TE, Ferrario CR. Sex specific effects of "junk-food" diet on calcium permeable AMPA receptors and silent synapses in the nucleus accumbens core. *Neuropsychopharmacology*. 2021;46(3):569-578. doi:[10.1038/s41386-020-0781-1](https://doi.org/10.1038/s41386-020-0781-1)
35. Panchal SK, Poudyal H, Iyer A, et al. High-carbohydrate, high-fat diet-induced metabolic syndrome and cardiovascular remodeling in rats. *J Cardiovasc Pharmacol*. 2011;57(5):611-624. doi:[10.1097/FJC.0b013e3181feb90a](https://doi.org/10.1097/FJC.0b013e3181feb90a)
36. Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW. The origin and neuronal function of in vivo nonsynaptic glutamate. *J Neurosci*. 2002;22(20):9134-9141. doi:[10.1523/JNEUROSCI.22-20-09134.2002](https://doi.org/10.1523/JNEUROSCI.22-20-09134.2002)
37. Scofield MD, Kalivas PW. Astrocytic dysfunction and addiction. *Neuroscientist*. 2014;20(6):610-622. doi:[10.1177/1073858413520347](https://doi.org/10.1177/1073858413520347)
38. Amaral VCS, Morais-Silva G, Laverde CF, Marin MT. Susceptibility to extinction and reinstatement of ethanol-induced conditioned place preference is related to differences in astrocyte cystine-glutamate antiporter content. *Neurosci Res* Published Online July 9. 2020:239-250. doi:[10.1016/j.neures.2020.07.002](https://doi.org/10.1016/j.neures.2020.07.002)
39. Peana AT, Muggironi G, Bennardini F. Change of cystine/glutamate antiporter expression in ethanol-dependent rats. *Front Neurosci*. 2014;8(SEP):1-9. doi:[10.3389/fnins.2014.00311](https://doi.org/10.3389/fnins.2014.00311)
40. Knackstedt LA, Melendez RI, Kalivas PW. Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. *Biol Psychiatry*. 2010;67(1):81-84. doi:[10.1016/j.biopsych.2009.07.018](https://doi.org/10.1016/j.biopsych.2009.07.018)
41. Rangel-Barajas C, Coronel I, Zhang Y, Hernández M, Boehm SL. Low-level developmental lead exposure does not predispose to adult alcohol self-administration, but does increase the risk of relapsing to alcohol seeking in mice: Contrasting role of GLT1 and xCT brain expression. *Neuropharmacology*. 2020;181:108339. doi:[10.1016/j.neuropharm.2020.108339](https://doi.org/10.1016/j.neuropharm.2020.108339)
42. Namba MD, Kupchik YM, Spencer SM, et al. Accumbens neuroimmune signaling and dysregulation of astrocytic glutamate transport underlie conditioned nicotine-seeking behavior. *Addict Biol*. 2020;25(5):139-148. doi:[10.1111/adb.12797](https://doi.org/10.1111/adb.12797)
43. Furlong TM, Jayaweera HK, Balleine BW, Corbit LH. Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *J Neurosci*. 2014;34(14):5012-5022. doi:[10.1523/JNEUROSCI.3707-13.2014](https://doi.org/10.1523/JNEUROSCI.3707-13.2014)
44. Anversa RG, Muthmainah M, Sketriene D, Gogos A, Sumithran P, Brown RM. A review of sex differences in the mechanisms and drivers of overeating. *Front Neuroendocrinol*. 2021;63(May):100941. doi:[10.1016/j.yfrne.2021.100941](https://doi.org/10.1016/j.yfrne.2021.100941)
45. Brown RM, Kupchik YM, Kalivas PW. The story of glutamate in drug addiction and of N-acetylcysteine as a potential pharmacotherapy. *JAMA Psychiatry*. 2013;70(9):895-897. doi:[10.1001/jamapsychiatry.2013.2207](https://doi.org/10.1001/jamapsychiatry.2013.2207)
46. Ramirez-Niño AM, D'Souza MS, Markou A. N-acetylcysteine decreased nicotine self-administration and cue-induced reinstatement of nicotine seeking in rats: comparison with the effects of N-acetylcysteine on food responding and food seeking. *Psychopharmacology (Berl)*. 2013;225(2):473-482. doi:[10.1007/s00213-012-2837-3](https://doi.org/10.1007/s00213-012-2837-3)
47. Murray JE, Everitt BJ, Belin D. N-Acetylcysteine reduces early- and late-stage cocaine seeking without affecting cocaine taking in rats. *Addict Biol*. 2012;17(2):437-440. doi:[10.1111/j.1369-1600.2011.00330.x](https://doi.org/10.1111/j.1369-1600.2011.00330.x)
48. Zhou W, Kalivas PW. N-acetylcysteine reduces extinction responding and induces enduring reductions in cue- and heroin-induced drug-seeking. *Biol Psychiatry*. 2008;63(3):338-340. doi:[10.1007/s11103-011-9767-z](https://doi.org/10.1007/s11103-011-9767-z)
49. Hurley MM, Resch JM, Maunze B, Frenkel MM, Baker DA, Choi S. N-acetylcysteine decreases binge eating in a rodent model. *Int J Obes (Lond)*. 2016;40(7):1183-1186. doi:[10.1038/ijo.2016.31](https://doi.org/10.1038/ijo.2016.31)
50. Giacometti LL, Barker JM. Sex differences in the glutamate system: Implications for addiction. *Neurosci Biobehav Rev*. 2020:157-168. doi:[10.1016/j.neubiorev.2020.03.010](https://doi.org/10.1016/j.neubiorev.2020.03.010)

How to cite this article: Sketriene D, Battista D, Lalert L, et al. Compulsive-like eating of high-fat high-sugar food is associated with 'addiction-like' glutamatergic dysfunction in obesity prone rats. *Addiction Biology*. 2022;27(5):e13206. doi:[10.1111/adb.13206](https://doi.org/10.1111/adb.13206)