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N-acetylcysteine reduces addiction-like behaviour towards high-fat high-sugar food in diet-induced obese rats

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TITLE: N-ACETYLCYSTEINE REDUCES ADDICTION-LIKE BEHAVIOUR TOWARDS HIGH-FAT HIGH-SUGAR FOOD IN DIET-INDUCED OBESE RATS

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Abstract

Compulsive forms of eating displayed by some obese individuals share similarities with compulsive drug taking behaviour, a hallmark feature of substance use disorder. This raises the possibility that drug addiction treatments may show utility in the treatment of compulsive overeating. N-Acetylcysteine (NAC) is a cysteine pro-drug which has experienced some success in clinical trials, reducing cocaine, marijuana and cigarette use, as well as compulsive behaviours such as gambling and trichotillomania. We assessed the impact of NAC on addiction-like behaviour towards highly palatable food in a rat model of diet-induced obesity. Adult male Sprague-Dawley rats were placed on a high-fat high-sugar diet for eight weeks and then assigned to diet-induced obesity-prone (DIO) or diet-induced obesity-resistant (DR) groups based on weight gain. DIO and DR rats were subjected to an operant conditioning paradigm whereby rats could lever press for high-fat high-sugar food pellets. This alternated with periods of signalled reward unavailability. Before treatment DIO rats ate more in their home cage, earned more food pellets in operant sessions, and responded more during periods that signalled reward unavailability (suggestive of compulsive-like food seeking) compared to DR rats. This persistent responding in the absence of reward displayed by DIO rats was ameliorated by daily injections of NAC (100 mg/kg, i.p.) for 14 days. By the end of the treatment period, lever-pressing by NAC-treated DIO rats resembled that of DR rats. These findings suggest that NAC reduces addiction-like behaviour towards food in rats and supports the potential use of this compound in compulsive overeating.

Introduction

Obesity is a major public health problem. Studying the factors underlying its development may complement treatments promoting weight loss. One factor contributing to the development of obesity is consumption of highly-palatable food in excess of energy requirements (Affenito *et al.*, 2012). In susceptible individuals, this can lead to compulsive-like overeating, characterized by the difficulty to inhibit behaviour despite known negative consequences, eating past satiety, and overall loss of control over high-fat high-sugar (HFHS) food intake (Davis, 2013). In this respect, compulsive overeating of HFHS food is strikingly similar to behavioural and substance addictions (Volkow & Wise, 2005; Gearhardt *et al.*, 2009; Smith & Robbins, 2013; Moore *et al.*, 2017; Sketriene *et al.*, 2020). Preclinical studies indicate that diet-induced obese rats have alterations in their brains akin to those observed in animal models of substance abuse (Brown *et al.*, 2017; Derman & Ferrario, 2018; Oginsky & Ferrario, 2019; Lau *et al.*, 2020). This raises an interesting question: do compounds used in drug addiction treatment have the potential to reduce aberrant food-seeking behaviours?

In this study, we investigated the potential of N-acetylcysteine (NAC) to reduce addiction-like behaviour towards highly palatable food, based on several observations. First, NAC attenuates some addictive behaviours including binge-like consumption, escalation in consumption, increased motivation and relapse towards cocaine (Baker *et al.*, 2003; Duailibi *et al.*, 2017), nicotine (Ramirez-Niño *et al.*, 2013), heroin (Zhou & Kalivas, 2008; Hodebourg *et al.*, 2019) and alcohol (Lebourgeois *et al.*, 2019; Mocelin *et al.*, 2019) in preclinical studies. Second, NAC successfully reduces cocaine-seeking (Woodcock *et al.*, 2020), craving and desire to use cocaine in clinical studies of cocaine abuse (Echevarria *et al.*, 2017), shows benefit in treatment of impulsive-control disorders such as trichotillomania (hair-pulling disorder; for review see Everett *et al.*, 2020), however, it should be noted that the literature is not always in agreement regarding the efficacy of NAC (see meta-analysis by Duailibi *et al.*, 2017). Third, this compound is considered safe for chronic treatment with almost no secondary effects and has been approved by the US Food and Drug Administration since 1963. Moreover, NAC has been shown to attenuate abnormal pro-inflammatory responses, limit oxidative damage, and reduce obesity-related complications (for review see (Dludla *et al.*, 2019). Together, these factors made NAC a perfect candidate to test whether possible addiction therapeutic is able to ameliorate addiction-like behaviour towards food in a model of diet-induced obesity.

Accordingly, we assessed the effects of sub-chronic NAC administration on addiction-like behaviour in a well-established diet-induced obesity model. In this naturalistic model only a

subpopulation of Sprague-Dawley rats exposed to palatable food develop obesity (Levin *et al.*, 1997). Moreover, exposure to a HFHS diet has a key role in the induction of addiction-like consumption in susceptible animals (Brown *et al.*, 2017). We confirmed and extended previous descriptions of addiction-like behaviour in this model and examined the impact of NAC on escalation of intake to binge-like levels over time and the persistence of HFHS food-seeking in the presence of cues that signal reward unavailability, indicative of compulsive-like behaviour. Compulsive behaviour is repetitive or perseverative in nature, despite adverse consequences (Robbins *et al.*, 2012). There are different ways to measure compulsivity, for example by introducing negative consequences (Moore *et al.*, 2020) or by perseverative tendencies (Izquierdo & Jentsch, 2012). We are focusing on the later aspect of compulsivity. We calculated an 'addiction score' (Belin *et al.*, 2009) and assessed if a history of overeating and predisposition to obesity can predict this score. Our aim was to explore the potential of NAC for the treatment of compulsive-like eating of HFHS food in a model of diet-induced obesity.

Materials and Methods

Experimental Subjects

Outbred male Sprague-Dawley rats (340±20 g, 9–10 weeks old, n=60; ARC, Canning Vale, Australia) were housed individually with nesting/enrichment material at a room temperature of 20±2°C on a reverse 12 h light/dark cycle (lights off at 7:00 a.m.). Rats were housed individually to be consistent with previous studies employing this model (e.g. Brown *et al*., 2017). Rats had *ad libitum* access to water and either standard rodent diet (Chow, 5% of fat and 20% of protein: total density = 3.01 kcal g^{-1}, Barastoc Rat and Mouse, Ridley, Australia) or HFHS diet (SF04-001, 23.5% fat and 22.6% of protein: total density = 4.54 kcal g^{-1}, Specialty Feeds, Glen Forrest, Australia). 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 (15-006).

Model of Diet-Induced Obesity

Animals had 8-weeks access to HFHS diet *ad libitum* in their home cage. Body weight was determined twice per week. Weight gain was calculated from weeks 3 to 8, to avoid weight gain due to normal development during the first two weeks. Animals were assigned to either DIO (top third of weight gain) or DR (bottom third) groups. The mid-range group was not used. For the remainder of the experiment, rats were placed back on the standard chow diet in the home cage and HFHS was only available in the form of 45mg food pellets during operant sessions (Fig.1A protocol timeline).

Operant Self-Administration and N-acetylcysteine Treatment

Operant self-administration experiments utilised operant conditioning chambers (Model ENV-007CT; Med Associates, Vermont, USA). The operant self-administration protocol was modified from a previously reported protocol designed to identify addiction-vulnerable versus addiction-resilient individuals (Belin *et al.*, 2009; Deroche-Gamonet *et al.*, 2004; Brown *et al.*, 2011, Brown *et al*., 2017). This consisted of alternating reward available (designated S+, 3×15 minutes) and reward-unavailable (designated S-, 3×5 minutes) periods paired with distinct discriminative stimuli (operant session = 60min total). During S+ periods (house light on), pressing the active lever resulted in the dispensing of a HFHS food pellet (F06162, 45 mg, 45% kcal fat; total density = 4.73 kcal g^{-1}; Bio-Serv, Flemington, NJ, USA) paired with a discrete cue light (5s right light on, house light off). A 5-s time-out period between active lever presses and reward delivery was maintained throughout the experiment. Responding on the active lever during S- (house light off) or responding on the inactive lever during either S+ or S- periods resulted in no programmed consequences. Fig. 1A shows the operant protocol and transition from fixed ratio (FR) 1 under only S1 conditions to FR1 with S2 period, to FR3 (3 days), followed by FR5 (remainder of the protocol). Five rats, 2 DR and 3 DIO, which pressed <10 times per session for 3 sessions in a row in the training period were excluded from the final analysis.

After 2 weeks of daily operant self-administration sessions, DIO and DR rats were randomly assigned into an experimental group receiving N-acetylcysteine (100 mg/kg; i.p., Sigma-Aldrich, St. Louis, MO, USA), or control group receiving vehicle (Veh, 0.9% saline; i.p.). NAC was prepared daily in saline and adjusted to pH = 7.2. NAC or Veh was administered 2 hr prior to the operant sessions.

A progressive ratio (PR) session to measure motivation was conducted once after at least 11 days of NAC injections. The PR breakpoint was taken as the last response completed before a

lapse of 2 hours during which no pellets were earned, or the last step completed in 6 hours, whichever occurred first.

Addiction scores

The 'addiction score' was calculated using an approach previously described (Belin *et al.*, 2009; Deroche-Gamonet *et al.*, 2004; Velázquez-Sánchez *et al.*, 2014; Brown *et al.*, 2017) for 3 behavioural criteria obtained from operant self-administration data: 1) binge-like eating: the rapid consumption of significantly larger than normal amounts; modelled by measuring kcal intake of palatable pellets in 1h operant session; 2) increased amount of work for palatable food; modelled by number of lever presses in S+ period; 3) lack of control to refrain from seeking the substance; modelled by measuring the persistence of lever-pressing during periods that signalled reward unavailability. Standardizing a score for each behaviour was calculated based on data 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. An initial addiction score (1) was calculated immediately after the training period in order to compare DR and DIO groups: average for the last day before injections and two first days of treatment. A second addiction score (2) was calculated for comparison between Vehicle and NAC groups at the end of two weeks of treatment: average for 3 last days of injections.

Locomotor and anxiety tests

Locomotor activity was measured as distance moved (cm) in one hour in the locomotor cells (MED-OFAS-RSU and Activity Monitor, Med Associates Inc). Anxiety-like behaviour before treatment was measured in the open field test: 10 min in 1 m^2 PVC arena 60cm (automated tracking software Cleversys Topscan). After the treatment 10 min Elevated Plus Maze test was performed in the custom-made Perspex consisting of two open arms (10cm x 44cm) and two enclosed arms (10cm x 44cm x 10cm) extending from a central platform (12 x12 cm) (automated tracking software Cleversys Topscan).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software Inc., San Diego, California). The time course of weight gain was analysed using a mixed effects model (time and weight gain as factors). Lever presses S+/S- and rewards before NAC

administration in DIO and DR groups were analysed using two-tailed Mann-Whitney test. After treatment lever pressing in S+ and S- periods and rewards earned were analysed by two-way repeated measures analysis of variance (ANOVA) with time and group as factors. Bonferroni's post-hoc analyses or t-tests were performed where indicated. PR breakpoint was analysed by Kruskal-Wallis test. A P-value <0.05 was considered to indicate statistical significance. Probability of Gaussian distribution was estimated to decide use on the use of parametric or non-parametric tests. 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).

RESULTS

Establishing Diet-Induced obese and Diet Resistant subpopulations

By week 2 of the HFHS homecage diet period DIO rats had gained significantly more body weight compared to DR. 2-way RM ANOVA revealed a main effect of time on diet ($F_{22, 814}$=419.21, P<0.0001), a main effect of group ($F_{1, 37}$=5.549, P=0.024) and a significant group x time on diet interaction ($F_{35, 1048}$=11.73, P<0.0001) Bonferroni's multiple comparisons revealed day 18 onwards DIO weighed significantly more than DR (P<0.01, Fig. 1B). At the end of the 8-week HFHS diet, DIO rats showed 45% more weight gain than DR rats (main effect of group, $F_{1, 33}$=29.45, P<0.0001).

Diet-induced obesity confers escalation of intake and loss of control over consumption of palatable food

After 8 weeks of *ad libitum* homecage access to HFHS diet, animals were placed on standard chow and HFHS access was restricted to 45 minutes daily during operant-self administration sessions (see Fig. 1A for protocol timeline). Initially DIO and DR groups responded similarly on FR1 and FR3 schedules, and no difference in learning of the instrumental task was observed (see Fig 4A for the timeline of lever presses). However, by day 10, when the response requirement increased to FR5, DIO rats escalated to pressing over 50% more than DR rats during S+ periods (Fig. 2A, active lever presses S+, 2-tailed Mann-Whitney, DR *Mdn*=245, DIO *Mdn*=466, $U_{18,17}$=83.50, P=0.021). This resulted in DIO rats consuming >45% more palatable food rewards when available during S+ periods, constituting binge-like consumption in this limited time period in comparison with their DR counterparts (rewards earned in 3 days before injections, DR *Mdn*=46, DIO *Mdn*=85, 2-tailed Mann-Whitney, $U_{18,19}$=83.50, P=0.023, data not shown).

Loss of control over behaviour to the point where it becomes compulsive is a hallmark feature of addiction. Compulsivity consists of two theoretically dissociable components – the persistence or perseveration of a behaviour and the lack of relationship that behaviour has to an overall goal (Dalley *et al.*, 2011). The persistence of lever-pressing during periods where the cue is not associated with reward delivery thus represents one aspect of compulsive-like behaviour and therefore models this feature of addiction-like behaviour in rodents (Bock *et al.*, 2013). As expected, DIO rats pressed significantly more than did DR animals during these periods of signalled reward unavailability (Fig. 2B; DR *Mdn*=3.5, DIO *Mdn*=6, 2-tailed Mann-Whitney, $U_{18,19}$=83.50, P=0.020). Accordingly, the addiction score was higher in DIO compare to DR animals (Fig 2C).

Weight gain on high-fat high-sugar diet predicts addictive-like behaviour towards food

Next, we tested the relationship between propensity for weight gain on HFHS diet and addictive-like behaviour toward palatable food. To that end we conducted correlational analyses on weight gain on the HFHS diet period and rats' performance on each of the 3 behaviours described above (baseline behaviours before NAC treatment). Each behaviour was positively correlated with weight gain: lever presses in FR5 reward-available period, S+ (Fig 2D, $F_{1,33}$=4.572, P=0.040), lever presses during FR5 reward-unavailable period, S- (Fig 2E, $F_{1,33}$=10.60, P=0.003), pellets consumed in FR5 reward available (S+) periods (data not shown, $F_{1,33}$=4.308, P=0.046) and addiction score 1 (Fig 2F, $F_{1,33}$=9.193, P=0.005). There were no significant differences in learning the operant task as measured by discrimination between inactive and active levers (2-tailed unpaired t-test, t_{33}=2.6, P=0.999, data not shown).

N-acetylcysteine prevents escalation of responding for HFHS in diet-induced obese rats

After establishing baseline behaviour of DIO and DR animals these groups were split into NAC (100mg/kg, i.p.) and vehicle treatment groups. For 14 days rats received daily systemic administration of NAC/VEH 2h prior to operant self-administration sessions. NAC treatment in the DR group did not change performance of any of the measured parameters as compared to baseline. By contrast, NAC treatment prevented escalation of lever pressing in DIO rats. Active presses in S+ period: DIO VEH group showed a significant increase in active lever presses with time; this escalation was not present in DIO rats administered NAC (Fig. 3A, P>0.05). 2-way RM ANOVA revealed a main effect of group ($F_{3,31}$=5.831, P=0.003), a main effect of treatment ($F_{1,31}$=8.104, P=0.008), and a significant group x treatment interaction

($F_{3,31}$=4.418, P=0.011). Bonferroni's multiple comparisons test revealed the only group with significantly higher responding at the end of treatment were the vehicle treated DIO animals (P<0.0007). This effect was mirrored in the lack of escalation the number of HFHS food pellets administered over the operant self-administration period (Fig. 3B). 2-way RM ANOVA revealed a main effect of group ($F_{3,31}$=6.281, P=0.002), a main effect of treatment, ($F_{1,31}$=5.513, P=0.025), and a significant group x treatment interaction ($F_{3,31}$=4.074, P=0.015). Bonferroni's multiple comparisons test revealed number of pellets administered significantly higher in vehicle-treated DIO rats (P=0.002) but not NAC treated DIO rats nor any DR group (P>0.05).

N-acetylcysteine improves loss of control over food-seeking behaviour in DIO and DR rats

Persistent lever-pressing during periods which signal reward unavailability is suggestive of compulsive-like food-seeking. Responding when food was unavailable was significantly higher in DIO animals than in DR animals (Fig. 2B). We observed that NAC significantly reduced loss of control over food seeking in both DIO and DR rats (Fig. 3C). 2-way RM ANOVA revealed a main effect of group ($F_{3,31}$=3.161, P=0.038), a main effect of treatment ($F_{3,31}$=6.380, P=0.0169) but no significant group x treatment interaction ($F_{3,31}$=1.207, P=0.324). T-tests performed within each of the 4 groups separately revealed a decrease in lever-pressing in DIO rats when comparing responding before versus after NAC treatment (P=0.0004), and a weak effect of NAC in DR rats (P=0.072).

However, the addiction score calculated after treatment showed that only DIO animals injected with NAC showed an improvement after 2 weeks of treatment, not DR (Fig. 3D). 2-way ANOVA revealed a main effect of group ($F_{1,31}$=18.58, P=0.0002), a trend towards a main effect of treatment ($F_{1,31}$= 3.999, P=0.054), and a significant group x treatment interaction ($F_{1,31}$=4.643, P=0.039). Bonferroni's multiple comparisons tests revealed addiction score was significantly decreased in DIO treated animals only (P=0.0126).

N-acetylcysteine may alter motivation for highly palatable food in DIO rats

A trend towards a significant difference in motivation to obtain a palatable food reward was observed between DIO and DR rats. Thus, a trend towards a significant difference was observed between groups with respect to breakpoint and total number of lever presses on the progressive ratio task (Fig.3E). 2-way ANOVA revealed a trend towards an effect of group

($F_{1,31}$=3.694, P=0.064) and an effect of treatment ($F_{1,31}$=4.121, P=0.051) but no interaction (group x treatment, $F_{1,31}$=0.090, P=0.767).

N-acetylcysteine does not change re-acquisition of self-administration, locomotion, or anxiety-like behaviour

Active lever presses and number of rewards earned did not change in any group in subsequent FR5 responding after the progressive ratio session, suggesting NAC had no impact on re-acquisition of self-administration (Fig.4 A). Locomotor activity and anxiety-like behaviour as tested in the open field and elevated plus maze tests, respectively, before or after diet was also not significantly different between groups (Fig.4 B, C, D).

Discussion

The primary goal of this study was to explore the potential of NAC, a well characterised and safe compound, for the treatment of addictive-like eating in a rat model. We found that sub-chronic administration of NAC attenuated escalation of intake as well as compulsive-like food-seeking in DIO rats, two hallmark features of addiction. These results are consistent with previous findings showing that NAC decreases other forms of addictive behaviour such as drug-seeking and relapse in substance use disorder and supports the exploration of NAC as an adjunct treatment for pathological forms of overeating such as binge eating and loss-of-control eating.

Diet-induced obese animals show addiction-like behaviour towards HFHS food

We extended earlier findings that DIO rats overeat and exhibit loss of control towards HFHS food (Brown *et al.*, 2017). Our results support previous findings that rats prone to obesity exposed to HFHS diet develop a loss of control over food-seeking behaviour. Furthermore, we showed a positive correlation between addictive-like behaviour and weight gain during eight weeks of HFHS diet, suggesting that susceptibility to overeating HFHS and thus diet-induced obesity may predict loss of control overeating behaviour.

NAC reduces addiction-like behaviour towards HFHS food

Next, we tested NAC's potential to reduce aberrant food-seeking behaviour in DIO and DR rats. Binge-like eating measured by palatable pellets consumption in 1h was reduced by 14d treatment with systemically administered NAC, consistent with previous findings which showed both systemic and central administration of NAC decreases binge-like eating of

HFHS food in rats without a sedating effect (Hurley *et al.*, 2016). The latter study employed a limited access binge model of 30min exposure of HFHS each day. We extend this by determining that NAC reduces addiction-like behaviour towards HFHS food. More specifically, NAC prevents escalation of palatable food intake in an operant paradigm and compulsive-like food seeking, measured as persistent lever-pressing despite reward unavailability (perseverative behaviour being one aspect of compulsivity). The latter finding is interesting, as NAC was able to reduce compulsive-like food seeking in both DIO rats and DR rats (though admittedly in the latter the effect was weaker ($p=0.072$ revealed by post hoc t-test), suggesting that NAC has the ability to reduce this type of behaviour in all individuals, irrespective of the severity observed. Of note, we cannot rule out that NAC is having a pro-cognitive effect, thereby restoring any potential deficit in learning discrimination for the S-context in DIO rats. This is unlikely however, given DIO and DR rats acquired the instrumental learning task in a similar fashion. Collectively, these data suggest that NAC could be beneficial for the treatment of compulsive forms of eating behaviour, such as binge eating, loss-of-control eating and addictive-like eating. Though the persistence of the effect observed in our study is yet to be determined, one study that administered NAC for 7d and observed an effect on cocaine-induced changes and behaviour 3 weeks after (Madayag *et al.*, 2007). Further studies could evaluate the potential of NAC as an adjunct treatment to assist in managing compulsive eating in conjunction with specific weight loss therapies.

The effect of N-acetylcysteine on compulsive-like food-seeking may be independent of any change in motivation for high-fat high-sugar food

NAC apparently modifies specific features of 'addictive behaviour' towards food however only a trend was observed towards affecting motivation as measured by progressive ratio. In line with this, Ducret *et al.* (2016) observed that NAC increases sensitivity to punishment in long (6h) vs short (1h) access model of compulsive cocaine uses despite having no impact on the motivation to self-administer cocaine (Ducret *et al.*, 2016). This speaks to a potential differential role for NAC in reducing compulsive-like behaviour towards reinforcers versus affecting their motivational properties, though as a trend to an effect was observed in our study this is not conclusive. Our data concurs with Madayag and colleagues who showed that NAC prevented escalation of responding for cocaine (Madayag *et al.*, 2007).

Possible mechanism by which N-acetylcysteine is acting to reduce addiction-like behaviour towards HFHS food

Although NAC's exact mechanisms of actions are unknown, we can speculate that it may act via a number of different ways to reduce addiction-like behaviour towards HFHS food. On the one hand, NAC provides cysteine, the rate-limiting substrate in the synthesis of glutathione, which plays a major role in the reduction of potentially toxic oxidizing reagents such as reactive oxygen species (Rae & Williams, 2017). Accordingly, studies have demonstrated that NAC attenuates abnormal pro-inflammatory responses, limits oxidative damage, as well as reduces obesity-related complications (for reviews see Deepmala *et al.*, 2015; Dludla *et al.*, 2019; Faghfouri *et al.*, 2020). Moreover, there is an established literature around oxidative stress and addiction (for review see Womersley *et al.*, 2019). Furthermore, NAC has been shown to have beneficial effects for metabolic disorders (Dludla *et al.*, 2019). Thereby, it is plausible that NAC is acting via an antioxidant mechanism to reduce food-seeking behaviour. Conversely, NAC is also converted to cysteine, a substrate for the glial cell glutamate/cysteine antiporter, meaning that NAC can act as a glutamate modulator. Cysteine uptake is followed by release of glutamate from glia into the extrasynaptic space where it seems to trigger the reduction of synaptic glutamate release (Moran *et al.*, 2005). Impairment of glutamate homeostasis has been proposed as a possible mechanism underlying the transition to addiction to drugs (for review see Reissner & Kalivas, 2010; Scofield *et al.*, 2016); preclinical studies of alcohol and cocaine abuse show NAC reduces drug relapse by restoring glutamate homeostasis (Reissner *et al.*, 2015; Lebourgeois *et al.*, 2019; Garcia-Keller *et al.*, 2020; Quintanilla *et al.*, 2020). For example, NAC ameliorates cue-induced reinstatement of cocaine-seeking via restoration of glial glutamate transporter-1 levels (Reissner *et al.*, 2015). However, another study shows that both chronic alcohol intake and relapse-like alcohol drinking are inhibited by NAC via actions on the cystine/glutamate antiporter and the presynaptic metabotropic mGlu2/3 receptor (Quintanilla *et al.*, 2020). Lastly, a recent study by Lau and colleagues shows diet-induced obesity disrupts glutamatergic synaptic plasticity within orbitofrontal cortex and this is restored by NAC (Lau *et al.*, 2020). Thus, it is possible, in the case of HFHS food at least, that NAC is working via one or several of these mechanisms which are yet to be confirmed. Further studies to confirm the exact mechanism by which NAC reduces compulsive-like food seeking are therefore warranted.

N-acetylcysteine has potential as an adjunctive pharmacotherapy to traditional weight loss treatments

In summary, the present study indicates that sub-chronic treatment with NAC can reduce addiction-like behaviour towards HFHS. Our data suggest that NAC is a promising adjunctive agent for the treatment of pathological eating behaviours, though further studies are required; it should be noted that the literature is not always in agreement regarding its efficacy for the treatment of other compulsive behaviours (for review see Duailibi *et al.*, 2017). This study also strengthens the view that the deleterious effects of diet-induced obesity and substance abuse can induce similar aberrant behaviour and that harnessing knowledge from addiction neuroscience to obesity research can be beneficial for both research fields.

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Competing Interests

The authors have no conflicts of interest to declare.

Author contributions

RMB and AJL designed the experiment. DB collected body weight and food intake data, as well as conducted operant self-administration experiments with the assistance of RMB and CP, under the supervision of RMB and AJL. DS analysed, interpreted data and wrote the first draft of the manuscript. PS assisted in the interpretation of the work. All authors critically reviewed the manuscript. All authors have approved the manuscript for submission.

Data Accessibility Statement

Data will be available upon request by correspondence with the corresponding author.

Abbreviations

DIO, diet-induced obesity; DR, diet-resistant; FR, fixed ratio; HFHS, high-fat high-sugar; NAC, N-acetylcysteine; PR, progressive ratio.

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Figure Captions

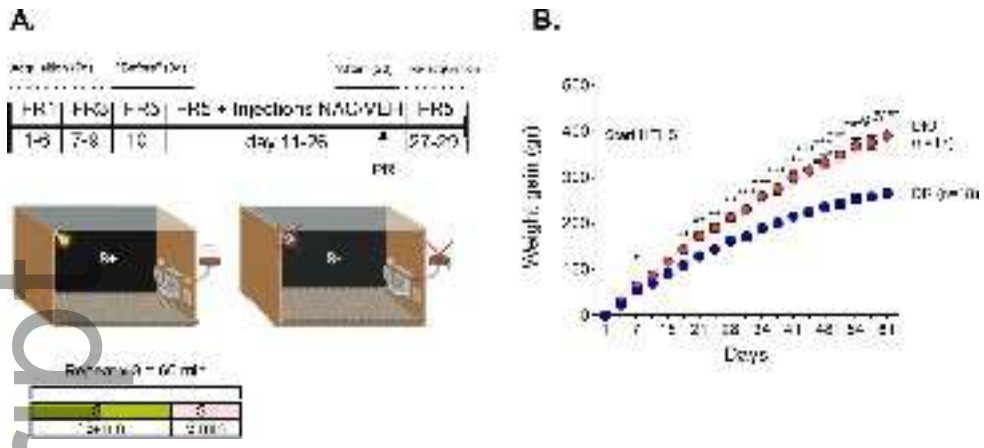
Figure 1. Experimental protocol and body weight of diet-induced obese (DIO) and diet-resistant (DR) rats over the free access palatable diet period and experimental protocol. A) Schematic representation of the operant box and timeline of the protocol. S+ period: reward available, house light on, discrete light cue paired with reward delivery. S-: reward is unavailable, house light off, flashing alternate cue light. Injections i.p. N-acetylcysteine (NAC; 100mg/kg, i.p.) or vehicle (VEH). B) Cumulative weight gain in grams over the free access diet period (2-way ANOVA; ***P*<0.01, ****P*<0.001, *****P*<0.0001) data = mean ±SEM.

Figure 2. Before treatment diet-induced obese (DIO) rats show increased addiction-like behaviour compared to diet resistant (DR) rats. A, B) Active lever presses in S+ (reward available) and S- (reward unavailable) periods, average for 3 days at 1 day before, and 2 days at the start of N-acetylcysteine (NAC) treatment, **P*<0.05, ***P*<0.01, Mann-Whitney rank sum test). Data = mean ±SEM. C) Addiction score 1: sum of scores for binge-like eating, loss of control and increased amount of effort on progressive ratio schedule. Data = mean ±SEM (***P*<0.01, unpaired t-test). D, E, F) Correlations between active lever presses in S+ and S- periods, addiction score, and weight gain on the free access high-fat high-sugar diet. Dashed line represents 95% confidence, simple linear regression, p values stated on figure (Spearman correlation test).

Figure 3. N-acetylcysteine (NAC) reduces addiction-like behaviour in diet-induced obese (DIO) but not diet resistant (DR) rats. A) Active lever presses in S+ period, average of 3 days ±SEM before versus after treatment with NAC or vehicle (VEH). DIO VEH group showed a significant increase in the number of active lever presses over time whereas this escalation was absent in DIO administered NAC (***P*=0.005, 2-way RM ANOVA with Bonferroni comparisons). B) Rewards earned before and after NAC/VEH administration, average of 3 days ±SEM. At the end of sub-chronic treatment, the DIO VEH group showed a significant increase in the number of rewards earned (***P*=0.002), yet this escalation was not present in NAC-treated DIO rats (2-way RM ANOVA with Bonferroni). (C) Active lever presses in period of signalled reward unavailability, average of 3 days ±SEM. DIO treated with NAC, but not vehicle showed a significant reduction in compulsive-like food seeking behaviour at the end of treatment (2-way RM ANOVA, paired t-tests). D) Addiction score after treatment:

sum of scores for binge like-eating, loss of control and increased amount of work, mean ±SEM (2-way ANOVA, Bonferroni's comparisons). E) Breakpoints on a progressive ratio schedule. Motivation towards rewards pellets was not significantly different between groups (2-way ANOVA).

Figure 4. N-acetylcysteine (NAC) does not change re-acquisition or locomotor or anxiety-like behaviour A) Active lever presses in S+ period, average of 3 days ±SEM. DIO vehicle (VEH) group showed a significant increase in the number of active lever presses over time whereas this escalation was absent in DIO administered NAC (2-way RM ANOVA). B) Open field test, before the treatment, time spent in the centre and in the corners (sec; 2-way ANOVA). C) Locomotor cells, ambulatory distance (m/h; 1-way ANOVA). D) Elevated Plus maze, after the treatment, time spent in the open and closed arms (sec; 2-way ANOVA).



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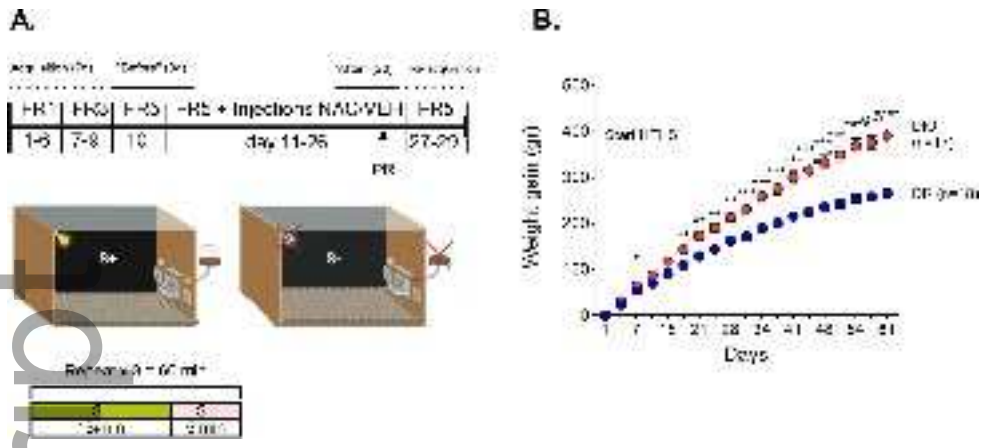

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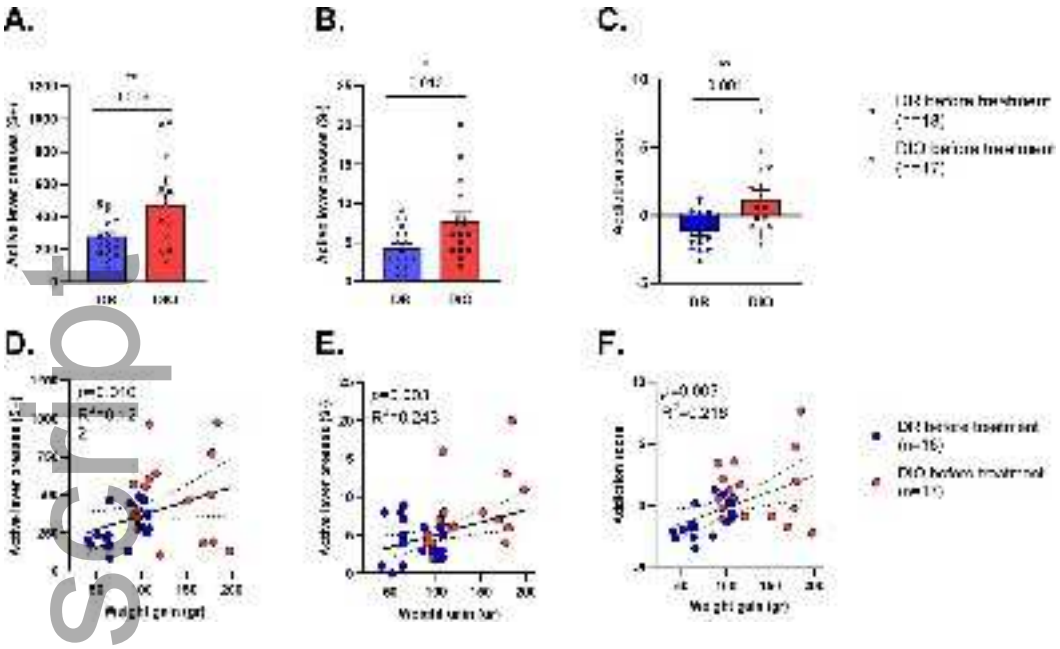

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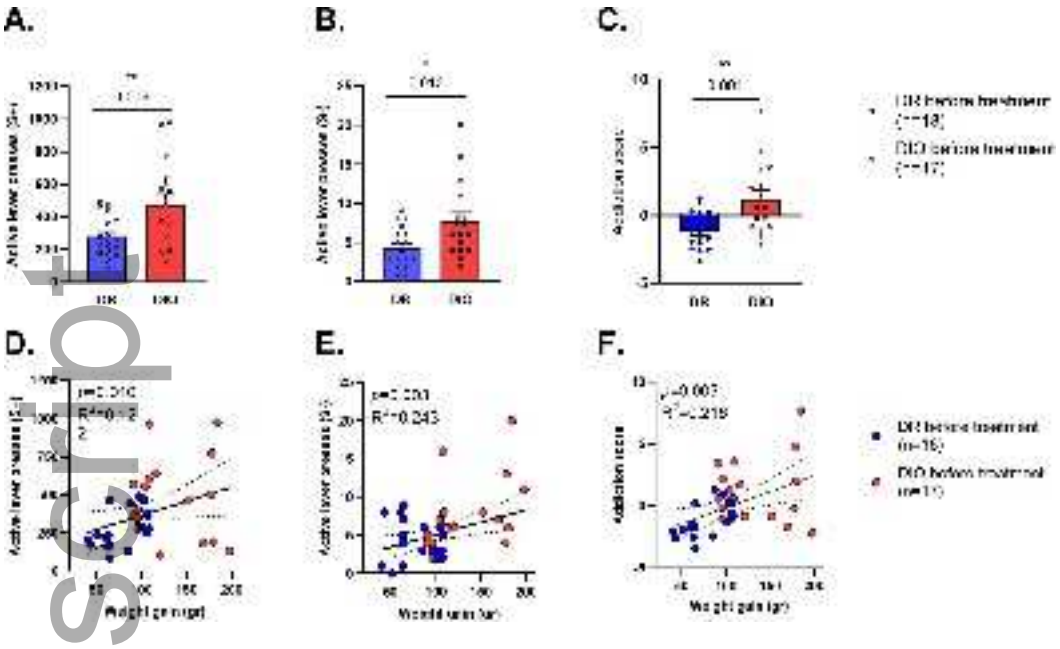

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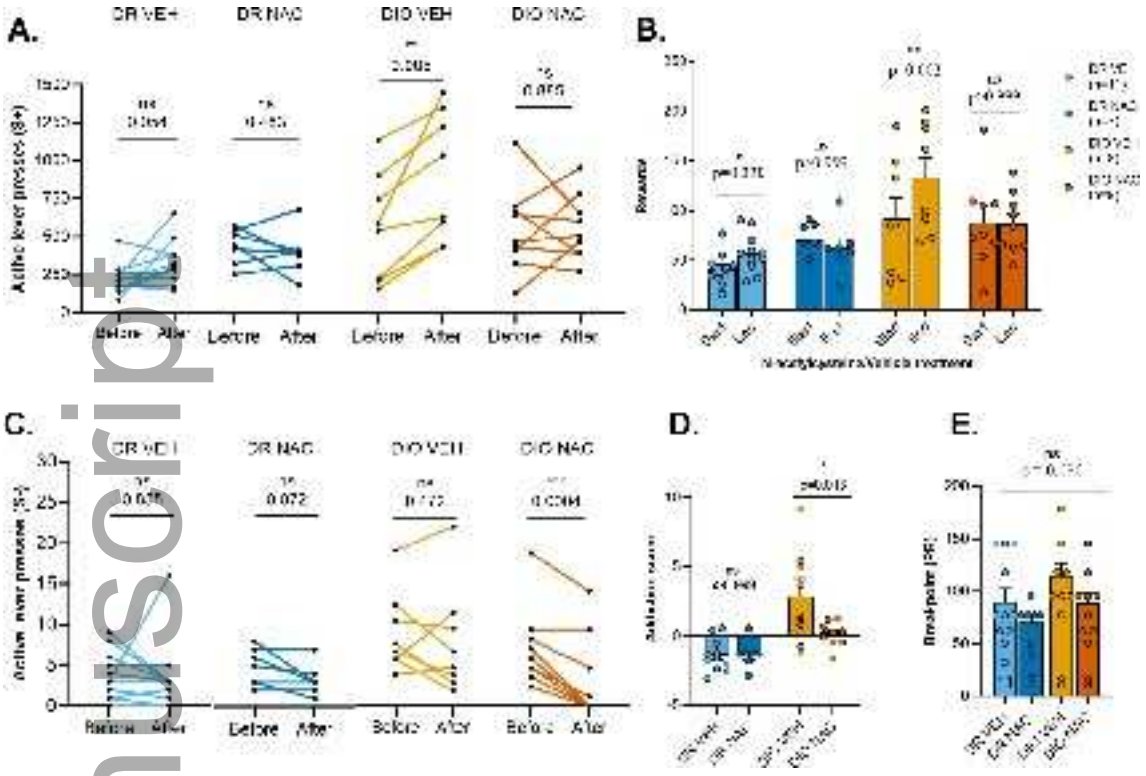

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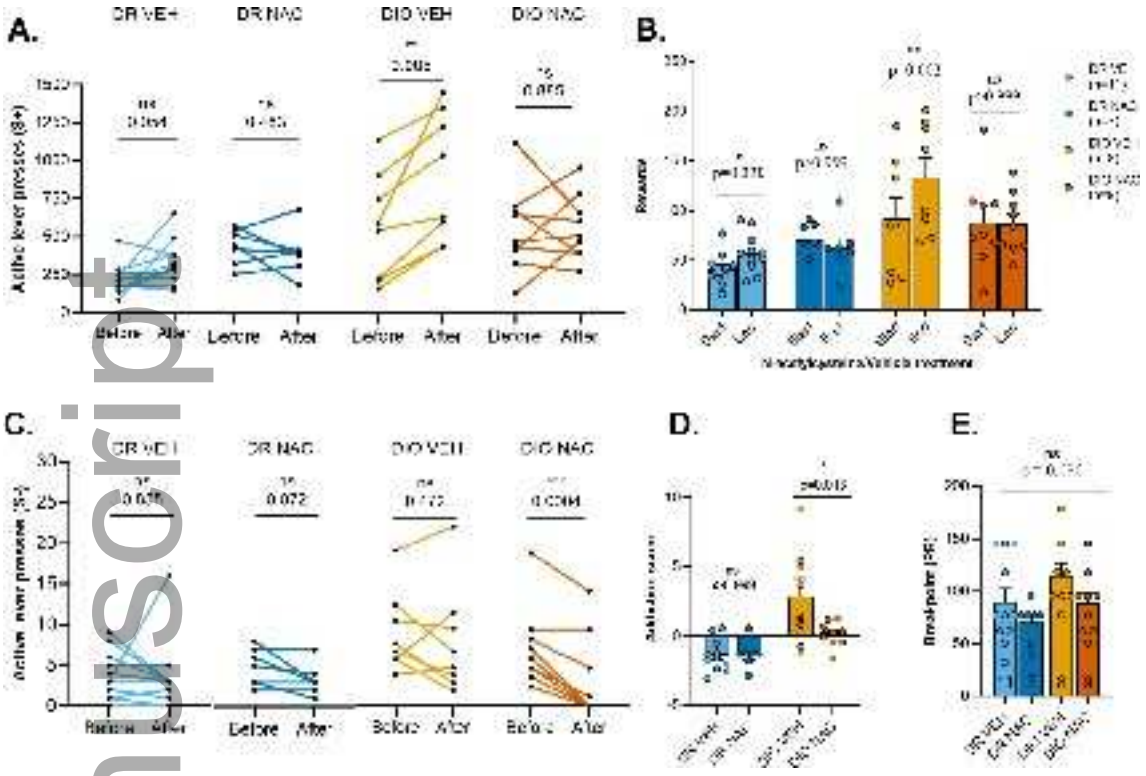

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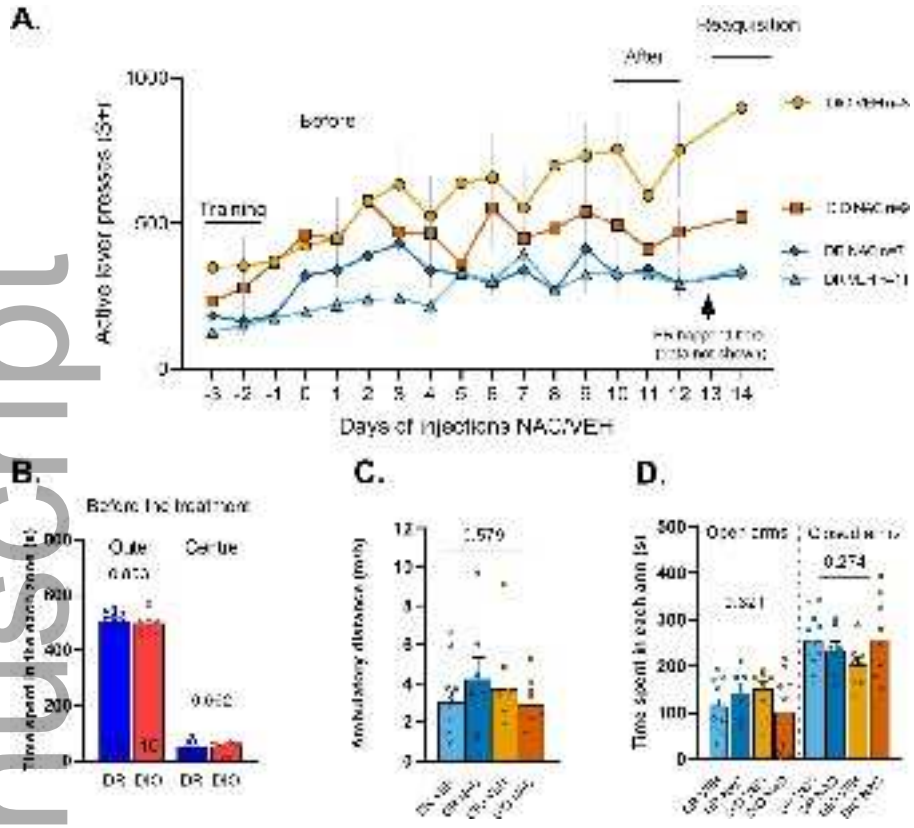

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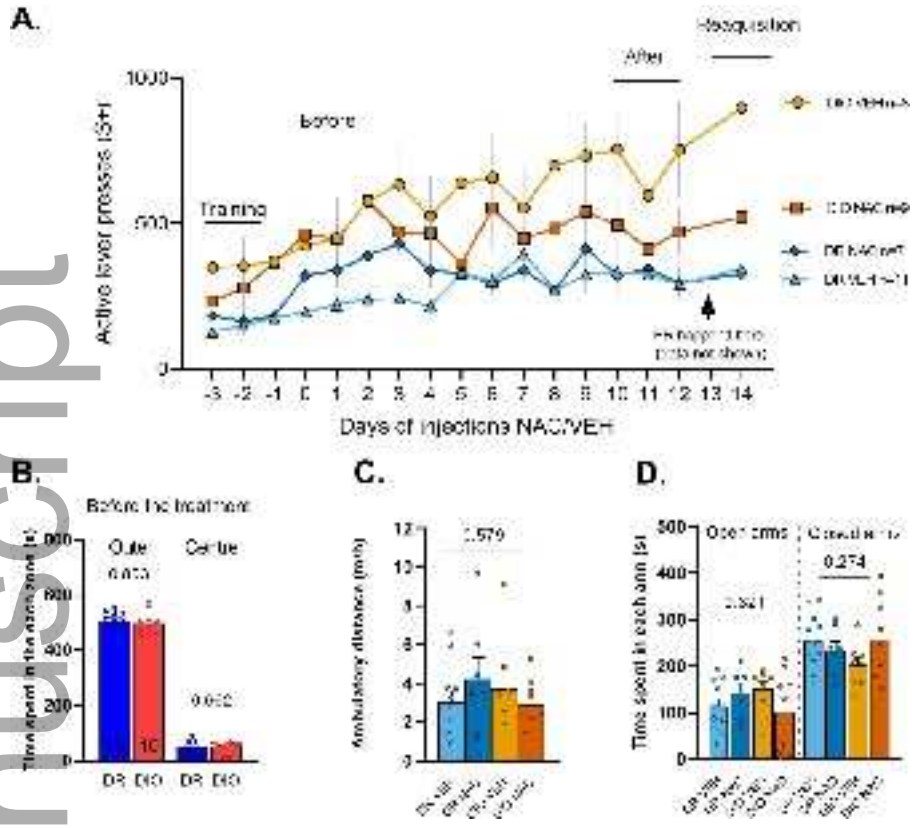

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