1-1-2016

# *N*-acetylcysteine Decreases Binge Eating in a Rodent Model

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Accepted version. *International Journal of Obesity*, Vol. 40, No. 7 (2016): 1183-1186. DOI. 

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*International journal of Obesity*, Vol. 40, No. 7 (July 2016): pg. 1183-1186. DOI. 

**Abstract:** Binge-eating behavior involves rapid consumption of highly palatable foods leading to increased weight gain. Feeding in binge disorders resembles other compulsive behaviors, many of which are responsive to *N*-acetylcysteine (NAC), which is a cysteine prodrug often used to promote non-vesicular glutamate release by a cystine–glutamate antiporter. To examine the potential for NAC to alter a form of compulsive eating, we examined the impact of NAC on binge eating in a rodent model. Specifically, we monitored consumption of standard chow and a high-fat, high carbohydrate western diet (WD) in a rodent limited-access binge paradigm. Before each session, rats received either a systemic or intraventricular injection of NAC. Both systemic and central administration of NAC resulted in significant reductions of binge eating the WD without decreasing standard chow consumption. The reduction in WD was not attributable to general malaise as NAC did not produce condition taste aversion. These results are consistent with the clinical evidence of NAC to reduce or reverse compulsive behaviors, such as, drug addiction, skin picking and hair pulling.

# Introduction

Binge eating is one of the most prevalent eating disorders in the United States, and contributes to the obesity epidemic that is currently endangering the health of ~30% of all Americans.[1, 2] Feeding in binge disorders is characterized by rapid consumption of large amounts of highly palatable food that occur in very short periods of time.[3] These maladaptive alterations in feeding behavior culminate in a destructive state that often parallels other compulsive disorders, such as drug addiction.[4, 5] In many instances, binge eating is reported to occur in the absence of hunger, suggesting that this compulsive feeding state could arise from excessive hedonic drive rather than unmet homeostatic needs.[6, 7]

Many compulsive disorders involve abnormal glutamatergic signaling.[8] Likely because of this, the cysteine prodrug *N*-acetylcysteine (NAC) has been shown to reduce many forms of compulsive behaviors ranging from addiction to trichotillomania.[9, 10] The mechanism of action of NAC typically involves increasing the activity of the cystine–glutamate antiporter system $xc^-$,[11] which has been shown to be a key component of glutamate homeostasis by maintaining extrasynaptic glutamate tone via non-vesicular release and, in turn, affecting receptor function (i.e., synaptic signaling) in regions implicated in hedonic motivated eating.[12, 13] System $xc^-$ has been shown to be an effective target to inhibit cocaine-induced behaviors and reinstatement in preclinical studies.[14] Because of the

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compulsive aspect of binge eating, an overeating behavior analogous to drug abuse, we examine the potential for systemic or intraventricular (ICV) administration of NAC to alter binge eating. In the current study, we utilized a frequently employed preclinical model of binge eating in which rodents are permitted daily limited access to a highly palatable food as an adjunct to their *ad libitum* standard chow (SC) and recapitulates several characteristics of human binge-eating disorder.[15]

# Methods

## *Animals*

Male Sprague–Dawley rats (225–250 g; 53–58-days-old; Harlan; Indianapolis, IN, USA) were housed individually under a 12 h light/dark cycle (2PM: Lights OFF; 2AM: Lights ON) with *ad libitum* access to a Harlan standard diet (8604 formula) for the duration of all experiments. Intake was monitored daily via a BioDAQ Food Intake Monitor (Research Diets, New Brunswick, NJ, USA) or by pre-weighing food bins before and after experimental sessions. Body weights were also recorded daily. All procedures using animals were approved by the Marquette University Institutional Animal Care and Use Committee.

## *Cannulation surgery and microinjections*

Animals were anesthetized with a ketamine/xylazine/acepromazine (77:1.5:1.5 $mg^{-1}$ $ml^{-1}$ $kg^{-1}$; intraperitoneal (i.p.)) cocktail and placed in a stereotaxic apparatus. A 26 gauge unilateral guide cannula (Plastics One; Roanoke, VA, USA) was placed into a lateral ventricle and secured to the skull via an acrylic resin. The stereotaxic coordinates were anterior/posterior, −0.8 mm from bregma; medial/lateral, ±1.5 mm from midline; dorsal/ventral, −3.0 mm from the surface of the skull, whereas the injector extended 0.5 mm further beyond the tip of the guide cannula. Stereotaxic coordinates were based on *The Rat Brain in Stereotaxic Coordinates*, 6th Edition.[16]

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## *Limited access binge-eating model*

Animals with *ad libitum* access to SC (Harlan 8604 formula; SC; 32% of total kilocalorie (kcal) protein, 54% of total kcal carbohydrate, 14% of total kcal fat; 3.0 kcal $g^{-1}$) were additionally allowed daily limited access (30 min $day^{-1}$) to a highly palatable western diet (WD; Research Diets; New Brunswick, NJ, USA; 17% of total kcal protein, 43% of total kcal carbohydrate, 41% of total kcal fat; 4.7 kcal $g^{-1}$) shortly after lights out (45 min). Chronic systemic (i.p.; 90 mg $kg^{-1}$) and intermittent central (ICV; 10 µg/ 5µl) NAC was administered 45–60 min before gaining limited access to WD based on previous work examining the preclinical therapeutic potential of systemic and centrally administered NAC in attenuating cocaine-relapse behavior in rodents.[17]

## *Chronic systemic NAC*

Animals ($n$=12–17/treatment) received chronic i.p. injections of either vehicle (saline) or NAC (90 mg $kg^{-1}$) 45–60 min before gaining limited access (30 min $day^{-1}$) to the pre-weighed bin of WD for 14 days.

## *Central NAC*

After a 1 week period of recovery from guide cannula surgery, animals ($n$=6–8/dose) were placed on the limited-access binge paradigm for 7 days during which NAC was injected into the cerebral ventricles on alternating days. On days 1, 3, 5 and 7 animals received central infusions of NAC (10 µg/5 µl) whereas control animals received an equal volume of vehicle (saline); microinjections were preformed 1 h before WD access. Animals were given daily access (30 min $day^{-1}$) to WD to avoid associating bin access with any protocol associated with microinjections.

## *Conditioned taste aversion*

A separate group of animals were habituated to restricted water access (1 h $day^{-1}$) and systemic injections (i.p.) of saline 45–60 min before water access for 1 week. Subsequently, animals were separated

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into three treatment groups ($n$=5/group): saline, NAC (90 mg kg$^{-1}$) or LiCl (2%; i.p.), and offered two bottles each containing a 0.15% saccharin solution for 24 h then returned to their previous restricted water conditions. Two days later, animals were provided a two-bottle choice test with one bottle containing 0.15% saccharin solution and the other containing water for 1 h.

### *Statistics*

Data are presented as means±standard errors of the mean, and were analyzed statistically (Sigma Plot 11; Systat Software Inc.; San Jose, CA, USA) by analysis of variance (with repeated measures when appropriate) or student's *t*-test. Fischer LSD analysis was used for all *post-hoc* group comparisons. *P* values <0.05 were considered statistically significant.

## Results

Daily systemic injections of NAC (90 mg kg$^{-1}$; i.p.) or vehicle (saline) injections were administered 45 min before limited access (30 min day$^{-1}$) to a highly palatable WD as an adjunct to *ad libitum* SC for 14 days. Animals treated with NAC ate significantly less WD (Figure 1a; treatment F(1,405)= 5.478; $P$<0.03), and significantly more SC (Figure 1b; treatment F(1,405)=4.893; $P$<0.04) over the 2 week study. As expected, NAC-treated animals displayed a significantly larger percentage of daily kcal ingested from SC and a concomitant reduction in WD intake than vehicle-treated rats by the 14th day of experimentation (Figure 1c; left; $P$<0.05). The compensatory increase in SC consumption paired with the attenuation in WD consumption resulted in no significant change in total calories consumed or body weight gain (Figures 1d and e). In a separate study, NAC administered centrally every 48 h (before WD access) at the onset of dark via ICV injections displayed a similar suppression in the escalation of WD consumption over a 7 day period (Figure 1f; treatment × time, F(6, 97)= 2.281; $P$<0.05).

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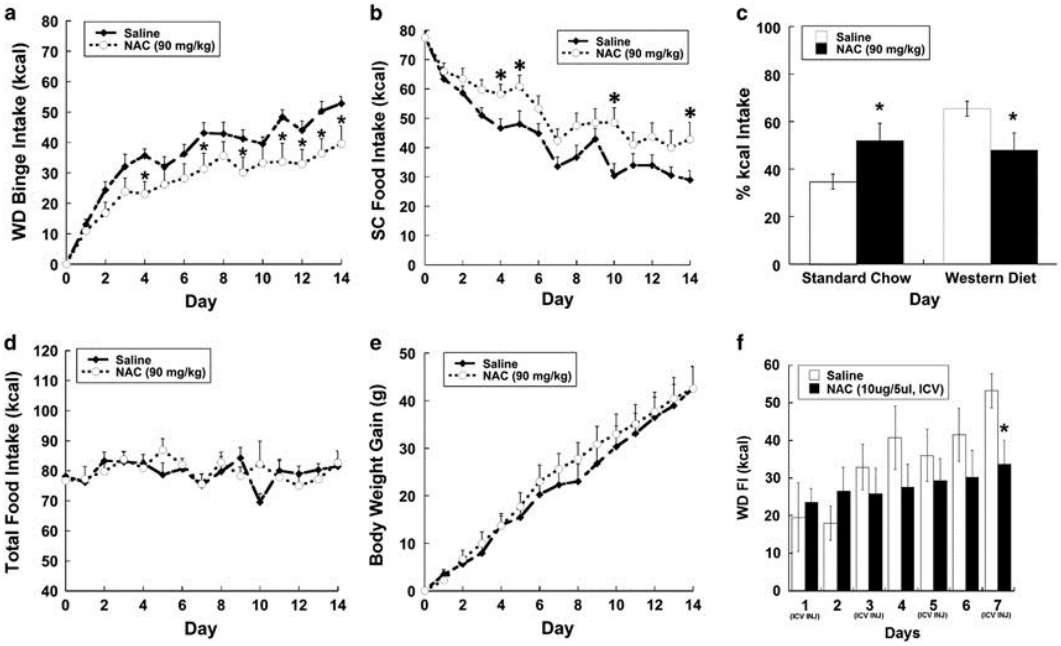
## Figure 1.




System (90 mg $kg^{-1}$; i.p.) and central (10 μg/5μl) NAC alters binge behavior produced by daily limited access to a palatable food. (**a**) NAC-treated animals ate significantly less WD (30 min $day^{-1}$) compared to saline-injected control animals. (**b**) Subsequently, NAC-treated animals ate significantly more SC over the duration of the study. (**c**) Percent kcal intake of SC on the 14th day is significantly higher for the NAC-treated group, whereas WD consumption was significantly decreased compared to the control group. (**d, e**) There is no significant difference in cumulative food intake or body weight gained between the two experimental groups. (**f**) In a separate study, animals received infusions (NAC or saline; ICV) 30 min before accessing WD, central administration of NAC significantly suppressed WD consumption over the 7 day study. Data expressed as mean±s.e.m. **P*<0.05 compared to control group.

The hypophagic effects of NAC appear to be specific to palatable WD consumption as it does not significantly alter the feeding behavior of animals maintained on *ad ibitum* SC only (Figure 2a). Moreover, there were no effects on SC consumption over the first 6 or 24 h after administration of NAC (data not shown). In addition, a conditioned-taste aversion revealed that suppression of WD by NAC was not due to malaise (Figure 2b).

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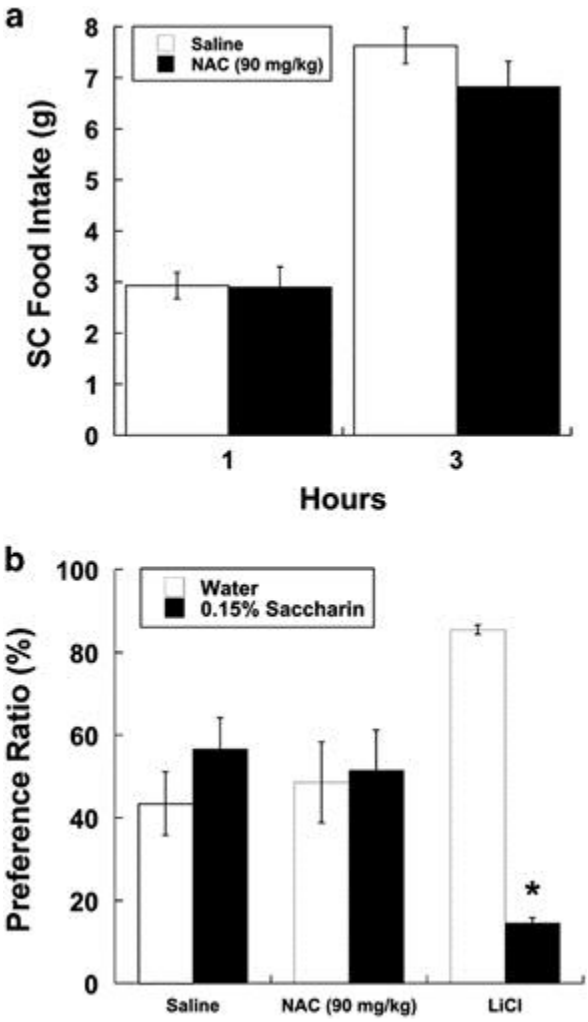
## Figure 2.




Systemic administration of NAC (90 mg $kg^{-1}$; i.p.) does not alter *ad libitum* SC consumption or produce any conditioned-taste aversion. (**a**) Animals maintained on *ad libitum* SC and not subjected to a limited-access binge paradigm show no changes in feeding behavior measured 1 and 3 h post i.p. injection. (**b**) Saccharin preference ratio (%) of control (saline), NAC and LiCl (2% body weight)-treated animals produced only a significant suppression of saccharin consumption in the LiCl-treated group. Data expressed as mean±s.e.m. **P*<0.05 compared to controls.

# Discussion

Chronic systemic NAC significantly attenuates binge eating of a WD over a 14 day study (Figure 1). Although chronic NAC administration did not alter total caloric intake or weight gain (Figures 1d and e), systemic or central administration was sufficient to reduce

intake of highly caloric and palatable food without inducing malaise (Figures 2b and c). This selectivity of NAC to regulate only WD consumption is quite notable as it suggests that system $xc^-$ activation does not impact overall caloric homeostasis but may lessen the hedonic drive for food. Future studies extending chronic NAC administration beyond 2 weeks may be necessary to demonstrate effects on long-term calorie consumption and body weight.

NAC, a cysteine prodrug, has been demonstrated to drive the activity of the cystine–glutamate antiporter system $xc^-$, which promotes non-vesicular glutamate release and consequently impact synaptic glutamate signaling, which is critical to the development and maintenance of a wide variety of compulsive behaviors.[18, 19] In the case of long-term use of drugs of abuse, there is a reduction in system $xc^-$ activity in the nucleus accumbens, which is thought to contribute to the pathological glutamate signaling that in turn contributes to the addiction.[20] Subsequently, both preclinical and clinical studies of NAC treatment have shown inhibition of cocaine-induced behaviors in animals and reduction in craving and/or use of cocaine and tobacco in humans.[9, 20, 21] The effectiveness of NAC to reduce binge eating of a highly caloric palatable food is consistent with other studies demonstrating the attenuation of compulsive behaviors, such as, drug taking, hair pulling and skin picking, and suggests that system $xc^-$ is a potential therapeutic target for the treatment of compulsive feeding disorders.[9, 10, 22] Pursuing effective treatments for compulsive eating disorders will require further understanding of the neural mechanisms that underlie hedonic drive.[7]

### Conflict of interest

The authors declare no conflict of interest.

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## Acknowledgements

This work was supported by the US National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; DK074734) and the US National Institute on Drug Abuse (NIDA: DA035088).