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The effects of noncontingent and self-administered cytisine on body weight and meal patterns in male Sprague-Dawley rats

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Abstract

Rationale—Increased appetite and weight gain after cessation is a deterrent for quitting smoking. Pharmacotherapies that can reduce this weight gain in ex-smokers would be invaluable, and yet are not well studied in this context.

Objective—To examine the effects of extended daily exposure to intravenous cytisine, an alpha4beta2 nAChR partial agonist used for smoking cessation in some European countries, on body weight and patterns of food intake in rats.

Methods—In the first experiment, programmed infusions of cytisine were administered over 15 h per day. Food intake, meal patterns, and weight change were examined relative to a vehicle-infused group during treatment, and in a post-cytisine phase. The second experiment examined the effects of cytisine on food intake, meal patterns, and weight change when substituted for nicotine in a self-administration protocol. Rats self-administered nicotine and cytisine during alternating four day periods, and changes in body weight, drug infusions, and meal patterns were compared between drugs and during an extinction phase.

Results—In the first experiment, cytisine-treated rats ate less and gained less weight than those that received the vehicle. This occurred primarily by a reduced frequency of meals. In the 12 day post-cytisine phase, animals maintained a lower body weight relative to controls throughout. In the second experiment, total pellet intake increased during cytisine substitution relative to nicotine and animals self-administered cytisine significantly less than nicotine. However, cytisine substitution maintained decreases in food intake and weight gain compared to baseline via decreases in total pellet intake and meal size.

Conclusion—Cytisine administration results in decreased weight gain and changes in meal patterns dependent upon mode and pattern of administration and a previous history of nicotine administration.

Keywords

Intravenous nicotine; cytisine; meal patterns; programmed infusion; self-administration; body weight; extended access

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1. Introduction

The majority of smokers express a desire to quit smoking (>70%), yet very few are successful in the long term (~5%). One of the biggest obstacles to quitting is a fear of gaining weight (Jeffery et al. 2000). Nicotine replacement therapies (NRTs), including skin patches, chewing gum, nasal spray, inhalers, and lozenges/tablets are somewhat successful at helping people quit smoking (Kralikova et al. 2009; Stead et al 2008; Wang et al. 2008), but are relatively unsuccessful at preventing long-term weight gain in ex-smokers (Klesges et al. 1989). Likewise, the effects of bupropion or naltrexone on weight loss are limited and do not seem to extend past the treatment phase, with subjects eventually gaining as much weight as untreated ex-smokers (Farley et al. 2012; Filofoz et al. 2004; Toll et al. 2010). Prevention of weight gain during and after treatment is an important objective for the development of new medications for smoking cessation.

Varenicline, a partial agonist at the high affinity $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChRs) in the brain, a weak agonist at $\alpha 3\beta 4$ and $\alpha 6$ containing receptors, and a full agonist at $\alpha 7$ nAChRs (Mihalak KB et al. 2006; Rollema et al. 2010), is currently the most successful pharmacotherapy for the treatment of nicotine addiction (Polosa and Benowitz 2011). Rats given varenicline show decreases in nicotine self-administration and reinstatement that are accompanied by a transient decrease in food intake at the highest dose (3.0 mg/kg; O'Connor et al. 2010). However, a double-blind study examining the effects of varenicline on smoking and eating behaviors found no effect on caloric intake or number of items consumed (Stoops et al. 2008) and, in a recent clinical trial, varenicline resulted in limited weight loss that was not maintained in the long-term (Farley et al. 2012). Moreover, varenicline has several negative side effects such as headaches, gastrointestinal complaints, and psychiatric disturbances (Freedman 2007; Kohen and Kremen 2007; Kuehn 2008; Polosa and Benowitz 2011; Purvis et al. 2009) that might limit its acceptance.

Cytisine is the parent compound of varenicline and has been used as a treatment for smoking cessation in east and central European countries for at least 50 years (Etter et al. 2006), although it is not currently approved for smoking cessation by the FDA. It is a partial agonist at $\alpha 4\beta 2$ nAChRs, and a partial to full agonist at $\beta 4$ containing receptors and $\alpha 7$ receptors (Harpsoe et al. 2013; Rollema et al. 2010; Stokes and Papke 2012; Tutka and Zatonski 2006; Tzankova and Danchev 2007). Cytisine use is associated with some of the same side effects as varenicline, but with notably decreased incidence of psychiatric symptoms (Moore et al. 2011; Tonstad et al. 2012; Tutka and Zatonski 2005), possibly due to differential activation of the $\alpha 3\beta 4$ nAChR receptor subunit (Stokes and Papke 2012). Quit rates achieved with cytisine are comparable to NRTs and bupropion (Walker et al. 2011; West et al. 2011), but cytisine is significantly less expensive (Stapleton and West 2012; Tutka and Zatonski 2005), making it attractive to low-income groups that are at a greater risk of weight gain (Klesges and Meyers 1989; Pistelli et al. 2009; Swan & Carmelli 1995).

Behavioral evidence for a partial agonist action of cytisine includes findings that mice will acutely self-administer cytisine (Rasmussen and Swedberg 1998) and injections into the ventral tegmental area result in a conditioned place preference (Museo and Wise 1994). Further, drug discrimination tests indicate that cytisine can substitute for nicotine from 20% (LeSage et al. 2009) to 65% (Reavill et al. 1990). In relation to food intake, Mineur et al. (2011) reported that food intake and body weight of mice were decreased during cytisine administration. Some human studies have reported slight increases in weight gain and appetite during cytisine treatment (Tutka and Zatonski 2006), but these effects occur in the context of smoking cessation and so may not be due to cytisine alone.

The primary goal of this study is to examine the effects of cytosine administration on weight gain and meal patterns in rats. A second goal is to examine the ability of cytosine to substitute for nicotine in a self-administration paradigm, allowing the study of cytosine's effects on feeding under more clinically relevant conditions. We hypothesized that, like nicotine (Grebenstein et al. *in press*), cytosine will reduce food intake and meal size, but compared with nicotine will have a reduced ability to support self-administration.

2. Materials and Methods

2.1 Animals and Housing

Male Sprague-Dawley rats initially weighing ~275 g at ~10 weeks old were used (n = 16 in experiment one; n = 15 in experiment two). All rats were purchased from Harlan (Indianapolis, IN). The principles described in the Guide for the Care and Use of Laboratory Animals were followed throughout, and the project was approved by the UF IACUC. Upon arrival, rats were housed individually in a conventional vivarium that was maintained at 22–26°C and 40–60% relative humidity, on a reverse 12:12 hr light:dark cycle (lights off at 10:00AM). Home cages were of polycarbonate, with Sani-Chips (Harlan) contact bedding. Purina 5001 Chow pellets were available *ad libitum* for the initial portion of the experiment when animals were housed in their home cages. During the test phase, animals were housed in operant chambers. Autoclaved tap water was available at all times from a standard bottle and sipper tube.

2.2 Surgery and Catheter Maintenance

Rats were anaesthetized with isoflurane and implanted with a ~9cm Micro-Renathane catheter (.037" O.D. × .023" I.D., #037; Braintree Scientific, Braintree, MA) sterilized in ethylene oxide as described elsewhere (Rowland et al. 2008; Grebenstein et al. *in press*).

2.3 Apparatus

Operant test chambers (ENV-008CT; Med Associates, St. Albans, VT) were used throughout the experiment. Chambers were located individually in sound-attenuating, ventilated boxes. A sipper tube containing autoclaved water was available at all times through a hole in one side of the chamber. Cytosine and vehicle (sodium phosphate buffer) were delivered to rats via polyethylene tubing (PE60), that was protected by a stainless steel spring and connected to an infusion pump (PHM-100 set at 3.33RPM; Med Associates) via a fluid swivel (375/22PS; Instech laboratories, Plymouth Meeting, PA) to allow unlimited movement within the chamber. A hole was located in the center of the ceiling of the chamber, which allowed passage of the polyethylene tubing out of the chamber. Another length of PE tubing connected the swivel to the infusion pump, which was located on a shelf mounted outside the cage. The pump held a 30mL syringe containing either sterile cytosine solution or the vehicle and fitted with an in-line nitrocellulose filter (0.22µm; Cameo #25ES; Osmonics). The timing and duration of the infusions were controlled by a computer program (MedPC-IV).

In experiment 1, the chambers were fitted with a single fixed lever on the wall opposite the water tube and next to a food receptacle. A single press on the lever caused a feeder to release one 45-mg nutritionally complete food pellet (Purified Rodent Tablet, 5TUL, Test Diet, Richmond, IN; energy content 12.7% fat, 20.5% protein, 66.8% carbohydrate) into a trough located next to the feeder. In experiment 2, the chambers were fitted with an additional retractable lever on the same side as the food lever. This lever was retracted during the baseline phase of the experiment, and was available once the treatment phase began. A single press on this lever resulted in a 30 sec time out during which one infusion of the drug was administered; presses on the lever during this time did not result in any

consequences. For the duration of the time out, a cue light located above the lever was turned on. Each chamber was interfaced to a computer through an input/output module (DIG-716; Med Associates). Rats lived in these chambers for 23 h/day, and were removed only for daily servicing. A 15-W house light (ENV-215M; Med Associates) was located ~2cm below the ceiling on the wall above the openings for the sipper tubes, running the same 12:12 lighting cycle as the vivarium. The max/min temperature inside the chambers was recorded periodically and typically was ~1°C above room temperature.

2.4 Drugs

Cytisine, (Sigma Chemicals, St. Louis MO) was dissolved in a vehicle of sodium phosphate buffer (pH~7.35; 0.1M), with 17mg heparin added per 100mLs. Concentrations of cytisine solution were adjusted to deliver doses of approximately 1.25mg/mL (5mg/kg per total daily dose). These doses were chosen because similar i.v. doses of cytisine in mice have been used to examine the pharmacokinetic and pharmacodynamic properties of the drug, and have been shown to enter the brain and activate nAChRs (Tutka and Zatonski 2006).

Nicotine hydrogen tartrate (Sigma Chemicals, St Louis, MO) was dissolved in the same buffer as above; concentrations were adjusted to deliver doses of approximately 0.01 or 0.06 mg/kg body weight per injection, calculated as the free base of nicotine.

2.5 Procedure

In both experiments, rats were placed into the operant chambers two days after catheterization. No specific lever training was conducted: all rats acquired the operant task within the first night. Baseline measures of body weight and food consumed were recorded for the next for 5 days.

After the baseline period in experiment 1, rats were divided into two groups matched for body weight. Testing continued with their catheters now attached to the infusion line. For the first 2 days all rats received programmed injections of vehicle. The programmed infusions (0.04ml) were 1-s in duration every 30 min for the entire dark phase (12-h) and for the last 3 h of the light phase. This resulted in a total of 30 injections over a 15 h period of the 23 h test session. This period coincided with the maximal feeding and active times of rats. Rats then entered a 12 day treatment phase during which one group of rats received vehicle injections and the other group received cytisine. The total daily dose of cytisine given to the treatment group, maintained for the 9 days of the treatment phase, was 5mg/kg (~.167 mg/kg/inf). After the treatment phase, rats entered a cessation phase during which nicotine infusions were replaced by vehicle. The cessation phase lasted 12 days. Body weight and food intake were recorded daily.

After the baseline period in experiment 2, rats were attached to the infusion lines, which were filled with the vehicle, for two days. During this period, the drug lever remained retracted and the animals were not allowed to respond on the lever for the drug. The following two days the vehicle was replaced with .01 mg/kg/infusion of nicotine, the drug lever was protracted, and animals were allowed to respond for the nicotine on an FR1 schedule of reinforcement. All subsequent drug self-administration was on this FR1 schedule of reinforcement. The dose of nicotine was then increase to .06 mg/kg/infusion, and animals were allowed to self-administered this dose. Once an animal reached stable self-administration, defined as 30 +/- 6 infusions of the high dose of nicotine for 3 days, the nicotine was changed to cytisine for 4 days. During this period, the animal was able to respond on the retractable lever to receive infusions of cytisine. Half of the animals received a low dose of cytisine (2.5mg/kg) while the other half received a high dose of cytisine (5mg/kg). After four days of cytisine self-administration, cytisine was replaced with the high dose

of nicotine until the animal again reached stable self-administration, at which time nicotine was again replaced with cytosine for four days. Animals that had received the high dose of cytosine were given the low dose during this period, while animals that had initially received the low dose were now given the high dose. This phase was followed by a final 4 days of nicotine self-administration. Nicotine was then replaced by vehicle, and lever pressing measured for 4 days. Body weight was recorded daily and meal parameters were recorded continuously throughout the experiment.

2.6 Data Analysis

For both experiments, the number of lever presses on the food lever was recorded every 10 min for each 23 h session. Meals were defined as 4 or more pellets in any 10 min bin with no more than a 10 min interval between pellets. Daily meal number as well as the number of meals taken during the dark (night) and light (day) phases were determined. Mean meal size was determined by dividing total pellets by number of meals and was computed separately for dark (12 h) and light (11 h) phases. In experiment 1, data for each rat were then subsequently averaged across days to yield seven principal phases: baseline (days 1–5), days 6–7 during which the animal received infusions of the vehicle, treatment days 8–11 and 12–16 during which cytosine was administered, and cessation days 17–20, 21–24 and 25–28 during which cytosine was replaced with vehicle. Body weights were recorded daily and will be reported as change from the initial value at the start of day 1. One-way ANOVAs were used to examine differences in body weight and meal patterns (food intake, meal number and meal size) between groups that received the vehicle or cytosine. Significance levels were set at $p < .05$. Post hoc tests were used to determine if there were significant increases in total pellet intake within groups, and were corrected for multiple comparisons via the Bonferroni method. For experiment 2, a priori analyses were conducted using paired samples t-tests, in order to examine differences in weight gain and meal patterns during the phases of the experiment we were most interested in. Therefore, changes in body weight gain, infusion number, and the meal pattern variables of interest were compared between the baseline phase and all other phases or the nicotine phase with all other phases. Significance levels were set to $p < 0.01$ to control the error rate for multiple comparisons. For clarity, all nicotine phases were averaged and are presented as a single treatment phase. The averages across animals for the last two days of each phase, with the exception of the baseline phase, were analyzed, to ensure only stable conditions were examined. Repeated measures ANOVAs were performed to test for any effects of treatment order between the two doses of cytosine, and none were found (Table 2). For simplicity, data from the 2.5mg/kg cytosine phase will be presented before the 5mg/kg cytosine.

3. Results

3.1 Experiment One

Body weight changes are shown in Figure 1. During the 5-day baseline period, the two groups of rats did not differ in weight gain, whereas during the treatment phase the group that received cytosine gained less weight than those that received vehicle. During the cessation phase, animals that received cytosine continued to weigh less than those that received the vehicle (Table 1; Figure 1). There was no significant change in the overall rate of weight gain between the two groups during the 12 days of the cessation phase. However, there was a difference in the rate of weight gain when only the first four days of cessation were compared ($F(1, 76) = 8.64$; $p < 0.01$), indicating a short term but temporary increase in weight gain in animals receiving cytosine. Further tests were done to assess if this increase in body weight was accompanied by an increase in total pellet intake. Within the cytosine group, pellet intake varied significantly across experimental phases ($F(1, 14) = 9.356$; $p < 0.001$), but this moderate increase in total pellet intake from the last few days of the

treatment phase to the first four days of the cessation phase was not significant (see Figure 2A). This trend was less apparent during the final two phases of cessation.

Total pellet intake and the number of meals are shown in Figures 2 and 3. During both baseline and the first two days of treatment when both groups received vehicle, there were no significant differences in total pellet intake and meal number during the dark or light periods, or over the entire 23h. The mean meal size did not significantly differ between the two groups during these phases throughout the dark period or over 23h, but the group destined to receive cytosine ate slightly larger meals during baseline (data not shown).

The treatment phase was separated into two periods following the two days of vehicle administration (days 8–11 and 12–16) during which animals either received cytosine or vehicle. Animals receiving cytosine ate significantly fewer pellets per 23h throughout the treatment phase than those that received the vehicle (Figure 2A) on days 8–11 ($F(1,15) = 23.46$; $p < .01$) and days 12–16 ($F(1,15) = 20.33$; $p < .01$). When analyzed separately for light and dark periods (Figure 2B–C), cytosine-treated rats ate significantly less during the dark throughout the treatment phase during the first group of days ($F(1,15) = 9.39$; $p < .01$) and the second group of days ($F(1,15) = 8.00$; $p < .05$), but did not differ from vehicle treated controls throughout the light period.

Examination of the number of meals revealed that cytosine treated rats ate significantly fewer meals than controls over 23h (Figure 3A) during days 12–16 of the experiment ($F(1,15) = 18.66$; $p < .01$), an effect which was not significant for days 8–11. When analyzed separately for light and dark periods (Figures 3B–C), the cytosine group ate fewer meals than the vehicle group during the dark on days 12–16 ($F(1,15) = 5.99$; $p < .05$), but not during days 8–11, and consumed fewer meals during the light period throughout the treatment phase on days 8–11 ($F(1,15) = 5.25$; $p < .05$) and 12–16 ($F(1,15) = 12.96$; $p < .01$). Examination of meal size revealed no overall effect over 23h, or when analyzed separately for day and night (data not shown).

During the cessation phase, there were no significant differences in total pellet intake and meal size between the cytosine-cessation and control groups during any of the 4-day segments throughout 23 h, the light period and the dark period (days 17–20, 21–24 and 25–28; Figures 2A and 2C). Animals in the cytosine group ate fewer meals during days 17–20 of the experiment ($F(1,13) = 4.80$; $p < .05$; Figures 3A), but there was no difference between groups on days 21–24 and 25–28 over 23 h, or for any of the days throughout the light and dark periods.

3.2 Experiment Two

Body weight changes are shown in Figure 4A. Compared to the average weight gained during the baseline phase, animals gained less weight in the nicotine phases ($t(1,11) = 7.329$; $P < 0.01$) and the 5mg/kg cytosine phase ($t(1, 11) = 3.460$; $p < 0.01$). Animals gained more weight during the 2.5mg/kg cytosine phase ($t(1, 11) = 3.848$; $p < 0.01$) and the extinction phase ($t(1, 12) = 4.986$; $p < 0.01$) compared to the nicotine phases. The number of self-administrations for the drug available (Figure 4B) did not differ as a function of phase of the experiment except during the extinction phase, where animals self-administered the vehicle significantly less than they self-administered nicotine ($t = 3.448$; $p < 0.01$).

Animals ate fewer pellets over 23h during the nicotine phases ($t(1,13) = 6.091$; $p < 0.01$), the 2.5mg/kg cytosine phase ($t(1, 12) = 4.767$; $p < 0.01$), the 5mg/kg cytosine phase ($t(1,11) = 4.979$; $p < 0.01$), and the extinction phase of the experiment ($t = 3.526$; $p < 0.01$) compared to the baseline phase (Figure 5A). In addition, animals ate more pellets during the 2.5mg/kg cytosine phase than they did during all nicotine phases ($t(1, 13) = 3.343$; $p < 0.01$).

When pellet intake was analyzed separately for light and dark effects, animals ate significantly fewer pellets during the nicotine phases compared to the baseline phase during the dark ($t(1, 12) = 3.496$; $p < 0.01$; Figure 5B) and the light period ($t(1, 12) = 4.789$; $p < 0.01$). There was a trend for animals receiving 5mg/kg cytosine to eat fewer pellets compared to baseline in the light period, but this difference was not significant. Animals also ate fewer pellets during extinction compared to baseline ($t = 3.567$; $p < 0.01$).

The total number of meals consumed over 23 hours (Figure 6A) was increased from baseline during the nicotine phases ($t(1,12) = 3.468$; $p < 0.01$), the 2.5mg/kg cytosine phase ($t(1,11) = 4.197$; $p < 0.01$), and the extinction phase ($t(1, 11) = 3.788$; $p < 0.01$). When total meal number was analyzed separately for light (Figure 6C) and dark effects (Figure 6B), animals consumed more meals during the nicotine phases ($t(1,12) = 4.079$; $p < .01$) and the extinction phase ($t(1,11) = 4.487$; $p < 0.01$) compared to the baseline phase in the dark period. There were no differences between the baseline or nicotine phases compared with all other phases during the light period.

There was a decrease in meal size over 23h (Figure 7A) from the baseline phase to the nicotine phases ($t(1,12) = 5.529$; $p < 0.01$), the 2.5mg/kg cytosine phase ($t(1,11) = 4.930$; $p < 0.01$), the 5mg/kg cytosine phase ($t(1,10) = 4.445$; $p < 0.01$) and the extinction phase ($t(4,11) = 4.631$; $p < 0.01$). When total meal size was analyzed separately for dark (Figure 7B) and light effects (Figure 7C), there was also a decrease in meal size during the dark period of the nicotine phases ($t(1,12) = 5.278$; $p < 0.01$), the 2.5mg/kg cytosine phase ($t(1,11) = 3.816$; $p < 0.01$), the 5mg/kg cytosine phase ($t(1,10) = 4.154$; $p < 0.01$), and the extinction phase ($t(1,11) = 3.495$; $p < 0.01$) compared to the baseline phase. Likewise, there was a decrease in meal size during the light period of the nicotine phases ($t(1,12) = 4.652$; $p < 0.01$), the 2.5mg/kg cytosine phase ($t(1,11) = 3.512$; $p < 0.01$), the 5mg/kg cytosine phase ($t(1,10) = 4.579$; $p < 0.01$), and the extinction phase ($t(1,11) = 4.150$; $p < 0.01$) compared to the baseline phase.

4. Discussion

4.1 Experiment One

Experiment 1 examined the effects of noncontingent intravenous administration of cytosine on body weight and meal patterns. In accordance with the effects of cytosine on body weight in mice reported by Mineur et al. (2011), administration of cytosine resulted in decreased weight gain in rats. Despite an initial increase in body weight, animals that received cytosine continued to gain less weight by the end of the 12 day cessation period, suggesting that any weight gain following cytosine is immediate and short-lived. The current study provides additional evidence and support for cytosine as a treatment option for individuals concerned about possible weight gain post-cessation. In contrast to other approved and experimental pharmacotherapies that result in either a slight weight gain during treatment or a large weight gain after treatment is stopped (Borrelli et al. 1999; Farley et al. 2012; Filofoz et al. 2004), cytosine administration appears to have lasting effects on body weight throughout treatment and post-cessation in rats.

The decrease in total food intake during cytosine administration was confined to the dark cycle. Meal pattern analysis revealed a decrease in meal number over 23h and in the dark and light cycles during the last few days of treatment. This reduction in meal frequency was not offset by an increase in meal size; therefore total food intake was reduced. This is in contrast to some studies with nicotine (Bellinger et al. 2003) in which a change in meal frequency is offset by a reciprocal change in meal size, so that total intake is essentially unchanged. Previously we found that nicotine, administered in the same temporal protocol as the present study, reduced meal size (Grebenstein et al. *in press*), while in the present

study, cytosine had no discernable effect. Little is known about the mechanism responsible for this effect in nicotine, though some evidence indicates that there are two separate systems that regulate each variable of interest (size and number). Consequently, a change in one variable results in a compensatory change in another, so as to maintain stable food intake (Meguid et al. 2000). It is possible that cytosine either differentially affects these systems or exerts its anorexic effects through entirely different mechanisms than nicotine.

There is evidence to indicate that cytosine has differential effects on human and rat nAChRs. Stokes and Papke (2012) characterized the responses of both human and rat $\alpha 3\beta 4$ receptors to cytosine, and found higher EC_{50} values and lower efficacy in human compared to rat receptors. Because Mineur et al. (2011) suggested that the $\beta 4$ receptor subunit is important for nicotine and cytosine's effects on body weight and food intake, results from rodent studies will need to be extrapolated to humans with caution. In contrast to species differences at $\alpha 3\beta 4$ receptors, the effects of cytosine at both $\alpha 4$ and $\alpha 7$ containing receptors are similar in humans and rats. Both $\alpha 7$ and $\alpha 4\beta 2$ receptors have been found throughout the ventromedial hypothalamus (Zoli and Picciotto 2012) and are important in nicotine-induced depolarization of NPY and POMC neurons (Huang et al. 2011). In addition, acetylcholine is an important neuromodulator of POMC's effects on food intake (Adan et al. 2008). Mineur et al. (2011) confirmed the presence of $\beta 4$ receptors on POMC neurons in the arcuate nucleus, and showed an increase in FOS-IR in POMC neurons in response to either nicotine or cytosine administration. Thus, since the relative importance of species differences in receptor sensitivity to the appetite-suppressant effect of cytosine is unclear, and the current evidence on weight gain in humans taking cytosine is inconclusive (Tutke and Zatonski 2006), the present results and those of Mineur et al. (2011) indicate that additional clinical research is warranted.

4.2 Experiment Two

In experiment 2, we examined the ability of cytosine to substitute for nicotine in a self-administration protocol and to characterize the effects of self-administered cytosine and nicotine on body weight and food intake. This approach more closely models the clinical situation of substituting cytosine for cigarettes during a quit attempt. Nicotine was self-administered robustly, and this behavior decreased during extinction, indicating the nicotine was reinforcing in our protocol. While the self-administration rates for nicotine and cytosine did not differ, the rates with cytosine also did not differ from extinction. This lack of difference between cytosine and vehicle self-administration demonstrates an inability of cytosine to serve as a reinforcer, which contrasts with the reinforcing effects of varenicline that have been previously reported (Rollema et al. 2007). These findings are consistent with previous research showing that cytosine only partially substitutes for nicotine in drug-discrimination tasks, while varenicline has shown partial to full substitution for nicotine in such assays (LeSage et al. 2009; Reavill et al. 1990).

We did not find any effect of cytosine dose on infusion number, and this was somewhat surprising. Because the cytosine doses were counterbalanced within animals, we tested to see if there were effects of treatment order on the variables of interest and none were found. One possibility for the lack of a dose effect is that animals were pressing for the light cues that became associated with nicotine infusions rather than for the cytosine itself. The level of infusions during the extinction period did not significantly differ from those seen when cytosine was administered, supporting the notion that stimuli associated with nicotine contributed to the maintenance of self-administration during cytosine substitution (Donny et al. 1999, 2003).

As expected, there was a significant decrease in body weight between the baseline period and the periods of nicotine self-administration. There was also a significant increase in body

weight gain during the extinction phase compared to the nicotine phases. These results support previous findings that nicotine administration results in decreased weight gain (Grunberg and Bowen 1985; Grunberg et al. 1985; Grunberg et al. 1986; Miyata 2001) followed by an increase in weight gain during cessation (Grunberg et al. 1986; Miyata et al. 1999, 2001). In contrast to our previous study (Greibenstein et al; *in press*) examining the effects of noncontingent intravenous nicotine administration on body weight, the present experiment was able to detect changes in weight gain within the first four days of the extinction phase, compared to the 10 days it took in the noncontingent experiment. These results indicate that self-administration might be a more sensitive model than noncontingent administration for detecting changes in food motivation post-cessation, despite comparable amounts of total self-administered nicotine between the two studies. Self-administration of nicotine resulted in specific changes in meal patterns that replicated results from our previous study (Greibenstein et al. *in press*) using noncontingent intravenous nicotine administration.

It is unclear from the present experiment whether weight loss or gain would have been observed had cytosine been given as the final administered substance. In light of results from experiment 1, it is possible that cytosine may result in decreased weight gain in the long-term. Future studies involving animals given the opportunity to self-administer nicotine for an extended period of time, followed by a period of cytosine self-administration, would provide insight into the weight loss benefits of cytosine following nicotine administration, in a manner more closely resembling human treatment protocols. The addition of a group that receives only cytosine would then allow for the control of any effects on weight gain due to the self-administration of multiple drugs. In addition, it will be important to determine what the lowest dose of cytosine is that produces effects on food intake and body weight in both smokers and nonsmokers. The current experiment demonstrates a nonspecific effect of cytosine on food intake, regardless of a history of nicotine administration, as a reduction in weight gain was seen in both nicotine naïve and nicotine exposed animals. Such effects of cytosine on body weight and food intake are due to the anorectic properties of drug itself, which does not appear to be specific to the nicotine withdrawal phase. Despite a lack of specificity, cytosine may still be used to reduce nicotine withdrawal-related weight gain, with a possible additional benefit of a reduction in nicotine intake.

The current study also examined changes in weight gain between periods of nicotine administration and subsequent periods of cytosine administration, and found that the decrease in weight gain during nicotine self-administration was not maintained when the low dose of cytosine was substituted for nicotine. While the 2.5mg/kg dose of cytosine resulted in increased weight gain compared to the weight gain seen during periods of nicotine self-administration, this effect did not occur at the 5mg/kg dose of cytosine. These results provide additional evidence in support of the higher dose of cytosine for weight loss purposes. Because of low brain concentrations of cytosine due to either possible increases in active brain efflux transporters (Rollema et al. 2010) or poor brain penetration (Reavill et al. 1990), treatment with cytosine involves frequent dosing of approximately 1.5 mg six times a day (Etter et al. 2006), and it is therefore not surprising that higher doses were required in order to see a substantial effect on weight gain. In addition, the possibility exists that the observed dose specific effects on body weight are due to the acute nauseating effects of a high dose of cytosine. This is unlikely however, because differential effects on food intake did not accompany the changes in body weight between the two doses. Future studies examining the dose response relationship between cytosine and food intake are warranted, as it was outside the scope of the present study to investigate.

In contrast to experiment 1, which found effects of cytosine specifically on meal number rather than meal size, self-administered cytosine seemed to mimic the effects of self-

administered nicotine via decreases in meal size in the dark and light periods with an increase in meal number throughout the dark period. Possible reasons for this discrepancy include the difference in the pattern of intake between the two experiments as well as the difference in the total amount of cytosine administered each day.

4.3 Conclusions

A higher dose of cytosine (5mg/kg) delivered either contingently or noncontingently, resulted in decreased weight gain and food intake comparable to the effect of nicotine, despite the fact that cytosine did not substitute for nicotine as a reward. Noncontingent administration of cytosine decreased food intake via changes in meal number, which was in contrast to the decrease in meal size observed with nicotine. These results indicate that cytosine might be useful to prevent weight gain during smoking cessation.

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Reference List

- Adan RA, Vanderschuren LM, la Fleur SE. Anti-obesity drugs and neural circuits of feeding. *Trends in Pharmacological Sciences*. 2008; 29(4):208–217. [PubMed: 18353447]
- Bellinger L, Cepeda-Benito A, Wellman PJ. Meal patterns in male rats during and after intermittent nicotine administration. *Pharmacology, Biochemistry and Behavior*. 2003; 74:495–504.
- Borrelli B, Spring B, Niaura R, Kristeller J, Ockene JK, Keuthen NJ. Weight suppression and weight rebound in ex-smokers treated with fluoxetine. *Journal of Consulting and Clinical Psychology*. 1999; 67(1):124–131. [PubMed: 10028216]
- Donny EC, Caggiula AR, Mielke MM, Booth S, Gharib MA, Hoffman A, Maldovan V, Shupenko C, McCallum SE. Nicotine self-administration in rats on a progressive ratio schedule of reinforcement. *Psychopharmacology*. 1999; 147:135–142. [PubMed: 10591880]
- Donny EC, Chaudhri N, Caggiula AR, Evans-Martin FF, Booth S, Gharib MA, Clements LA, Sved AF. Operant responding for a visual reinforcement in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. *Psychopharmacology*. 2003; 169:68–76. [PubMed: 12774186]
- Etter JF, Lukas RJ, Benowitz NL, West R, Dresler CM. Cytisine for smoking cessation: a research agenda. *Drug and Alcohol Dependence*. 2008; 92:3–8. [PubMed: 17825502]
- Farley, AC.; Hajek, P.; Lycett, D.; Aveyard, P. Interventions for preventing weight gain after smoking cessation. Vol. 2012. *The Cochrane Library*; 2012.
- Filozof C, Fernandez Pinilla MC, Fernandez-Cruz A. Smoking cessation and weight gain. *Obesity*. 2004; 5:95–103.
- Freedman R. Exacerbation of schizophrenia by varenicline. *American Journal of Psychiatry*. 2007; 164:1269. [PubMed: 17671295]
- Grebenstein PE, Thompson IE, Rowland NE. The effects of extended intravenous nicotine administration on body weight and meal patterns in Sprague-Dawley rats. *Psychopharmacology*. 2012 in press.
- Grunberg NE, Bowen DJ. The role of physical activity in nicotine's effects on body weight. *Pharmacology Biochemistry and Behavior*. 1985; 23:851–854.
- Grunberg NE, Bowen DJ, Maycock VA, Nespor SM. The importance of sweet taste and caloric content in the effects of nicotine on specific food consumption. *Psychopharmacology*. 1985; 87:198–203. [PubMed: 3931146]
- Grunberg NE, Bowen DJ, Winders SE. Effects of nicotine on body weight and food consumption in female rats. *Psychopharmacology*. 1986; 90:101–105. [PubMed: 3094051]

- Harpsoe K, Hald H, Timmerman DB, Lensen ML, Dyhring T, Nielson EO, Peters D, Balle T, Gajhede M, Kastrip JS, Ahring PK. Molecular determinants of subtype-selective efficacies of cytosine and the novel compound NS3861 at heteromeric nicotinic acetylcholine receptors. *Journal of Biological Chemistry*. 2013; 288(4):2559–2570. [PubMed: 23229547]
- Huang H, Xu Y, van den Pol AN. Nicotine excites hypothalamic arcuate anorexigenic proopiomelanocortin neurons and orexigenic neuropeptide Y neurons: similarities and differences. *Journal of Neurophysiology*. 2011; 106:1191–1202. [PubMed: 21653710]
- Jeffery RW, Hennrikus DJ, Lando HA, Murray DM, Liu JW. Reconciling conflicting findings regarding postcessation weight concerns and success in smoking cessation. *Health Psychology*. 2000; 19(3):242–246. [PubMed: 10868768]
- Klesges RC, Meyers AW, Klesges LM, LaVasque ME. Smoking, body weight, and their effect on smoking behavior: a comprehensive review of the literature. *Psychological Bulletin*. 1989; 106(2): 204–230. [PubMed: 2678202]
- Kohen I, Kremen N. Varenicline-induced manic episode in a patient with bipolar disorder. *American Journal of Psychiatry*. 2007; 164:1269–1270. [PubMed: 17671294]
- Kralikova E, Kozak JT, Rasmussen T, Gustavsson G, Le Houezec J. Smoking cessation or reduction with nicotine replacement therapy: a placebo-controlled double blind trial with nicotine gum and inhaler. *BMC Public Health*. 2009; 9:433. [PubMed: 19943947]
- Kuehn BM. FDA warns of adverse events linked to smoking cessation drug and antiepileptics. *Journal of the American Medical Association*. 2008; 299(10):1121–1122. [PubMed: 18334683]
- LeSage MG, Shelley D, Ross JT, Carroll FI, Corrigan WA. Effects of the nicotinic receptor partial agonists varenicline and cytosine on the discriminative stimulus effects of nicotine in rats. *Pharmacology, Biochemistry and Behavior*. 2009; 91:461–467.
- Meguid MM, Fetissov SO, Varma M, Sato T, Zhang L, Laviano A, Rossi-Fanelli F. Hypothalamic dopamine and serotonin in the regulation of food intake. *Nutrition*. 2000; 16:843–857. [PubMed: 11054589]
- Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ neuronal nicotinic receptors. *Molecular Pharmacology*. 2006; 70:801–805. [PubMed: 16766716]
- Mineur YS, Abizaid A, Rao Y, Salas R, DiLeone RJ, Gundisch D, Diano S, Biasi MD, Horvath TL, Gao X, Picciotto MR. Nicotine decreases food intake through activation of POMC neurons. *Science*. 2011; 332:1330–1332. [PubMed: 21659607]
- Miyata G, Meguid MM, Fetissov SO, Torelli GF, Kim H. Nicotine's effect on hypothalamic neurotransmitters and appetite regulation. *Surgery*. 1999; 126:255–263. [PubMed: 10455892]
- Miyata G, Meguid MM, Varma M, Fetissov SO, Kim H. Nicotine alters the usual reciprocity between meal size and meal number in female rat. *Physiology and Behavior*. 2001; 74:169–176. [PubMed: 11564465]
- Moore TJ, Furberg CD, Glenmullen J, Maltsberger JT, Singh S. Suicidal behavior and depression in smoking cessation treatments. *PLoS ONE*. 2011; 6(11):e27016. [PubMed: 22073240]
- Museo E, Wise RA. Place preference conditioning with ventral tegmental injections of cytosine. *Life Sciences*. 1994; 55(15):1179–1186. [PubMed: 7934614]
- O'Connor EC, Parker D, Rollema H, Mead AN. The $\alpha 4\beta 2$ nicotinic acetylcholine-receptor partial agonist varenicline inhibits both nicotine self-administration following repeated dosing and reinstatement of nicotine seeking in rats. *Psychopharmacology*. 2010; 208:365–376. [PubMed: 19967529]
- Polosa R, Benowitz NL. Treatment of nicotine addiction: present therapeutic options and pipeline developments. *Trends in Pharmacological Sciences*. 2011; 32(5):281–289. [PubMed: 21256603]
- Purvis TL, Mambourg SE, Balvanz TM, Magallon HE, Pham RH. Safety and effectiveness of varenicline in a veteran population with a high prevalence of mental illness. *The Annals of Pharmacotherapy*. 2009; 43:862–867. [PubMed: 19383936]
- Reavill C, Walther B, Stolerman IP, Testa B. Behavioural and pharmacokinetic studies on nicotine, cytosine and lobeline. *Neuropharmacology*. 1990; 29(7):619–624. [PubMed: 2385332]
- Rollema H, Shrikhande A, Ward KM, Tingley FD, Coe JW, O'Neill BT, Tseng E, Wang EQ, Mather RJ, Hurst RS, Williams KE, De Vries M, Cremers T, Bertrand S, Bertrand D. Pre-clinical properties of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonists varenicline, cytosine and

- dianicline translate to clinical efficacy for nicotine dependence. *British Journal of Pharmacology*. 2010; 160:334–345. [PubMed: 20331614]
- Rowland NE, Robertson K, Soti F, Kem WR. Nicotine analog inhibition of nicotine self-administration. *Psychopharmacology*. 2008; 199:605–13. [PubMed: 18536909]
- Stapleton JA, West R. A direct method of ICER tables for the estimation of the cost-effectiveness of smoking cessation interventions in general populations: application to a new cytisine trial and other examples. *Nicotine and Tobacco Research*. 2012; 14(4):463–471. [PubMed: 22232061]
- Stead LF, Perera R, Bullen C, Mant D, Lancaster T. Nicotine replacement therapy for smoking cessation. *Cochrane Database of Systematic Reviews*. 2008; 3(1)
- Stokes C, Papke RL. Use of an $\alpha 3\beta 4$ nicotinic acetylcholine receptor subunit concatamer to characterize ganglionic receptor subtypes with specific subunit composition reveals species-specific pharmacologic properties. *Neuropharmacology*. 2012; 63:538–546. [PubMed: 22580377]
- Stoops WW, Vansickel AR, Glaser PE, Rush CR. The influence of acute varenicline administration on smoking and eating behavior in humans. *Pharmacology, Biochemistry and Behavior*. 2008; 91:165–169.
- Swan GE, Carmelli D. Characteristics associated with excessive weight gain after smoking cessation in men. *American Journal of Public Health*. 1995; 85(1):73–77. [PubMed: 7832265]
- Toll BA, White M, Wu R, Meandzija B, Jatlow P, Matuch R, O'Malley SS. Low-dose naltrexone augmentation of nicotine replacement for smoking cessation with reduced weight gain: a randomized trial. *Drug and Alcohol Dependence*. 2010; 111:200–206. [PubMed: 20542391]
- Tonstad S, Davies S, Flammer M, Russ C, Hughes J. Psychiatric adverse events in randomized, double-blind, placebo-controlled clinical trials of varenicline. *Drug Safety*. 2010; 33(4):289–301. [PubMed: 20297861]
- Tutka P, Zatonski W. Cytisine for the treatment of nicotine addiction: from a molecule to a therapeutic efficacy. *Pharmacological Reports*. 2006; 58:777–798. [PubMed: 17220536]
- Tzankova V, Danchev N. Cytisine – from ethomedical use to the development as a natural alternative for smoking cessation. *Biotechnology and Biotechnical Equipment*. 2007; 21(2):151–160.
- Wang D, Connock M, Barton P, Fry-Smith A, Aveyard P, Moore D. 'Cut down to quit' with nicotine replacement therapies in smoking cessation: a systematic review of effectiveness and economic analysis. *Health Technology Assessment*. 2008; 12(2)
- Walker N, Howe C, Bullen C, McRobbie H, Glover M, Parag V, Wiiliman J, Veale R, Nosa V, Barnes J. Study protocol for a non-inferiority trial of cytisine versus nicotine replacement therapy in people motivated to stop smoking. *BMC Public Health*. 2011; 11:880–889.
- West R, Zatonski W, Cedzynska M, Lewandowska D, Pazik J, Aveyard P, Stapleton J. Placebo-controlled trial of cytisine for smoking cessation. *The New England Journal of Medicine*. 2011; 365:1193–1200. [PubMed: 21991893]
- Zoli M, Picciotto MR. Nicotinic regulation of energy homeostasis. *Nicotine and Tobacco Research*. 2012; 14(11):1270–1290. [PubMed: 22990212]

Highlights

- Noncontingent cytosine results in decreased weight gain via changes in meal number
- The effects of noncontingent cytosine on body weight persist for at least 12 days post-treatment
- Meal patterns seen between self-administered and noncontingent nicotine are similar
- Cytosine is self-administered less than nicotine
- Self-administered and noncontingent cytosine differentially effect meal patterns

Fig. 1.

Changes in body weight over treatment and cessation phases for vehicle and cytosine treated groups in Experiment 1. Shown are mean $\pm$ SE cumulative change in body weight (g) on consecutive days of treatment and cessation phases, in rats that received either cytosine ($n = 8$) or vehicle ($n = 8$). * $P < 0.1$, ** $P < 0.01$ difference between vehicle and cytosine

Fig. 2.

Total number of pellets consumed in the various phases of Experiment 1. Shown are mean $\pm$ SE lever presses for pellets consumed, averaged over the ranges of days indicated during 23 hours (a), the dark period (b), or the light period (c), in rats that received either vehicle ($n = 8$) or cytisine ($n = 8$) during the treatment phase. * $P < 0.05$, ** $P < 0.01$ difference between vehicle and cytisine

Fig. 3.

Total meal number during the various phases of Experiment 1. Shown are mean $\pm$ SE number of meals, averaged over the ranges of days indicated during 23 hours (a), the dark period (b), or the light period (c), in rats that received either vehicle ($n = 8$) or cytisine ($n = 8$) during the treatment phase. * $P < 0.05$, ** $P < 0.01$ difference between vehicle and cytisine

Fig. 4.

Body weight and number of infusions across treatments in Experiment 2. A: mean $\pm$ SE cumulative change in body weight (g) for the last two days of each treatment phase, and B: mean $\pm$ SE number of lever presses for infusions for the last two days of each treatment phase. The consecutive phases for panels A and B are: baseline ($n = 14$), all nicotine ($n = 15$), 2.5 mg/kg cytisine ($n = 14$), 5 mg/kg cytisine ($n = 13$), and extinction ($n = 14$). * $P < 0.01$ difference between the indicated and baseline phases; # $P < 0.01$ difference between the indicated and nicotine phases.

Fig. 5.

Total number of pellets across treatments. Shown are mean $\pm$ SE lever presses for pellets, averaged over the last two days of the various treatments during 23 hours (a), the dark period (b), or the light period (c). The consecutive phases for panels A, B and C are: baseline ($n = 14$), all nicotine ($n = 15$), 2.5 mg/kg cytisine ($n = 14$), 5 mg/kg cytisine ($n = 13$), and extinction ($n = 14$). * $P < 0.01$ difference between the indicated and baseline phases; # $P < 0.01$ difference between the indicated and nicotine phases.

Fig. 6.

Total meal number across treatments. Shown are mean $\pm$ SE number of meals, averaged over the last two days of the various treatments during 23 hours (a), the dark period (b), or the light period (c). The consecutive phases for panels A, B and C are: baseline ($n = 14$), all nicotine ($n = 15$), 2.5 mg/kg cytisine ($n = 14$), 5 mg/kg cytisine ($n = 13$), and extinction ($n = 14$). * $P < 0.01$ difference between the indicated and baseline phases; # $P < 0.01$ difference between the indicated and nicotine phases.

Fig. 7.

Total meal size across treatments. Shown are mean $\pm$ SE number of pellets per meal, averaged over the last two days of the various treatments during 23 hours (a), the dark period (b), or the light period (c). The consecutive phases for panels A, B and C are: baseline ($n = 14$), all nicotine ($n = 15$), 2.5 mg/kg cytisine ($n = 14$), 5 mg/kg cytisine ($n = 13$), and extinction ($n = 14$). * $P < 0.01$ difference between the indicated and baseline phases; # $P < 0.01$ difference between the indicated and nicotine phases.

Table 1

^a

	Day	F	Vehicle		Cytisine	
			Mean	SE	Mean	SE
Treatment						
	1	.43	2.63	.50	1.1	2.22
	2	.05	9.5	1.38	8.9	2.57
	3	3.84	14.8	1.22	10.4	1.87
	4	5.88*	18.9	1.73	12.5	1.98
	5	7.69*	24.9	1.87	16.1	2.21
	6	7.60*	28.6	1.91	18.3	3.25
	7	18.97**	33.8	2.28	18.6	2.62
	8	18.68**	38.9	2.46	21.0	3.18
	9	15.91**	41.0	2.51	26.5	2.63
	10	16.81**	45.3	2.54	28.8	3.12
Cessation						
	11	15.09**	47.8	2.94	28.9	3.87
	12	18.94**	49.8	3.00	35.3	3.81
	13	3.65	52.1	3.73	42.6	3.28
	14	4.93*	57.3	3.24	46.4	3.61
	15	6.35*	59.3	3.43	43.9	4.86
	16	7.13*	62.1	3.20	46.6	4.87
	17	8.65*	64.7	3.82	47.1	4.64
	18	8.57*	68.3	3.78	49.9	5.04
	19	6.27*	72.3	4.51	53.0	6.25
	20	6.67*	75.0	5.47	53.0	6.33
	21	6.05*	77.3	4.92	57.0	6.36
	22	4.80	77.5	5.30	57.8	7.24

F values for the changes in body weight over each day of the treatment and cessation phases between the vehicle and cytosine groups in Experiment 1.

* p < .05.
** p < .01

Table 2*b*

Variables	Phase F	Order F
Body Weight	5.22**	.65
Infusions	4.66*	6.09
Total Pellets	7.75**	.20
Night Pellets	3.07*	.45
Day Pellets	4.72**	.02
Total Meals	2.14	.15
Night Meals	2.78*	.02
Day Meals	.84	7.64
Total Meal Size	4.10**	1.97
Night Meal Size	4.10**	1.49
Day Meal Size	2.78*	.36

b F values for the effects of drug order and phase of the experiment on the variables of interest in experiment 1.

*
p < .05.

**
p < .01