DIFFERENTIAL SUPPRESSION OF HYPERGLYCEMIC, FEEDING, AND NEUROENDOCRINE RESPONSES IN ANOREXIA

by

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ABSTRACT
We have used the anorexia shown by rats provided with hypertonic saline to drink to investigate central mechanisms that can inhibit feeding. Rats dehydrated (DE) in this manner for 3 or 5 days showed a severe attenuation of the compensatory feeding seen after an overnight fast, compared to control euhydrated (EU) rats or rats whose food was restricted to match the intake of anorexic rats. Food intake following injections of 2-deoxy-D-glucose (2DG) was also significantly decreased in DE animals compared to that eaten following a 2DG injection given before DE. However, all the DE animals demonstrated a robust eating response after water was returned, irrespective of whether they had received injection of 2DG or vehicle. Despite a profound reduction in 2DG-induced feeding, other glucoregulatory responses to 2DG remained intact in DE animals. After 2DG injection, both corticosterone secretion and blood glucose were significantly elevated from pre-injection values regardless of whether or not animals were DE. Thus, the mechanisms responsible for anorexia in DE-animals specifically target stimulatory feeding pathways but leave intact other counter-regulatory gluometabolic motor events.

Key Words: corticosterone, plasma glucose, 2-deoxyglucose, overnight fast, dehydration
INTRODUCTION

Anorexia, the loss of appetite for food, is evoked by many different mechanisms. Although it is a
significant complication to a variety of clinical pathologies, the underlying neural mechanisms
responsible for anorexia are largely unknown. To investigate these neural substrates we have taken
advantage of a simple way to stimulate anorexia in animals. When their drinking water is replaced
with hypertonic saline rats not only become progressively dehydrated, they also exhibit a profound
anorexia, which is maintained until access to water is restored (50). In an effort to conserve water and
limit the addition of osmoles to an already compromised fluid compartment, dehydration (DE)
implements a series of adaptive responses that target gastrointestinal function. Thus, salivation, gut
motility, and spontaneous circadian-driven feeding are all reduced in an attempt to resolve or
minimize fluid perturbations at the expense of energy balance (11, 17, 54). In this way, DE anorexia
offers a useful paradigm for investigating how the normal compensatory mechanisms used to trigger
feeding during negative energy balance can be inhibited.

We have already demonstrated that both dehydrated animals and animals that are food restricted
(FR) to match the intake of anorexic rats show the same attributes of negative energy balance (54).
These include body weight loss, diminished circulating leptin and insulin, and increased blood
glucocorticoid concentrations and neuropeptide Y (NPY) gene expression in the arcuate nucleus of
the hypothalamus (ARH). Normally these neural and endocrine processes stimulate compensatory
feeding mechanisms aimed at increasing caloric intake to match expenditure. In this way, weight loss
triggers hunger to restore body energy stores through food intake. Elevated glucocorticoids and
decreased insulin levels can independently stimulate food intake (4, 44), while falling plasma leptin
levels after starvation (9, 19) or the deficiency of leptin in ob/ob mice (8) both stimulate eating.

Feeding behavior is mediated in certain circumstances by the increased activity of those NPY-
producing neurons in the ARH (1, 37) that express leptin receptors (6) and project to the
paraventricular nucleus of the hypothalamus (PVH) and the lateral hypothalamic area (LHA) (6, 15).
NPY injections in the PVH or LHA elicit robust feeding (26, 45, 46), while chronic infusions of NPY leads to hyperphagia and obesity (57). Based on their neuropeptide and endocrine profile, we have proposed that in DE-anorexic animals, some component of these NPY-mediated compensatory mechanisms is inhibited until released by subsequent water intake (52).

The present study was designed to determine whether DE-anorexic rats show reduced feeding responses to two challenges that invoke feeding responses thought to involve NPY-mediated mechanisms: 2-deoxy-D-glucose (2DG) and overnight starvation. NPY appears to be a key mediator of fasting-induced hyperphagia in that food deprivation increases NPY mRNA in the ARH (9), and elevates both NPY levels and release in the PVH (24, 37, 56). 2DG is a glucose analogue that leads to cytoglucopenia by competitively inhibiting glucose utilization. Evidence implicates NPY in the feeding response that follows either central or peripheral administration of 2DG (2, 21, 22, 27). 2DG feeding is mediated by central catecholamine neurons (31, 33, 34), which colocalize NPY and project to the PVH (40). Immunoneutralization of NPY in the PVH impairs 2DG feeding (22), while 2DG-induced glucoprivation increases Fos expression in ARH NPY neurons (27).

2DG offers a further advantage as an experimental tool for delineating the neural circuits underlying anorexia because, in addition to stimulating feeding, it rapidly elicits two other motor responses: sympathetic activation of epinephrine secretion from the adrenal medulla, which leads to hyperglycemia (41); and corticosterone secretion from the adrenal cortex (55), which is driven by increased activation of neuroendocrine CRH neurons in the PVH. Together, these behavioral, autonomic, and neuroendocrine motor events serve to replenish and redistribute metabolic fuels. Examining how other non-behavioral motor control systems function during DE should provide a broader view of how adaptive neural mechanisms function in anorexia.

Some of these results have been already presented in abstract form (38).
MATERIALS AND METHODS

ANIMALS AND PROCEDURES

Adult male Sprague-Dawley rats weighing 235-260 grams were obtained from Harlan Laboratories and housed in suspended plexiglass cages with sanitized wood chips. They were maintained in a temperature controlled room on a 12h:12h light:dark schedule with lights on at 06.00h. Rats were provided with continuous access to food (Teklad rodent diet 8604) and water throughout experiment, except where stated. In some animals, drinking water was replaced with 2.5% saline solution for up to 5 days; in others, the amount of food available was restricted to match that eaten by DE animals (54). We have previously shown that five days of drinking hypertonic saline increases plasma osmolality by approximately 6% (53, 54). Body weights and nocturnal food intake were measured daily throughout the experiment. All procedures have been approved by the local IACUC.

SURGICAL PROCEDURES

Rats were handled daily for approximately 4 days before any surgical intervention and daily thereafter. On the fourth day after arrival, rats designated for 2DG injections were anesthetized with an intramuscular 100μl/kg injection of a 50% solution of Ketamine (100mg/ml) plus xylazine (20mg/ml) and sterile intra-atrial catheters inserted by way of the external jugular vein. Catheters were threaded subcutaneously to the dorsal surface, exteriorized between the scapulae, and then sutured in place. Catheters were flushed daily with sterile heparinized 0.9% saline. Animals were allowed to recover to pre-surgical weight before further manipulation. Only rats with stable weights gains and consistent nocturnal intakes were included in the study.

OVERNIGHT STARVATION

Five groups of animals were used in this experiment. Food intakes were measured in all groups for three days prior to testing. Drinking water was replaced in two groups with 2.5% saline for either 3 (DE-3d, n=5) or 5 days (DE-5d, n=5). Body weights and food intake were then measured twice daily
between 08.00-09.00h and 16.00-17.00h. Food was removed from all cages at 17.00h on the evening of the final night of saline. The following morning at 08.00h a measured amount of food was returned and the food remaining in the cage measured to the nearest 0.1 gram each hour for a total of four hours. At the conclusion of the feeding test, saline was replaced with drinking water and food intake measured after a further hour.

A food restriction (FR) schedule was provided for two other groups of animals maintained on drinking water. Animals were weight-matched to animals DE for 3 (FR-3d, n=5) or 5 days (FR-5d, n=5). They were then given an amount of food at the beginning of each light and dark period equal to that eaten by DE animals (54). The amount of food was calculated for each rat as a percentage of the food eaten per 100g of mean body weight (for two days before beginning the food restriction). On the evening of the second or the fourth day of FR, food was removed completely. Food was returned to animals the following morning and intake measured each hour for the next four hours. A fifth group of euhydrated (EU) animals was allowed continuous access to food (ad-lib, n=5) and water. Food intake was measured in this group for four hours the morning following an overnight fast.

**RESPONSES TO 2-DEOXY-D-GLUCOSE**

Four experiments were performed to determine the effect of DE on the responses to 2DG. Experiment 1 established a dose-feeding response curve for 2DG. Experiment 2 determined the effect of 2 doses of 2DG given 7 days apart to test the validity of using each animal as its own control before and during DE. Experiment 3 then determined the effects of DE on feeding responses to 2DG. In Experiment 4, we measured the effects of DE on the plasma glucose and corticosterone responses to 2DG.

All feeding tests were conducted between 08.00h and 13.00h as follows. At the beginning of the experiment all food and sawdust was removed from cages, animals were weighed and a measured amount of food placed in the test cage for approximately one hour. Equal volumetric doses of vehicle or 2DG (0.1 ml/kg) were then injected into the jugular catheter. Some rats in Experiments 3 and 4
were given a subcutaneous 2DG injection because of blocked jugular catheters. There was no significant difference in response between catheter and subcutaneous injections, so these data were pooled. Following the injection, food consumption was measured by weighing the food remaining in each cage to the nearest 0.1 gram each hour for the next four hours.

Experiment 1

To establish appropriate doses of 2DG for investigating the effects of DE on the feeding response to 2DG, animals maintained with continuous access to water were injected with either vehicle (0.9% saline) or one of four doses of 2DG; 50 mg/kg (n=5), 100 mg/kg (n=4), 200 mg/kg (n=5), and 250 mg/kg (n=6). Food intake was then measured as just described.

Experiment 2

One group of animals (n=5) was tested three times over the next 9 days for food intake after injections of either vehicle or 2DG. On day one, vehicle injections were given to measure baseline food consumption in the test cage. The following day each animal was given a 200mg/kg 2DG injection. Food intake was again measured on day 9 after a 200mg/kg 2DG injection.

Experiment 3

Rats (n=28) were divided into three groups and tested three times over the next 9 days for food intake after injections of either vehicle or 2DG. Vehicle injections were given on day one to all animals to establish baseline food consumption in the test cage. The following day (day 2) each animal was given a vehicle, 200, or 250 mg/kg 2DG injection. On day four, drinking water was replaced with 2.5% saline. Food intake was again measured on day 9, the fifth day of DE after a vehicle, 200, or 250 mg/kg 2DG injection. Each animal received the same treatment on days 2 and 9. At the conclusion of the four-hour feeding test, drinking water was returned to all DE animals and food intake measured during the following hour.
Experiment 4

The design of this experiment was virtually identical to Experiment 3 except that blood samples were taken from the jugular catheter for plasma glucose and corticosterone determinations. Animals were tested three times after equal volumetric injections of 0.9% saline vehicle or 2DG (0.1ml/kg). Venous blood samples (150µl) were collected from the jugular catheters of all animals immediately before and 15, 30, 60, and 120 minutes after injection. Food was removed for 2 hours before baseline blood collection and not returned until after the final blood sample.

On day one, all animals received an injection of vehicle to determine baseline responses; on day three, animals received an intra-atrial injection of vehicle (n=4) or 200 mg/kg 2DG (n=8). Drinking water was replaced with 2.5% saline at 12.00h on the following day. On the morning of the fifth day of DE, rats were injected with vehicle or 200 mg/kg of 2DG. Drinking water and food was replaced at the conclusion of the blood sampling. Only animals that exhibited feeding response of more than 3g to drinking water were included in the study.

Blood samples were immediately placed on iced, centrifuged, plasma removed, and stored at 20ºC until assayed. Plasma glucose concentration was assayed using a YSI 2700 auto-analyzer (Yellow Springs, OH). Plasma corticosterone concentrations were determined by double antibody radioimmunoassay (48) using a commercial available kit (ICN Pharmaceuticals). All samples were assayed in duplicate in single assays. Internal controls were within appropriate ranges.

STATISTICS

Data are expressed as means ± SEM. Changes in body weight during DE, food intake suppression, food intake after overnight starvation, and amount eaten after return of water were compared between groups using one-way ANOVA. For analysis of plasma glucose and corticosterone responses, incremental increases were calculated for each animal by subtracting the maximum concentration attained from the pre-injection value. Baseline EU tests were compared to EU 2DG tests in experimental animals using ANOVA with repeated measures at each time point. Data from EU and
DE animals were compared using a 2 x 2 repeated-measure ANOVA. Bonferroni’s multiple comparison test was used to measure individual differences. The critical level for significance was set at p<0.05 for all comparisons.
RESULTS

FEEDING RESPONSES TO OVERNIGHT STARVATION

Overnight starvation elicited significantly different amounts of compensatory feeding in DE, FR, and ad lib groups (at hour 4, F [df 4,25] =45.92, P< 0.001). Figure 1A shows that the amount of food eaten after 4 hours by FR-5d animals was significantly greater than that consumed by ad lib animals (p<0.05). However, both groups of DE animals ate significantly less after overnight starvation than both FR and al lib animals, with the DE-5 animals eating significantly less than the DE-3 animals (p< 0.01). This represented a 60% and 80% reduction compared to that displayed by the ad lib fed EU group (Fig. 1B). However, all DE animals displayed a robust eating response during the hour after access to drinking water was restored (Fig. 1A), the size of which was not significantly different between the two DE groups.

FEEDING RESPONSES TO 2-DEOXY-D-GLUCOSE

Changes in body weight over the course of the feeding tests are shown in Table 1. There was no significant difference in rate of weight gain among the animals in the three treatment groups before or during the testing period. At the end of DE, rats in each treatment group lost a similar amount of body weight and exhibited equivalent anorexia (Table 1).

Experiment 1

Figure 2 shows the amount of food eaten after injection of increasing doses of 2DG. 50 mg/kg or 100 mg/kg 2DG elicited a feeding response that was no different than vehicle at all time points except at hour 4, when the response to 100 mg/kg was significantly greater (p<0.05) than vehicle or 50 mg/kg (Fig. 2B). After either 200 or 250 mg/kg 2DG animals ate significantly more than vehicle at all times (p<0.01). Feeding responses to vehicle injection and 50 mg/kg of 2 DG were indistinguishable. Based on the results of this experiment, 200 or 250mg/kg were used to test the effects of DE on feeding responses in subsequent experiments.
Experiment 2

EU animals given two injections of 200mg/kg 2DG separated by 7 days responded to the second injection in a manner indistinguishable from the first (Fig. 3). One-way repeated measure ANOVA indicated a significant effect of 2DG on food intake (F [df 2,12] = 16.33, P< 0.001). Both the first and second dose of 2DG elicited significantly more food intake than vehicle at all time points (p<0.01). However, the amount of food intake eaten after the first injection was not significantly different from the amount of food intake after the second injection at any time.

Experiment 3

Injection of vehicle did not elicit a significant eating response in any of the three groups of animals (Fig 4A), but after administration of either 200 mg/kg or 250 mg/kg of 2DG these animals ate significantly more (Fig. 4B; F [df 2,37] = 48.33, p<0.001). Figure 4C shows DE significantly reduced the ability of 2DG to elicit food intake. Two-way repeated measure ANOVA revealed a main effect of DE (F [df 2, 25]= 44.25, P<0.001) and an interaction effect between 2DG and DE (F [df 2, 25] = 12.3, P<0.001). Finally, all DE animals ate similar amounts of food when drinking water was returned at the end of the 2DG-feeding test, irrespective of whether they were injected with vehicle or one of the two doses of 2DG four hours previously (Fig. 4D).

Experiment 4

Effects of DE on the Responses of Plasma Glucose Concentrations to 2DG. Plasma glucose results are presented in Table 2 and Figure 5. Vehicle injections did not significantly increase plasma glucose concentrations in any treatment group on day 1 (Fig. 5A). On day 3, animals were injected with either vehicle or 200mg/kg 2DG (Fig. 5B). There was no significant response to vehicle, but 2DG elicited a significant increase in plasma glucose from pre-injection values (F [df 4,35] = 13.25, p < 0.001). On day 9, pre-injection plasma glucose concentrations were no different to pre-injection values on days 1
and 3 (Table 2). After injection of vehicle or 2DG, mean plasma glucose concentrations in DE animals were again unaffected by vehicle injection, but were significantly increased by 2DG from pre-injection values (Fig. 5C; F [df 4,33] = 9.90, p < 0.001).

Two-way ANOVA with repeated measures indicated that 2DG administration was a significant main effect at all time points after injection (at 60 minutes, F [df 1,10] = 85.78, p < 0.001). However, DE was not significant as either a main or an interactive effect at any time point measured. DE did not significantly alter the incremental increase in glucose elevation elicited by 2DG from that seen in EU animals at any time point. Additionally, there was no significant difference in changes of plasma glucose after vehicle injection between animals in the EU and DE states.

Although DE did not significantly alter the magnitude of the plasma glucose response to 2DG, there was an apparent difference in the kinetics of the response. Peak mean glucose concentrations were seen at 15 minutes on day 3 (EU), but at 120 minutes on day 9 (DE). This was partly due to very high values in the 15min samples from two animals on day 3. Removing these values reduced the variance of the mean at 15mins in such a way that peak values were now seen at 60mins in these animals.

**Effects of DE on the Responses of Plasma Corticosterone Concentrations to 2DG.** Corticosterone (CORT) responses to vehicle or 2DG are illustrated in Table 2 and Figure 6. Vehicle injections did not significantly increase plasma corticosterone concentrations from pre-injection values in any treatment group on days 1, 3 or 9 (Fig. 6A). However, 2DG injections significantly increased plasma corticosterone concentrations on day 3 in EU animals (F [df 4,35] = 9.61, P< 0.001, Fig. 6B).

Following 5 days of DE on day 9, pre-injection levels of CORT were significantly elevated from those measured at day 3 (F [df 1,10] = 6.8, P< 0.05, Table 2). However, 2DG injections still significantly increased plasma corticosterone concentrations from pre-injection values in DE animals (F [df 4,33] = 3.95, p< 0.01, Fig. 6C).

Two-way ANOVA with repeated measured revealed a significant main effect of 2DG on plasma corticosterone concentrations at the time (60 mins) when mean maximum value was attained after
2DG injections (F [df 1,10] = 82.12, p<0.001, Fig. 6C). Finally, there was no significant interaction effect at any time point. There was also no significant difference between the incremental increase of CORT in animals receiving 2DG, regardless of hydration state at any time point after the pre-injection measurement.
DISCUSSION

Our present results demonstrate two points regarding the control of energy balance in DE-anorexic rats. First, that normal plasma glucose concentrations are maintained during DE-anorexia, presumably because metabolism is now biased towards increased glycogenolysis and lipolysis. This observation, taken together with the fact that DE-anorexic and paired-FR animals have virtually identical endocrine and neuropeptidergic responses to negative energy balance (54), shows that DE-anorexic animals maintain a normal metabolic response to reduced food intake. Of course, the critical difference between FR and DE-anorexic animals is the decreased drive to eat in the latter.

Second, we show that DE for as little as three days results in a severe attenuation of the compensatory food intake that normally occurs after an overnight fast. DE animals also eat less in response to doses of 2DG that elicit feeding in the same rats before DE, and have been shown by others to produce eating (35, 43). These observations are consistent with a previous study showing 2DG-induced food intake is attenuated in water-deprived rats (49). DE animals therefore do not seek to repair either an actual (from overnight starvation) or a perceived (from 2DG) caloric deficit until after access to water has been restored. At this point DE-anorexic animals reliably begin robust compensatory feeding within ten minutes from being given back drinking water (50). This rapid eating response clearly demonstrates that DE animals will eat with appropriate stimulation, and that the mechanisms responsible for inhibiting feeding to a variety of stimuli are quickly counteracted by drinking water. The mechanisms responsible for the reversal of this anorexia are currently unknown.

2DG-induced glucoprivation rapidly elicits a triad of compensatory motor responses aimed at mobilizing glucose stores and replenishing energy supplies: increased secretion of epinephrine to produce hyperglycemia, glucocorticoid release, and feeding. These complimentary processes are activated more or less simultaneously and promote glucose delivery to the brain. However, the fact that under certain circumstances they can be uncoupled, demonstrates that their control mechanisms are not tightly linked. For example, phlorizin and alloxan, which inhibit glucose transport and glucose
oxidation respectively, both elicit eating behavior but not hyperglycemia when injected into the fourth ventricle (18, 36). Similarly, area postrema lesions impair feeding after 2DG administration but leave intact both hyperglycemic and the corticosterone secretory response (13). In this regard, we show that DE-anorexics retain the ability to mount both a hyperglycemic and a glucocorticoid secretory response to the same dose of 2DG that fails to stimulate eating. DE therefore specifically targets pathways associated with stimulating food intake, while leaving intact those mechanisms responsible for neuroendocrine and sympathetic glucometabolism-related motor events.

Repeated daily 2DG administration can impair the feeding response to 2DG (39), possibly as a result of a chronic elevation of circulating glucocorticoids (10). In the present study we have confirmed previous reports that dehydrated animals show increased plasma corticosterone levels in the morning (51). However, the mechanisms responsible for suppressing feeding in these animals are most likely different from those arising after repeated daily 2DG, and three observations suggest that this suppression is probably not a consequence of these increased plasma corticosterone concentrations. First, we show that non-DE control rats receiving two 2DG challenges 7 days apart show identical feeding responses to each challenge. Second, the morning elevation in plasma corticosterone levels seen in DE animals remains well below the peak values attained after 2DG injection in EU animals (present study; (51). Finally, unlike DE, repeated daily injections of 2DG not only attenuate the feeding response, they also abolish the 2DG-induced hyperglycemia (39).

The neural mechanisms that control feeding following glucoprivation or deprivation and are not fully understood, but the large body of data implicating NPY/catecholaminergic neurons in the hindbrain and NPY/agouti-related protein-containing neurons located in the ARH provides a framework for discussing our results with regard to the neural substrates of anorexia.

Injections of an anti-dopamine β-hydroxylase-saporin conjugate (D-SAP) into the terminal regions of catecholaminergic neurons will specifically destroy these neurons (31). Ritter and colleagues have recently taken advantage of this specificity to show that D-SAP injected into the PVH blocks both the
feeding and corticosterone responses to 2DG, but leaves intact the hyperglycemic response (31, 32). In contrast, D-SAP injected into the spinal cord destroys catecholaminergic neurons with descending connections and blocks the hyperglycemic response to 2DG while leaving the feeding and corticosterone response intact (31, 32). These data demonstrate that different subsets of hindbrain catecholaminergic neurons mediate the behavioral, autonomic, and neuroendocrine components of the glucoprivic response.

We show that DE-anorexic rats have hyperglycemic and corticosterone responses to 2DG that are indistinguishable from controls. This demonstrates that the inhibitory mechanisms present in DE-anorexia rats do not impact either those ascending and descending catecholamine pathways that target CRH neuroendocrine neurons and mediate glucocorticoid responses, or those pre-ganglionic neurons in the spinal cord that mediate hyperglycemia. In addition, our results do not support the view that neuroendocrine CRH neurons in the PVH are involved with compensatory feeding behaviors (54); corticosterone secretion remains viable in DE animals while feeding is markedly impaired. This notion is also supported by the fact that electrolytic lesions of the PVH do not hinder glucoprivic feeding (7, 42). Collectively, these data suggest that 2DG-induced feeding requires sets of hypothalamic neurons located outside the PVH, and that these systems are potential targets for DE-generated inhibition.

In conclusion, we have shown previously that the anorexia that develops after drinking hypertonic saline inhibits spontaneous nocturnal feeding (50). The present study shows this anorexia also involves an inhibition of two other types of feeding; compensatory feeding in response to overnight starvation, and the feeding that usually follows glucoprivation. The fact that both hyperglycemic and the glucocorticoid responses to 2DG remain intact in DE-anorexic animals shows that DE specifically targets those mechanisms that control the motor events of feeding behavior, but not neuroendocrine or autonomic motor responses.
Evidence suggests specific alterations to feeding mechanisms in the ARH are not responsible for DE-anorexia (54). Instead, DE-anorexia appears to be generated by activity in separate inhibitory circuits. These circuits may involve CRH, neurotensin or oxytocin neurons found in those parts of the perifornical LHA and PVH that are targeted by plasma osmolality-sensitive rostral hypothalamic afferents (25, 28, 29, 54). In this regard, several lines of evidence support the idea that a critical component for the development of DE-anorexia is located within the LHA, particularly its perifornical part (LHApf). NPY-containing projections from the ARH to the LHApf are important for stimulating those types of feeding initiated by changes in the levels of circulating hormones such as leptin (15, 16). Similarly, hindbrain adrenergic and noradrenergic neurons activated by 2DG co-localize NPY (40) and project to both the PVH and LHA (14, 47). Furthermore, neurons within the LHA express NPY receptors (12, 20) and injections of NPY into the LHApf produce strong feeding responses (46). We suggest that DE in some way inhibits the output of those NPY-containing circuits that normally elicit food intake in response to caloric deficits. This hypothesis is consistent with certain other types of anorexia where animals exhibit a suppressed feeding response to central injections of NPY, have increased NPY gene expression in ARH, and show increased NPY release in the PVH (3, 5, 23, 30). However, unlike these other models of anorexia, DE-anorexia is rapidly and completely reversed within minutes simply by restoring access to drinking water, making it a particularly useful model with which to investigate the neural substrates of anorexia.

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TABLES

Table 1: The effects of drinking 2.5% saline on body weights and mean nocturnal food intake.

There were no significant differences between each treatment group in either the mean (± S.E.M.) body weights at the start of the experiment, the rate of increase before DE, or the decline in body weight after DE. Similarly, DE was associated with a similar decrease in mean (± S.E.M.) nocturnal food intake in all three groups of animals.

Table 2: The effects of drinking 2.5% saline on plasma glucose and corticosterone responses to injections of vehicle or 2-deoxy-D-glucose.

Injection of vehicle (VEH; 0.9% saline) did not increase mean (± S.E.M.) plasma glucose and corticosterone either before (Days 1 and 3 EU) or after drinking 2.5% saline (DE; Day 9). Similarly, 2-deoxy-D-glucose (2DG; 200mg/kg) injections significantly increased plasma glucose and corticosterone concentrations in all treatment groups. Pre-injection values (pre) were determined on blood samples taken before injection of VEH or 2DG. The maximum absolute value attained after injection (max.) is given for each treatment group. See text for levels of statistical significance.
**FIGURE LEGENDS**

**Figure 1:** Hypertonic saline ingestion (DE) inhibits feeding in response to overnight starvation. Mean (± SEM) cumulative weight of food eaten after overnight starvation (A) following 5 days of DE (open triangles), 3-day DE (open squares), ad lib (open circles), 3-day food restriction (FR; closed squares), and 5-day FR (closed triangles). When water was returned to DE animals there was a robust eating response measured 1 hour later (dashed lines). (B) Mean (± SEM) total weight of food eaten 4 hours after food replacement by FR (solid line) or DE (dashed line) animals after overnight starvation and various times of FR or DE. Data are expressed as a percentage of the response shown by ad-lib fed animals (100%). See text for levels of statistical significance.

**Figure 2:** The feeding response to various doses of 2-deoxyglucose injections. (A), Mean cumulative intake (± SEM) each hour for four hours after injection of vehicle (open circles), 50 (closed squares), 100 (closed triangles), 200 (closed circles) or 250 (closed diamonds) mg/kg of 2-deoxyglucose (2DG). (B), Relationship between dose of 2 DG and food eaten 2 hour after injection. See text for levels of statistical significance.

**Figure 3:** The feeding response to doses of 2-deoxyglucose injections repeated at 7 day intervals. (A) Mean cumulative intake (± SEM) each hour for four hours after injection of vehicle (open circles), or (B and C) 200 mg/kg 2DG. The second dose of 2DG in C) was given 7 days after the first (B).

**Figure 4:** The effects of 5 days of hypertonic saline ingestion on the feeding response to 2-deoxyglucose injections. Mean (± SEM) cumulative food intake in the same group of animals after injection of vehicle (open symbols), 200 mg/kg 2DG (squares) or 250 mg/kg 2DG (triangles). Food intake is shown after injection of vehicle (A), 2DG given 1 day later (B), and 2DG given after 5 days of hypertonic saline ingestion (C). Food intake is also shown in animals given vehicle injection using the same time schedule (B and C, open circles). (D) illustrates the mean (± SEM) food intake of the same animals shown in C) 1 hour after water was returned at the conclusion of the 4 hour 2DG test.
**Figure 5:** The incremental increase in mean (± SEM) plasma glucose concentrations above pre-injection values (0 mins) in animals after injection of vehicle (A), 200 mg/kg 2DG given 1 day later (B, closed squares), and 200 mg/kg 2DG given after 5 days of hypertonic saline ingestion (C, closed squares). The change in mean plasma glucose concentrations after vehicle injection are shown in animals before (B, open circles) and after 5 days of hypertonic saline ingestion (C, open circles).

**Figure 6:** The incremental increase in mean (± SEM) plasma corticosterone concentrations above pre-injection values (0 mins) in animals after injection of vehicle (A), 200 mg/kg 2DG given 1 day later (B, closed squares), and 200 mg/kg 2DG given after 5 days of hypertonic saline ingestion (C, closed squares). The change in mean plasma corticosterone concentrations after vehicle injection are shown in animals before (B, open circles) and after 5 days of hypertonic saline ingestion (C, open circles).
Table 1: The effects of drinking 2.5% saline on body weights and mean nocturnal food intake.

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<td>baseline (2 day ave)</td>
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<td>18.6 ± 0.6</td>
<td>18.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>decrease with DE (%)</td>
<td>59.7 ± 4.6</td>
<td>65.8 ± 4.8</td>
<td>64.9 ± 4.2</td>
<td></td>
</tr>
<tr>
<td><strong>Animals per group</strong></td>
<td>8</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: The effects of drinking 2.5% saline on plasma glucose and corticosterone responses to injections of vehicle or 2-deoxy-D-glucose.

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mg/dl)</th>
<th>Corticosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre.</td>
<td>max.</td>
</tr>
<tr>
<td><strong>Day 1 (EU)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEH</td>
<td>104.6 ± 3.0</td>
<td>112.0 ± 3.0</td>
</tr>
<tr>
<td><strong>Day 3 (EU)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEH</td>
<td>112.0 ± 4.3</td>
<td>114.3 ± 5.6</td>
</tr>
<tr>
<td>2DG</td>
<td>105.5 ± 1.2</td>
<td>270.0 ± 34.3</td>
</tr>
<tr>
<td><strong>Day 9 (DE)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEH</td>
<td>98.5 ± 11.0</td>
<td>113.5 ± 25.9</td>
</tr>
<tr>
<td>2DG</td>
<td>106.4 ± 4.6</td>
<td>246.3 ± 29.6</td>
</tr>
</tbody>
</table>
Figure 1

A) Cummulative food eaten (g) vs. Time (h) with error bars indicating standard deviation.

B) Food eaten in 4h (% of ad lib) vs. Time (days) with error bars indicating standard deviation.

water returned to dehydrated animals
Figure 2
Figure 3
Figure 4
Figure 5