Glucoregulatory Feeding by Rats after Intraventricular 6-Hydroxydopamine or Lateral Hypothalamic Lesions

Abstract. *Rats given intraventricular injections of 6-hydroxydopamine or bilateral electrolytic lesions of the lateral hypothalamus do not show the normal increase in food intake in response to large decreases in glucose utilization or exposure to severe cold stress. However, they will eat more during chronic glucoprivation that is less intense, or during exposure to more moderate cold stress. Thus, the feeding deficits of these lesioned rats may not reflect an inability to respond to certain qualitatively different stimuli, but rather an inability to respond to quantitatively different intensities of the same stimulus.*

Lesions of the lateral hypothalamus, or of extrahypothalamic sites, which interrupt the dopamine-containing neurons of the nigrostriatal bundle produce aphagia and adipsia in rats (*1*). When these animals resume voluntary feeding and drinking behaviors, it has been reported that, unlike neurologically intact rats, they do not increase their food intake in response to the sudden decreases in glucose utilization (glucoprivation) that occur after treatment with insulin (*2*) or 2-deoxy-D-glucose (2-DG) (*3*). This finding has had an important influence on theories of hunger, because it suggests (i) that there are specific controls for glucoregulatory feeding in the brain, (ii) that lesions which destroy dopamine-containing neurons in the brain abolish feeding responses to glucoprivation but not to other stimuli for hunger, (iii) that there are multiple stimuli for hunger which are separable, and (iv) that the well-known stimulus for food intake that is provided by glucoregulatory needs (*4*) may not be essential for intake of freely available food. Our studies presented here dispute all these inferences by providing evidence that lesioned animals can, in fact, increase their food intake in response to moderate glucoregulatory needs. These and other results further suggest that the long-term impairments of lesioned rats may involve the disruption of nonspecific components of motivation rather than individual regulatory behaviors.

Male albino rats (300 to 350 g) of the Sprague-Dawley strain (Zivic-Miller, Pittsburgh), were housed and tested in individual wire-mesh cages. They were allowed free access to Purina Chow pellets and tap water unless otherwise noted. Two experimental groups were prepared for study, each with appropriate controls. The first group (n = 26) received bilateral electrolytic lesions of the lateral hypothalamus (*5*), while the second group (n = 6) were given intraventricular injections of 6-hydroxydopamine (*6*), in order to permanently deplete brain catecholamines (*7*). The six rats given large electrolytic lesions became aphagic for 2 to 9 days, lost at least 20 percent of their body weights, and had to be maintained by intragastric tube feeding and access to palatable foods and fluids until, after 12 to 137 days, they again ate dry chow and drank water. The other lesioned animals became hypophagic and hypodipsic, and lost 6 to 14 percent of their body weights, for 1 to 2 days after lesioning but quickly recovered normal ingestive behaviors.

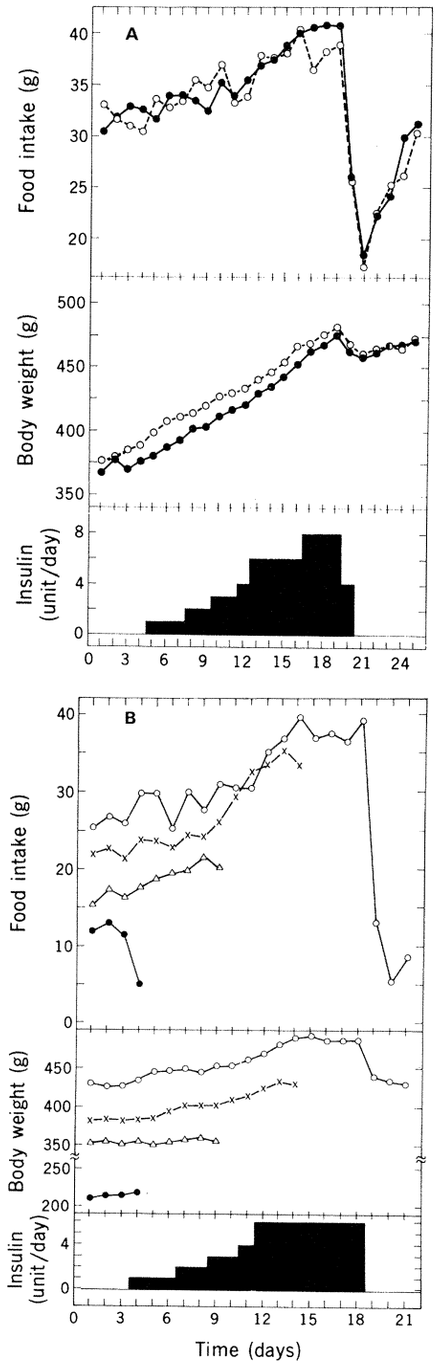

Fig. 1. (A) The effects of daily injections of protamine-zinc insulin on food intakes and body weights of vehicle-injected control rats (○) and rats that had received two intraventricular injections of 6-hydroxydopamine (200 μg each) (●). Each point represents mean values from five animals. Not included are the comparable results from a sixth rat with biochemical lesions, which died after 13 days of insulin treatment. (B) The effects of daily injections of protamine-zinc insulin on food intakes and body weights of rats that had been given bilateral electrolytic lesions of the lateral hypothalamus. Each symbol depicts a representative animal. Of 13 lesioned rats that were tested, only four survived the entire 15 days of treatment; the others lasted for 1, 4, 4, 6, 6, 8, 10, 11, and 13 days before the experiments were terminated. All four sham-lesioned control rats survived and showed increases in food intake and body weight that were comparable to those of control rats in (A).

Testing began 2 to 3 weeks after the return of voluntary food and water ingestion. For three successive days, food intake (with water present) was measured every hour for 7 hours (9:30 a.m. until 4:30 p.m.). On day 4, after the first hour of testing, each rat was injected intraperitoneally with 750 mg of 2-DG per kilogram of body weight, a dose that stimulates maximal food intake by intact rats (*8*). Food intake was monitored hourly for the following 6 hours. Control rats increased their feeding within the first hour after injection and consumed significantly more food during the test session than they had on previous days (mean difference = + 4.4 g; n = 9; $P < .001$). In contrast, none of the lesioned rats showed a significant feeding response to 2-DG (mean difference = + 0.4 g for six rats with biochemical lesions; −0.8 g for 26 rats with electrolytic lesions) (*9*). Since most of these animals did not show pronounced initial impairments, these results indicate that the feeding deficits of lesioned rats need not be a residue of prior aphagia.

Within 1 week after demonstration of failure to respond to acute decreases in glucose utilization, rats were made chronically hypoglycemic with periodic injections of protamine-zinc insulin. In order to reduce the possibility of lethal hypoglycemia, the insulin was administered in doses no greater than 1 to 2 units per subcutaneous injection, and no more frequently than at 6-hour intervals. Rats received increasing doses, from 1 to 8 units per day, over a 2- to 3-week period. In accord with previous reports (*10*), the food intakes and body weights of nine control rats showed progressive increases after the onset of insulin treatment. When the treatments were terminated, there was a temporary reduction in food intake, and body weights declined abruptly toward normal. Changes in food intake and body weight that were identical to those found in control animals were ob-

served in rats given biochemical (Fig. 1A) or electrolytic lesions (Fig. 1B). Although these experiments often were terminated by the incapacitation of the animals and other signs of hypoglycemic shock, hyperphagia and body weight gain were observed in each lesioned rat while the tests lasted, with the exception of three animals given large electrolytic lesions, which died during the first 4 days of insulin treatment.

These findings indicate that lesioned animals which do not increase their food intakes in response to single injections of 2-DG nevertheless are capable of responding to glucoregulatory needs presented less abruptly. Their ability to respond to glucoprivation produced by protamine-zinc insulin seems to be related both to the size of lesions and the magnitude of regulatory imbalance; that is, rats seem more likely to respond if both lesion and need are small, but become less likely to eat as either is increased. If the behavioral deficits produced by lesions reflect both the animal's residual capacities and the situational demands, then the failure of rats with large lesions to eat after severe glucoprivation (*2, 3*) would seem to be based on the magnitude of the stress, rather than its quality, in such animals.

Impairments in the feeding responses of lesioned rats after injections of insulin or 2-DG often are contrasted with the increased food consumption that occurs when the need for caloric intake is increased during exposure to a cold environment (*2, 3*). In confirmation of these findings, we observed that ten rats with small electrolytic lesions of the lateral hypothalamus, which did not increase their food intakes in response to 2-DG at 750 mg/kg, did increase their food intakes by more than 20 percent during the first few days of exposure to 5°C and maintained their hyperphagia throughout a 3-week period of testing (Fig. 2). Such results have been interpreted as indicating that the lesions had differential effects on glucoregulatory and thermoregulatory controls of feeding (*2, 3*). However, 1 month later, when these animals were shaved before being placed into the cold again, seven ate little and died within the first 2 days, while the other three initially ingested normal amounts of food and did not become hyperphagic for 4 to 7 days. Five control rats that were shaved and deprived of food also died within the first 2 days; however, five additional shaved control rats that were given food increased their intakes by the second or third day (Fig. 2). Furthermore, two rats with larger electrolytic lesions of the lateral hypothalamus did not increase their food intakes when exposed to 5°C without being shaved and lost body weight continually for 10 days, at which point testing was terminated; a third such rat, which did become hyperphagic and maintained body weight for 12 days of exposure, suddenly reduced its food intake and lost body weight precipitously until testing was terminated 10 days later (*11*).

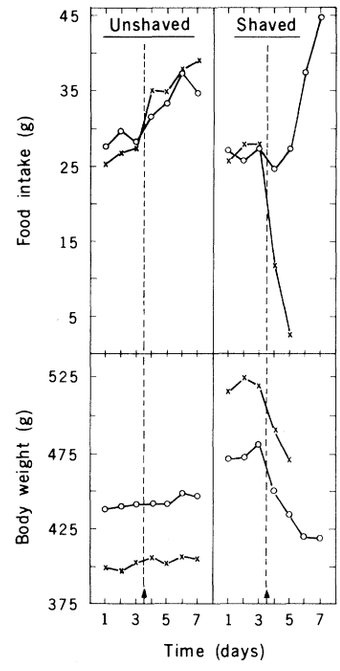

Fig. 2. The effects of continuous exposure to 5°C on food intakes and body weights of rats that had been given bilateral electrolytic lesions of the lateral hypothalamus. Values are for a representative control (○) and lesioned rat (×). Rats were either unshaved (left) or shaved (right) just before being placed in the cold environment (arrow).

These results indicate that lateral hypothalamic lesions do not fractionate separate controls of feeding behavior. As with acute glucoprivation, lesioned rats will not increase their food intake soon after being exposed to a cold environment if their lesions are unusually large or if the cold stress is made severe (in this case, by prior removal of the insulation provided by the fur). Thus, the permanent feeding deficits of lesioned rats after their apparent recovery may not reflect an inability to respond to certain qualitatively different stimuli, but rather to quantitatively different intensities of the same stimulus. Consequently, the failure of brain-damaged rats to eat during metabolic emergencies should not imply that they will be unable to respond appropriately to more moderate and gradually developing homeostatic imbalances such as arise when food is continuously available in the normal laboratory environment.

Insulin and 2-DG treatments appear to elicit feeding due to cerebral glucoprivation, an extreme and unusual stress (*8, 12*). Our results suggest that rats with large lesions cannot respond behaviorally to such stimuli. In retrospect, it seems clear that other experimental treatments that have been effective in revealing residual deficits in animals bearing extensive damage to central catecholamine-containing neurons also involve the abrupt onset of large nutritional needs, often requiring maximal behavioral responses in intact rats. For example, the first reports of permanent deficits in rats with lateral hypothalamic lesions described their inability to show a normal rapid drinking response after acute dehydration of the intracellular or intravascular fluid compartments. These early observations were interpreted as indicating that osmo- and volume-regulatory controls of thirst had been abolished by the lesions, and thus ad libitum drinking was attributed to nonregulatory mechanisms (*13*). However, our recent work has indicated that lesioned rats which do not drink at first will increase their water intakes 10 to 15 hours later if the dehydrational stimuli are prolonged (*14, 15*). These results complement our findings reported here and indicate that specific regulatory controls of drinking behavior are not abolished by electrolytic or biochemical lesions of central catecholamine-containing neurons.

Recent studies also have demonstrated that after hypothalamic lesions or intraventricular 6-hydroxydopamine there are disruptions in a much wider range of motivated activities than feeding and drinking, including mating, maternal and thermoregulatory behaviors, and avoidance of punishment. While it is possible that separate clusters of catecholaminergic neurons are involved in the mediation of each of these behaviors, it seems more likely that the neurons collectively are involved in some function that is common to all of the behaviors. In this regard, there is considerable evidence supporting a role of brain catecholamines in cerebral and behavioral states of arousal (*15, 16*), and thus these may have been the nonspecific contributions to voluntary behavior that were impaired by the biochemical and electrolytic lesions in our experiments.

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5. Eact rat was placed in a stereotaxic instrument with its skull positioned flat, and the electrode was placed 6.0 mm anterior to the interaural line, 2.0 mm lateral to the sagittal sinus, and 8.0 mm ventral to the dura of the cortex. Lesions were made with a monopolar stainless steel electrode by passing a 1-ma anodal current for 10 seconds (n = 20), or a 2-ma anodal current for 20 seconds (n = 6).
6. 6-Hydroxydopamine hydrobromide (Regis Chemical) was used. Rats received 20 μl of either 200 μg of 6-hydroxydopamine (the dose given is of the free base) or the vehicle (0.9 percent NaCl, 0.1 percent ascorbic acid) into the cerebrospinal fluid by way of the left lateral ventricle. Three days later the same injections were delivered by way of the right lateral ventricle of each rat.
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11. At the conclusion of the experiments, the remaining five rats with biochemical lesions were killed by decapitation, and the brain of each animal was rapidly removed from the skull and dissected on Dry Ice. Subsequent fluorometric analysis revealed that mean levels of norepinephrine and dopamine after 6-hydroxydopamine treatment were 0.02 and 0.34 μg per gram of fresh telencephalic tissue, respectively, in comparison to control values of 0.22 and 0.83 μg/g. The remaining ten rats with electrolytic lesions were killed with an overdose of anesthetic and perfused with 10 percent formalin, and their lesions were subsequently located by microscopic examination of stained brain sections. Fairly symmetrical bilateral destruction of the most lateral portions of the lateral hypothalamus, at the level of the ventromedial nucleus, was observed in each brain, with significant damage invading the internal capsule; with the larger lesions, perifornical hypothalamic tissue and much of the zona incerta and subthalamus also were destroyed.
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Glucagon Release: Paradoxical Stimulation by Glucose During Calcium Deprivation

Abstract. *During calcium deprivation, the rate of glucagon release by the isolated perfused rat pancreas is positively related to the glucose concentration of the perfusion medium. It is suggested that such a paradoxical behavior, which is reminiscent of the abnormality of glucagon secretion recently disclosed in diabetic subjects, results from a perturbation in the normal structural and functional bridging between pancreatic alpha and beta cells.*

Whereas calcium is an essential requirement for most exocrine and endocrine secretory processes (*1*), the release of Parathormone (*2*) and, more recently, that of glucagon (*3*) were shown to be enhanced during calcium deprivation. The latter finding was documented by incubating pieces of pancreas obtained from duct-ligated rats in media containing a fixed concentration of glucose (8.3 m*M*). In the experiments reported here, we further investigated the effect of lowered calcium levels on the secretion of glucagon, using the more sensitive and dynamic technique of perfusion of the rat pancreas and performing the experiments at various glucose concentrations (3.3, 5.5, 8.3, and 16.6 m*M*). This procedure led us to discover a paradoxical stimulation of glucagon release by glucose during calcium deprivation. It is proposed that such a phenomenon, which is reminiscent of the situation encountered in human diabetes mellitus, may shed light on the significance of the structural and presumably functional coupling between different types of endocrine pancreatic cells, as recently revealed by the freeze-etching technique (*4*).

Fed female albino rats, with a mean body weight of 220 g, were used. The pancreases were dissected under sodium pentobarbital anesthesia (48 mg per kilogram of body weight, intraperitoneally) by the procedure described by Loubatières *et al.* (*5*); all of the adjacent organs, including the duodenum, were excluded. The pancreases were perfused in situ, through the coeliac and superior mesenteric arteries by means of a cannula inserted in the aorta, the effluent being collected without recycling from the portal vein. The flow rate ranged between 1.7 and 2.0 ml/min. The perfusion medium was a Krebs-Ringer bicarbonate buffer (*p*H 7.4) containing bovine albumin (4 g/100 ml; Pentex, fraction V, Miles Laboratories), equilibrated against a mixture of O_2 and CO_2 (95 : 5), constantly filtered (pore size, 1.2 μm), and warmed to 37°C at the entrance of the pancreas (*6*). Glucose (3.3, 5.5, 8.3, or 16.6 m*M*) was present throughout the 80 minutes of perfusion, the first hormonal measurements being performed 25 minutes after the onset of the perfusion (– 15 minutes in Figs. 1 and 2). Calcium was added to the perfusion medium by a sidearm syringe to attain a theoretical concentration of 2 m*M* during the initial 40-minute equilibration period. Calcium deprivation was induced by stopping this calcium infusion (Fig. 1). Upon assay, the total calcium concentration averaged 1.92 ± 0.21 m*M* (n = 14) during the equilibration period and 0.17 ± 0.01 m*M* (n = 14) during calcium deprivation (*7*). The effluent was collected every minute in chilled tubes containing 2000 kallikrein inhibitor units of Trasylol (*8*). Glucagon and insulin were estimated by radioimmunoassay, with beef and pork glucagon and rat insulin as standards (*9*).

Glucose in high concentration (8.3 and 16.6 m*M*) stimulated insulin release at the high calcium level (the equilibration period), this stimulant action being inhibited during the period of calcium deprivation (Fig. 1, C and D). The true degree of inhibition of insulin release at the two highest glucose concentrations was more marked than that suggested in Fig. 1; indeed, in control experiments in which the high calcium level was maintained throughout, the rate of insulin release evoked by glucose progressively increased during the second part of the experiment (Fig. 2). The output of glucagon during the equilibration period, that is, at the high calcium level, was inversely related to the glucose concentration of the perfusion medium (Fig. 1; Table 1, minutes – 15 to 1). These findings are in agreement with the known effects of glucose and calcium on the pancreatic beta

Table 1. Mean glucagon output by the perfused rat pancreas during the control period and the early and late periods of calcium deprivation, at various glucose concentrations; n, number of experiments.

Glucose (m*M*)	n	Mean glucagon output (pg/min)		
		Control period	Calcium deprivation	
		Minutes – 15 to 1 (mean ± S.E.M.)	Minutes 2 to 7 (mean ± S.E.M.)	Minutes 8 to 30 (mean ± S.E.M.)
3.3	5	694 ± 128	1049 ± 183	377 ± 57
5.5	8	451 ± 35	804 ± 74	470 ± 81
8.3	7	256 ± 68	459 ± 55	615 ± 104
16.6	6	203 ± 62	704 ± 52	1048 ± 125