7. The homologues of neuron 4 are electrically coupled [S. B. Kater, A. D. Murphy, J. R. Rued, *J. Exp. Biol.* **72**, 91 (1978); F. Bahls, S. B. Kater, R. Joyner, *J. Neurobiol.* **11**, 365 (1980)] and, for the purpose of this analysis, are treated as a functional unit. In a typical preparation a single neuron 4 was tested for novel coupling to both the ipsilateral and the contralateral neuron 5. No specific pathway for coupling can be inferred from these measurements.
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Sustained Intracerebroventricular Infusion of Brain Fuels Reduces Body Weight and Food Intake in Rats

Abstract. *Long-term infusion of glucose, β-hydroxybutyrate, and glycerol into the third ventricle of the rat brain caused a stabilization of body weight at a lower than normal level. Among the glucose- and glycerol-treated animals this weight loss was caused in part by temporary hypophagia. Among the animals treated with β-hydroxybutyrate the weight loss was unaccompanied by a reduction in food intake. The results are consistent with the view that the systems controlling food intake and body weight are sensitive to the availability of brain fuels. They are not consistent, however, with the view that these control systems monitor calories independently of their source.*

The body weight and food intake of animals appear to be controlled within a fairly narrow range, but the mechanisms involved in their control are not well understood. Although ablation and stimulation studies indicate that an important part of the control system is located in the hypothalamus, they cannot elucidate the nature of the information that is processed. Such clarification can be obtained, however, by determining the effects of chemicals believed to participate in the central control of food intake and body weight. The food intake control system may directly monitor the concentrations of circulating nutrient metabolites and may alter food intake in response to either a surfeit or a deficit in these substances. Hypothalamic electrical activity can be altered by intravenous or iontophoretic glucose (*1*) and by insulin administered in sufficient amounts to significantly lower the concentration of blood glucose (*2*). Also, injections of glucose and amino acids into the hypothalamus decrease food intake (*3*) and injections of insulin antibodies (*4*) or 2-deoxy-D-glucose (*5*) into the same general region of the brain increase food intake. It has not been known whether these substances have parallel effects on body weight.

This report describes the results of a study designed to determine the effects of long-term intracerebroventricular infusions of glucose, glycerol, and β-hydroxybutyrate on both food intake and body weight (*6*). Glucose and β-hydroxybutyrate were chosen because they are the major metabolites of carbohydrate and lipid metabolism which have been shown to be utilized by the brain as energy sources (*7*). Glycerol was selected for three reasons: (i) it can be metabolized by the brain (*8*), (ii) when administered peripherally by a variety of routes it is capable of suppressing food intake (*9–12*), and (iii) its long-term administration leads to a reduction in body weight (*12, 13*). Infusions were made into the third ventricle because the surrounding hypothalamic regions are involved in the control of food intake and body weight and because substances that cross the blood-brain barrier poorly, such as β-hydroxybutyrate, are taken up maximally in this region (*14*). It is obvious that weight regulation can only be investigated with long-term treatments, and it is likely that a system concerned with the long-term control of food intake would be more responsive to a sustained rather than short-term change in the availability or utilization of energy-producing metabolites.

The subjects were male albino rats (Charles River) weighing an average of 300 g at the beginning of the experiment. They were maintained in individual cages on a 12-hour light-dark cycle. A guide cannula made of 21-gauge stainless steel tubing was implanted, aimed at the third ventricle (*15*). The stereotaxic coordinates were 6.4 mm anterior to bregma, 2.6 mm below the surface of the brain, and on the midline. The animals were allowed to recover for at least 3 weeks, and were then given unrestricted access to a pellet diet (*16*). Food intake, water intake, and body weight were measured daily at about 8 a.m. and 6 p.m.

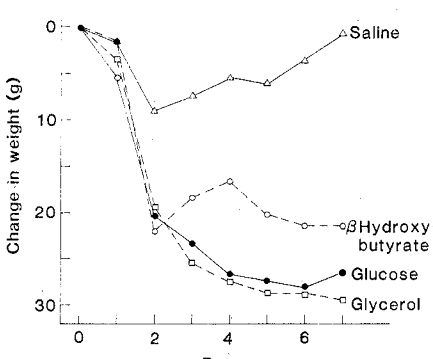

Fig. 1. Changes in body weight from initial weight. The label at the end of each curve indicates the substance infused.

After at least 7 days, each animal was fitted with an Alzet minipump (*17*) filled with the test solution. The pump was connected by a length of polyethylene tubing (inner diameter, 0.015 inch), also filled with the test solution, to an insert cannula made of 27-gauge stainless steel tubing trimmed so that when it was inserted into the guide cannula it would terminate in the third ventricle. The insert cannula was cemented in place while the animal was under light ether anesthesia. The pump and tubing were then inserted into a subcutaneous channel opened under the skin on the animal's back. The pump rested just over the scapula when in place. The incision was closed with surgical silk and the animal was returned to its cage. The animals weighed between 350 and 420 g at the time of pump implantation.

The Alzet pump delivered its contents at a rate of 1 μl per hour for 7 days. On the seventh day the tubing emerging from the insert cannula was cut and the end was heat-sealed. Food and water intake and body weight were measured in the morning and evening during the entire time that the pump was connected and for at least 7 days after the tubing was cut.

The rats were infused with D-glucose, glycerol, or DL-β-hydroxybutyrate at a concentration of $0.15M$ and an osmolar concentration (adjusted with saline) of 300 milliosmoles per liter. The substances were delivered to the ventricle at a rate of 1.5×10^{-7} mole per hour. A $0.15M$ NaCl solution was infused into members of a control group to evaluate the effects of infusing the vehicle into the ventricle.

There was a small weight loss, probably due to surgical trauma, in the animals infused with saline, but they gained weight at a normal rate thereafter (Fig. 1). The other compounds caused sustained, significant changes in body weight [$F(3, 20) = 6.9$, $P < .005$]—the greatest loss occurring during the first few days of infusion. Post hoc comparisons showed that these three compounds

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Table 1. Changes, from baseline, in intake of food and water.

Treatment	N	Food intake (% change)			Water intake (total % change)
		Days 1 to 3	Days 4 to 7	Total	
Saline	6	−6.7*† ± 1.7	−15.2*† ± 4.0	−1.9† ± 3.8	−2.5 ± 6.2
β-Hydroxybutyrate	7	−8.6*† ± 3.2	−17.2*† ± 3.1	−2.1† ± 5.0	−3.8 ± 2.9
Glucose	5	−20.4†‡§ ± 2.0	−33.4‡§ ± 7.6	−10.3 ± 2.3	−2.5 ± 7.2
Glycerol	6	−31.2*‡§ ± 1.3	−43.8‡§ ± 3.4	−21.8 ± 2.2	−8.1 ± 2.1

*Value is significantly different from corresponding value for glucose. †Value is significantly different from corresponding value for glycerol. ‡Value is significantly different from corresponding values for saline. §Value is significantly different from corresponding value for β-hydroxybutyrate.

produced significantly larger weight losses than saline ($P < .05$, Newman-Kuels procedure) and that the magnitude of the effect did not differ significantly between compounds. Glycerol tended to produce the largest effect; β-hydroxybutyrate the smallest.

Over the 7-day infusion period, food intake differed significantly between groups [$F(3, 20) = 23.5$, $P < .001$)] but water intake did not (Table 1). Post hoc comparisons revealed that glycerol produced a significantly larger reduction in food intake than any of the other compounds ($P < .01$). Glucose produced a larger effect than saline or β-hydroxybutyrate; the latter two did not differ in their effect. In all cases, food intake was depressed maximally during the first few days after pump implantation and returned toward normal thereafter (Table 1). During the last 4 days of infusion, food intake was still significantly depressed only in the glycerol-treated animals but had recovered substantially. The changes in food intake thus paralleled the changes in weight, suggesting that feeding begins to return to normal as animals stabilize at a lower body weight (*18*).

The reduction in food intake in the glucose- and glycerol-treated animals was related to the diurnal cycle. During the dark phase the mean reduction in intake (from baseline) was 24.4 ± 2.7 percent for the glucose-treated animals and 29.6 ± 5.3 percent for the glycerol-treated animals. During the light phase food intakes were more variable, with the glucose-treated animals showing a 16.5 ± 15.6 percent increase and the glycerol-treated animals an 8.8 ± 9.2 percent decrease. Thus the infusion of either substance had a large effect on nighttime intake and no significant effect on daytime intake. Similar effects have been reported for subcutaneously administered glycerol (*9, 12*).

It is unlikely that the effects observed here are secondary to a nonspecific or toxic effect of the infusions since (i) food but not water intake was affected, (ii) food intake was altered only during the night, and (iii) food intake recovered as body weights began to stabilize.

All the metabolites had effects on body weight and two of the three had effects on food intake. Glucose provided the most potential calories and glycerol the fewest. Since the magnitude of the effects on food intake did not follow this ordering, our results do not provide support for the theory of Ugolev and Kassil (*19*) that the ability of metabolites to reduce food intake is related to their ability to serve as substrates of energy metabolism. Of course, differences in diffusion or metabolism rates may complicate this interpretation.

Animals infused with β-hydroxybutyrate lost significant amounts of weight but ate virtually the same amount of food as saline-treated animals. Their weight loss, therefore, must have been caused by an increase in activity or heat production. A number of other studies have also demonstrated that changes in body weight are not always accompanied by changes in food intake (*20*).

Glucose and glycerol reduced both weight and food intake. It is likely that part of this weight loss was due to the hypophagia produced by the infusions, but metabolic alterations may have contributed to the effect. The effects of glycerol on food intake were significantly larger than those of glucose, in agreement with results obtained with subcutaneous, intraportal, and intraduodenal infusions of glycerol (*9–13*). These results suggest that glycerol may play a special role in the system controlling food intake and body weight. The effects of intraventricular glycerol infusions on weight and food intake are dose-related and can be obtained with infusion rates as low as 0.075 μM per hour (*21*).

We conclude that these effects are mediated by the brain rather than peripheral organs because the changes are as large as or larger than those obtained when much larger doses of the same substances are given peripherally. For example, in this study glycerol administered intracerebroventricularly at a rate of 3.6×10^{-6} mole/day led to a weight reduction of approximately 30 g. In a previous study (*13*), glycerol administered subcutaneously at a rate of 6.7×10^{-4} mole/day led to a weight reduction of approximately 20 g. Glucose given intracerebroventricularly at a rate of 3.6×10^{-6} mole/day led to nearly 30-g weight reduction, whereas when glucose was given subcutaneously at a rate of 3.6×10^{-4} mole/day (*13*) there was no detectable weight loss. Thus, even if all the metabolites that were administered to the brain had diffused peripherally (a most unlikely occurrence), they would not have been at a concentration sufficient to produce the effects that are observed when they are administered peripherally. Furthermore, glycerol administered to the fourth ventricle at a rate of 7.2×10^{-6} mole/day has no effect on food intake or body weight, indicating that the effect is mediated in tissue in close proximity to the third ventricle.

These results support the view that direct monitoring of metabolites by the brain may play a role in the control of food intake and body weight and demonstrate that the effects obtained are not simply related to the potential caloric value of the various compounds. In addition, these results support the idea that significant changes in weight can occur without significant alterations in food intake and that glycerol may function as a feedback signal informing the brain of the lipid reserves in the body (*13*).

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Dominance Hierarchies in *Leptothorax* Ants

Abstract. *The social organization of* Leptothorax allardycei *is unique among ant species thus far studied. The workers form linear dominance hierarchies characterized by routine displays of dominance, avoidance behavior, and even fighting. The high-ranking ants are favored in liquid food exchange, have greater ovarian development, and produce 20 percent of the eggs.*

The typical social system of ant species is based on a strict division of labor: the queen is specialized for reproduction and the workers gather food, rear progeny, and defend the colony (*1*, *2*). My research on *Leptothorax* (= *Macromischa*) *allardycei* has revealed that the social behavior of this neotropical myrmicine differs from that of all other known species of ants. Reproductive competition among the workers is resolved through the formation of a dominance hierarchy involving routine displays of dominance, avoidance behavior, and even fighting.

An agonistic encounter between two workers of *L. allardycei* begins with the dominant ant touching the submissive ant with its antennae (*3*). There is brief pause before the dominant either rapidly turns or lunges toward the subordinate (Fig. 1). It then pummels the gaster, thorax, or head of the subordinate with its mandibles. The encounter may continue, with the dominant ant advancing onto the top of the subordinate, sometimes climbing clear of the nest floor and standing on the subordinate. The typical response of the subordinate is to crouch and freeze, with the antennae drawn back to the sides of the head. It stays in this position until the dominant moves away.

Newly eclosed adults do not always conform to the above generalizations, and sometimes their responses result in fights. The intensity of these encounters can cause the combatants to fall and struggle on the nest floor. However, injuries have not been observed as a result of these encounters. *Leptothorax allardycei* workers possess a well-developed sting but do not use it in dominance encounters.

The ants also exhibit avoidance behavior. In my examination of this phenomenon, a behavioral sequence was labeled avoidance only if an individual stopped abruptly while moving through the nest, directed its antennae toward a particular ant, and then darted away in the direction from which it came. In each such instance a lower ranking worker was avoiding a higher ranking worker ($P <$ .001, modified binomial test; $N = 24$).

Tables 1 and 2 show examples of dominance hierarchies in two colonies of *L. allardycei*. In general the hierarchies are almost perfectly linear. Less than 0.5 percent of the interactions are reversals. The only change in rank results from the assimilation of newly eclosed workers into the hierarchy (*4*). Dominance activity parallels rank (*5*). The highest ranking worker accounted for 43 percent of all displays of dominance and the second ranking worker accounted for 28 percent.

High-ranking workers gain a twofold advantage from their domination of coworkers: they receive more liquid food from nest mates and they gain a reproductive advantage by laying male-producing eggs.

It is typical for liquid food to be mutually exchanged among workers of social insect colonies (*6*). However, in *L. allardycei* food transfer is unidirectional; high-ranking workers receive food from low-ranking workers but do not reciprocate (*7*). From observations of liquid food transfer between workers of known rank, I calculated that the probability that rank is not associated with direction of food transfer is .0012 (Kendall's rank correlation). While this trophic advantage is an important feature of the social structure, there is no fitness advantage to the worker unless it is translated into egg laying.

A fitness advantage to unfertilized,

Table 1. Dominance hierarchy constructed from observation of 200 interactions in a queenright colony over 18.2 hours. Each entry is the number of times an interaction occurred between the ants indicated.

Dominant ants	Subordinate ants																Total
	oe	he	po	sa	lo	mi	lp	pa	da	ch	fa	do	li	bl	le	ri	
oe	–	43	19	18	1	2	2			1				1	1	2	90
he		–	45	20	3	1	5			1		1	1			1	78
po			–	3	1	5	3	2		1							15
sa				–	2	3	2	1		2	1						11
lo					–	1											1
mi						–	2			1			1				4
lp							–										
pa								–									
da									–							1	1
ch										–							
fa											–						
do												–					
li													–				
bl														–			
le															–		
ri																–	

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