Brain Research, 55 (1973) 229–233

A comparison of the eating in response to hypothermic and glucoprivic challenges after nigral 6-hydroxydopamine and lateral hypothalamic electrolytic lesions in rats

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(Accepted February 26th, 1973)

The lateral hypothalamus is critical to the control of feeding and drinking. Bilateral damage to this area produces aphagia and adipsia in rats[1,17] and electrical excitation can evoke eating[2] or drinking[7]. Yet, the neural systems mediating these effects on eating and drinking have not been identified conclusively. The primary pathway through the lateral hypothalamus, the median forebrain bundle, is not essential for the maintenance of feeding, because its interruption at points either rostral or caudal to the lateral hypothalamus has little effect on feeding[11]. Electrolytic lesions in a region lateral to the median forebrain bundle, bordering on the medial edge of the internal capsule, produce the most severe aphagia and adipsia[6,12].

Fluorescent histochemical techniques have revealed an ascending nigroneostriatal dopamine pathway which passes through this far-lateral hypothalamic region[19]. Ungerstedt suggested that damage to this pathway may be responsible for the syndrome of feeding and drinking impairments which result from electrolytic lateral hypothalamic lesions. By applying 6-hydroxydopamine to the substantia nigra, he found that selective destruction of this bundle produced aphagia and adipsia[18,20].

More information concerning the deficits produced by damage of the nigroneostriatal bundle is necessary before its equivalence with the lateral hypothalamic syndrome is established. Much is known about the feeding impairments and their recovery after electrolytic lateral hypothalamic lesions. Rats recover from aphagia and adipsia in a predictable sequence of stages, culminating in the ability to maintain their weight on pellets and water[16]. Immediately after a lesion, they display sensory neglect[10], *i.e.*, they do not localize olfactory or visual stimuli in space or tactile stimulation of the body surface. Even after they are regulating their body weight on pellets and water, they show persistent abnormalities in responding to regulatory challenges[3]. We have been examining the behavior of rats with 6-hydroxydopamine damage to the nigrostriatal bundle and comparing their deficits with those seen after lateral hypothalamic lesions. One aspect of that comparison will be presented in this paper.

A normal rat overeats when it is made hypoglycemic by an injection of insulin[9] or when cellular glucose availability is decreased by injecting 2-deoxy-D-glucose[15]. A

normal animal also eats more when placed in a cold environment[5]. The rat with electrolytic damage to the lateral hypothalamus shows a selective impairment in its feeding. When placed in the cold, it overeats in a normal manner[4]. Yet, it does not overeat in response to either insulin[4] or 2-deoxy-D-glucose[3,21]. We have examined the response of rats recovered from the aphagia and adipsia produced by nigral 6-hydroxydopamine injections to see whether they show a similar selective impairment in feeding.

Stereotaxic surgery was performed on animals of the Charles River or Carworth (CFE or CFN) albino strains. In one group of 3 animals, electrolytic damage to the lateral hypothalamus was produced by positioning a stainless steel electrode at the following coordinates (with reference to a flat skull): 6.0 mm anterior to the inter-aural line, 2.0 mm lateral to the exposed superior sagittal sinus, and 8.0 mm ventral to the dura of the cortex. Then, 1 mA of direct current was passed for 10–15 sec on each side. In a second group of 5 animals, 6-hydroxydopamine was injected into the substantia nigra through a 26-gauge cannula positioned 1.8 mm lateral to the superior sagittal sinus, 2.0 mm dorsal and 3.5 mm anterior to the inter-aural line (with the toothbar 2.5 mm ventral to the inter-aural line)[8]. 6-Hydroxydopamine (Regis Chemicals) was dissolved in a solution of 0.1% (w/v) ascorbic acid in isotonic saline to a concentration of 2 mg/ml, then 2–8 μl was injected into each side at a rate of 1 μl/min. Postoperatively, rats were fed intra-gastrically with liquid diet, and offered palatable foods during recovery[16]. Rats with nigral damage recovered the ability to maintain their body weight on pellets and water from 5 to 98 days after surgery; those with hypothalamic lesions, from 24 to 141 days postoperatively.

After it had regained the capacity to regulate its body weight on pellets and water, each rat was subjected to regulatory challenges. Glucoprivation was induced by injecting 750 mg/kg of a 10% w/v solution of 2-deoxy-D-glucose (Sigma Chemicals) in distilled water. Food intake in the following 6 h was measured and compared with the amount of food eaten on a previous day in the 6 h following an injection of isotonic saline of equal volume. Eating in response to the cold was tested as follows. Records of food eaten, water drunk, and body weight were kept for 3 days while the animal was at room temperature (23 °C). Then, the rat was placed into a 5 °C cold box for 3 days, during which time feeding, drinking, and body weight were again recorded daily. Finally, the rat was returned to room temperature for 3 more days of measurement. After the cold test, each rat was retested for response to 2-deoxy-D-glucose.

At the end of the experiment, the brains of rats with nigral 6-hydroxydopamine injections were removed, and catecholamine levels were analyzed, using the Alice Hogans photofluorimetric procedure[14]. Striatal dopamine was found to be depleted to 15% of normal values (range = 0.4–33.0%), whereas norepinephrine in the diencephalic–mesencephalic region is normal (112% of normal control values). The telencephalon of rats with 6-hydroxydopamine is depleted of norepinephrine to 11% of normal values.

The results of this experiment indicate that rats with damage to the nigrostriatal dopamine bundle show the same selective feeding impairments as do those with lateral hypothalamic lesions. As shown in Table I, normal rats overeat in response to 2-deoxy-D-glucose ($P < 0.01$) and in the cold ($0.05 < P < 0.10$). None of the rats with

TABLE I

FOOD INTAKE OF LESIONED RATS

Weight (g) of pellets eaten during hypothermic and glucoprivic (2-DG) challenges. Both absolute intake and the difference in intake between the control and experimental conditions are indicated for each animal. A positive value in the difference column indicates a response typical of the normal animal. $\overline{D}$, mean of difference values; Iso. sal., isotonic saline (control); 2-DG, 2-deoxy-D-glucose.

	Rat No.	*Pre-cold 2-DG*			*Cold test*			*Post-cold 2-DG*		
		Iso. sal.	*2-DG*	*Difference*	*23 °C*	*5 °C*	*Difference*	*Iso. sal.*	*2-DG*	*Difference*
Nigral 6-OH-DA	12	—	—	—	14.8	22.3	7.5	2.5	0.9	—1.6
	14	3.9	2.2	—1.7	17.5	24.3	6.8	3.6	0.0	—3.6
	18	6.5	1.2	—5.3	19.2	27.0	7.8	5.8	1.0	—4.8
	19	1.0	0.5	—0.5	19.3	20.0	0.7	1.5	1.5	0.0
	23	4.0	3.4	—0.6	19.4	30.7	11.3	7.8	0.0	—7.8
$\overline{D} \pm S_{\overline{D}}$				—2.0 ± 1.1			+6.8 ± 1.7			—3.6 ± 1.1
Lateral hypothalamic electrocoagulation	51	0.9	0.0	—0.9	17.5	22.0	4.5	5.5	1.6	—3.9
	57	0.1	0.2	0.1	26.2	41.7	15.5	13.0	5.7	—7.3
	153	1.7	1.4	—0.3	16.7	20.0	3.3	2.5	1.8	—0.7
$\overline{D} \pm S_{\overline{D}}$				—0.4 ± 0.2			+7.8 ± 3.9			—4.0 ± 1.9
Normal		5.0	8.0	3.0	21.6	27.3	5.7			
		3.0	5.0	2.0	20.2	23.5	3.3			
		1.0	5.0	4.0	26.3	26.8	0.5			
		5.3	4.8	—0.5	21.4	26.8	5.4			
		2.9	4.0	1.1						
		0.2	5.4	5.2						
		0.3	5.4	5.1						
$\overline{D} \pm S_{\overline{D}}$				+2.8 ± 0.7			+3.7 ± 1.2			

6-hydroxydopamine injection into substantia nigra ate more in the 6 h following 2-deoxy-D-glucose than following isotonic saline. Yet, all these rats ate more in the cold than at room temperature ($P < 0.02$). For comparison, those rats with electrolytic lateral hypothalamic lesions were given both thermal and glucoprivic challenges. In confirmation of the earlier work, we found that they all ate more in the cold, but not in response to 2-deoxy-D-glucose. Because of the small sample size, the overeating of the rat with lateral hypothalamic lesions in the cold does not reach statistical significance.

Because the thermal test was typically conducted after the 2-deoxy-D-glucose injection, it could be argued that the ability to overeat in the cold simply resulted from a general recovery of the rat's feeding capacities. To test this, all brain-damaged rats were given the glucoprivic test again after the hypothermic test had been completed. All rats, those with nigral 6-hydroxydopamine as well as those with electrolytic lateral hypothalamic damage, still failed to overeat in response to 2-deoxy-D-glucose.

As Smith and Epstein[15] observed, 2-deoxy-D-glucose at these high doses (750 mg/kg) typically results in ataxia and stupor soon after its injection. One might argue that brain-damaged rats may be more susceptible to the ataxia-inducing effects of 2-deoxy-D-glucose, and eat less food simply because of a more profound ataxia and stupor. However, our observations suggest that such an hypothesis is incorrect. First, the latency and duration of the ataxia produced by 2-deoxy-D-glucose is equivalent in normal rats and those with nigral 6-hydroxydopamine injections. All the rats became ataxic within 15 min after the intraperitoneal injection and had recovered by 2 h. Second, even after the ataxia and stupor had disappeared (*i.e.*, between the second and sixth hour after the injection), rats with nigral damage still ate less than did normal rats. The intake of normal rats during this 4-h period was 2.98 g, while that of rats with 6-hydroxydopamine injections was 0.93 g ($t = 2.74$; $df = 6$; $P < 0.05$). These results suggest that the lack of eating in response to the glucoprivic challenge seen in brain-damaged rats is not simply secondary to motor abnormalities.

Our additional testing of these rats with nigral 6-hydroxydopamine injections indicates that they recover their food and water regulation in the same sequence of stages as do rats with lateral hypothalamic lesions, that they have similar impairments in drinking to cellular dehydration or hypovolemia, and that they are somewhat finicky to adulteration of their water with small amounts of quinine. This work is still in progress, and will be described more fully in a later publication.

Although other workers, using electrolytic lesions[13] or ventricular 6-hydroxydopamine injections[22], have indicated the importance of dopamine destruction in producing severe feeding deficits, the present work is the first to show that injection of 6-hydroxydopamine into the nigro-striatal bundle produces a selective deficit in food regulation identical to that produced by lateral hypothalamic lesions.

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