Facilitation of Recovery by α-Methyl-p-Tyrosine after Lateral Hypothalamic Damage

Abstract. *Rats with bilateral lateral hypothalamic lesions die of starvation in approximately 7 days after surgery. Rats that were treated with α-methyl-p-tyrosine for 3 days prior to lateral hypothalamic surgery spontaneously eat, drink, and gain weight after surgery. These data suggest that recovery of function after lateral hypothalamic damage involves denervation supersensitivity.*

Bilateral lesions of the lateral hypothalamus in the rat produce a well-known syndrome of aphagia and adipsia (*1*). If kept alive by intragastric tube feedings for several days after surgery, lateral hypothalamic rats may eventually recover the ability to spontaneously eat and drink (*1, 2*). Berger, Wise, and Stein (*3*) suggested that recovery of feeding after lateral hypothalamic lesions is dependent on recovery of a noradrenergic reward system. According to a denervation supersensitivity model (*4*) suggested to account for other recovery phenomena (*5*), time-dependent supersensitivity of partially denervated neurons to other intact inputs would mediate recovery. Lateral hypothalamic lesions might result in removal of intrahypothalamic connections to remaining tissue. If the recovery process involves supersensitivity of noradrenergic neurons and if such neurons were functionally denervated and made supersensitive at some time prior to lateral hypothalamic damage, then facilitation of recovery after the latter might be expected. Using α-methyl-*p*-tyrosine, a drug that selectively interferes with catecholamine synthesis, we now present data supporting this hypothesis.

Fourteen rats (*6*) under methohexital anesthesia received bilateral lesions in the lateral hypothalamus by a direct anodal current of 2 ma for 30 seconds. Coordinates were 0.8 to 1.0 mm anterior to bregma, 2.5 mm lateral to the midline, and 2.0 mm from the base of the skull (*7*). Three sham-operated controls had the electrodes lowered but did not receive lesions. All of the rats with lesions were treated prior to surgery (pretreated) with either saline or DL-α-methyl-*p*-tyrosine methyl ester hydrochloride (αMT). Pretreatment began 3 days before surgery and consisted of two injections (0.5 ml per injection) per day (9:00 a.m. and 5:00 p.m.) for 2 days and one injection on the third day (9:00 a.m.). Surgery was conducted 1 day after the last injection. Four rats were pretreated with saline, three with 10 mg/kg of αMT, three with 75 mg/kg of αMT, and three with 100 mg/kg of αMT. Three rats similarly treated with injections of 150 mg/kg of αMT all died several hours after the fourth injection. All rats were weighed prior to the operation during drug administration and on each day after the operation. Rats were allowed free access to food and water, both prior to and after the operation. Purina laboratory chow (pellets only) were always present on the floor of the cage of each rat. No special procedures after the operation for maintaining the rats were conducted. The rats were killed (death was considered imminent) when a righting response and locomotion could not be elicited by a tail pinch (*8*).

The results of pretreatment with αMT were quite clear. Rats with lateral hypothalamic lesions alone were always aphagic and adipsic and died in approximately 7 days (mean = 7.4). Rats pretreated with 10 mg/kg of αMT were also always aphagic and adipsic and died in 7.9 days. All of the rats pretreated with either 75 or 100 mg/kg of αMT (*9*) recovered. Such rats were aphagic and adipsic for 2 to 6 days after surgery and then began eating, drinking, and gaining weight (*10*) before being killed for lesion verification. Percent weight changes after lateral hypothalamic lesions are shown in Table 1 (*11*).

After this experiment, three rats were again pretreated with 100 mg/kg of αMT as well as two rats with saline. However, instead of conducting surgery a day after the fifth injection, these rats were killed at that time. We extracted the brains and then assayed them for the concentration of norepinephrine (*12*). The brains of rats treated with αMT had approximately 50 percent less norepinephrine than the saline controls. The αMT dosage regimens used to facilitate lateral hypothalamic recovery should then have had significant effects on the activity of central catecholamine neurons.

Our data are therefore consistent with the initial denervation supersensitivity hypothesis. Administration of αMT should have pharmacologically produced a partial denervation of neurons subserving recovery. In the absence of any preliminary treatment, it would normally take many days for rats with bilateral hypothalamic lesions to recover from the aphagia and adipsia. And such recovery would occur only if such rats were initially kept alive with tube feedings (*1*). The duration of this recovery period would be a function of the number of remaining neurons involved in eating and drinking and the degree of the supersensitivity to remaining inputs. Indeed, rats with extremely large lesions would not be expected to recover under any circumstances (*2*). By administering αMT prior to surgery, the neurons subserving recovery should be sufficiently supersensitive sooner after surgery for recovery to occur. Termination of drug treatment before surgery should allow intact inputs remaining after surgery to become functional again. The time

Table 1. Mean weight changes (percent of weight prior to surgery) after lateral hypothalamic lesions.

Days after surgery	Body weight change (% of weight prior to surgery)				
	Lesions saline	Lesions αMT (10 mg/kg)	Lesions αMT (75 mg/kg)	Lesions αMT (100 mg/kg)	Sham-operated
1	86.8	86.2	89.3	87.5	99.2
2	80.9	80.4	87.3	85.2	101.8
3	75.0	74.6	85.9	83.6	103.8
4	70.1	69.7	83.6	84.1	105.6
5	65.2	65.0	81.8	88.7	108.1
6	61.4	61.6	79.8	90.5	110.0
7	*	58.6	80.1	92.2	111.3
8	*	*	82.3	95.3	111.2
9	*	*	84.4	95.6	111.8
10	*	*	85.9	96.0	114.1

* Group means were computed only for days on which all rats of a group were still surviving.

course of the entire process is thereby facilitated.

Although this investigation began with the premise that noradrenergic neurons were involved in the lateral hypothalamic syndrome (*3*), evidence suggests that the same syndrome may be all, or in part, attributable to interruption of a dopaminergic pathway involving the substantia nigra and the corpus striatum (*13*). Evidence implying that denervation supersensitivity follows lesions of this pathway has also been reported (*14*). Our data may, therefore, have also demonstrated the significance of supersensitivity for recovery of a dopaminergic system. Further studies should help to clarify the relative importance of noradrenergic and dopaminergic neurons in the early deficits as well as the later recovery after lateral hypothalamic lesions.

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References and Notes

1. P. Teitelbaum and A. N. Epstein, *Psychol. Rev.* **69**, 74 (1962).
2. Whether recovery occurs or not appears to depend on the size and the lateral extent of the lesions [see P. J. Morgane, *Amer. J. Physiol.* **201**, 420 (1961); G. D. Ellison, C. A. Sorenson, B. L. Jacobs, *J. Comp. Physiol. Psychol.* **70**, 173 (1970)].
3. B. D. Berger, C. D. Wise, L. Stein, *Science* **172**, 281 (1971). It should also be noted that lateral hypothalamic lesions reduce the telencephalic concentration of norepinephrine [see M. J. Zigmond, J. P. Chalmers, J. R. Simpson, R. J. Wurtman, *J. Pharmacol. Exp. Ther.* **179**, 20 (1971)].
4. S. K. Sharpless, *Annu. Rev. Physiol.* **26**, 357 (1964).
5. S. D. Glick and B. Zimmerberg, *J. Comp. Physiol. Psychol.*, in press.
6. The rats (220 to 280 g) were female albinos (Sprague-Dawley).
7. The skull was inclined according to the atlas of L. J. Pellegrino and A. J. Cushman [*A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967)]. Far-lateral coordinates were purposely chosen to ensure that lack of treatment of rats with lateral hypothalamic lesions would result in death by starvation. See (*2*) and a → S. Balagura, R. H. Wilcox, D. V. Coscina, *Physiol. Behav.* **4**, 629 (1969).
8. Two rats treated with saline and one rat treated with 10 mg/kg of αMT prior to surgery died before being killed. When death was considered imminent, rats were killed so that adequate perfusion with formalin and more accurate histology could be performed. Brains were sectioned at 40 μm. This report includes only rats with bilaterally symmetrical lesions in the lateral hypothalamus at the level of the posterior part of the anterior hypothalamus and the ventromedial hypothalamic nucleus. Damage to the medial border of the internal capsule and to the ventral subthalamus was also observed.
9. Reducing the body weight of rats by 20 percent at the time of lateral hypothalamic surgery has been found to shorten the duration of aphagia after the operation; see T. L. Powley and R. E. Keesey, *J. Comp. Physiol. Psychol.* **70**, 25 (1970). Rats pretreated with either 75 or 100 mg/kg of αMT typically lost 5 to 10 g of body weight after the first day of drug administration and then gained weight normally. At the time of surgery, the mean body weights of all groups were within a range of 7 g. The recovery results cannot, therefore, be attributed to weight changes prior to surgery, per se.
10. Rats began eating, drinking, and gaining weight spontaneously and simultaneously; the mean duration for this to occur was 3.8 days. No intermediate steps, for example, consumption of very palatable foods, were necessary for recovery to occur. This indicates that the four classical stages of lateral hypothalamic recovery (*1*) are not sequentially necessary. That is, eating wet palatable food does not necessarily have to precede eating dry food and water. Our data have no bearing, however, on whether the four stages can be elicited during this facilitated recovery. It is indeed possible that rats showing recovery would have consumed palatable food during the earlier period of apparent aphagia and adipsia.
11. After the first day, all differences between the groups pretreated with 75 or 100 mg/kg of αMT and the groups pretreated with either 10 mg/kg of αMT or saline were significant (*t*-tests, $P < .01$ to .001).
12. The rats were decapitated, the whole brain was removed, and the cerebellum was dissected out and discarded. The brains were homogenized (1 g/10 ml) in cold 0.4*N* perchloric acid and were centrifuged at 15,000 rev/min for 15 minutes. Samples (5 ml) of each brain supernatant were then removed and 0.5 ml of 10 percent ethylenediametetraacetic acid were added to each. The *p*H of the supernatants was then adjusted to *p*H 8.4 with 5*N* NaOH, 1*N* NaOH, and dilute 1*N* NaOH. Each supernatant was then passed over an alumina column. Norepinephrine concentrations were measured by the fluorimetric method of H. Weil-Malherbe [*Methods Biochem. Anal.* **16**, 293 (1968)]. Emission spectra were read from 450 to 490 nm. The results (average microgram of norepinephrine per gram of brain) were as follows: saline, 0.627; αMT, 0.325. The difference was significant (*t*-test) at $P < .05$.
13. U. Ungerstedt, *Acta Physiol. Scand.*, Suppl. 367, 95 (1971).
14. ———, *ibid.*, p. 69.
15. Supported by NIMH grant 1 RO1 MH21156-01 to S.D.G. We thank S. Wilk for his help in conducting the norepinephrine assays.

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Reflection Spectra of Lunar Dust Grains with Amorphous Coatings

Bibring *et al.* (*1*) report that a significant fraction of grains of lunar fines are coated with a low-density, amorphous substance about 500 Å thick, which is probably host grain material metamictized by solar wind bombardment. In one portion of their report Bibring *et al.* suggest that the low albedos and reddish spectra of the fines may be due in part to the coatings. In support of this suggestion they calculate the Fresnel reflection coefficient as a function of wavelength for an electromagnetic wave vertically incident on the plane surface of a semi-infinite, nonabsorbing substance with index of refraction $n_2 = 1.98$, coated with a layer of material 500 Å thick with $n_1 = 1.80$. The calculated reflection spectrum superficially resembles the lunar spectrum. Unfortunately, the calculation is irrelevant to the problem of the lunar albedo because it ignores the refracted ray. Although the coatings are very interesting, it can be shown that they have only a marginal effect on the lunar reflectivity.

The reflectivity R of an optically thick powder, consisting of grains with single-scattering abedos w_0, is given to a first approximation by the Kubelka-Munk, or two-stream, expression (*2*):

$$R = [1 - (1 - w_0)^{1/2}]/[1 + (1 - w_0)^{1/2}]$$

For the moon, R is .07, so that the effective, average, grain albedo is $w_0 = .25$. Contributing to w_0 are both rays that are reflected directly from the surface of the grain (the so-called regular or specular component of reflection) and rays that are refracted into the grain to be partially absorbed and volume-scattered so that they leave the grain in arbitrary directions. For silicates, with typical indexes of refraction around 1.6, the rays reflected from the surface contribute only about .06 to w_0, and thus the refracted rays must account for the remaining .19. Coating the grains will decrease the intensity of the rays reflected from the surface in the manner discussed by Bibring *et al.*, but will increase the intensity of the refracted rays because of smaller internal reflection on exiting, so that R will be only slightly affected.

The relative contributions of the surface-reflected and volume-scattered rays may also be estimated from polarization measurements on the lunar fines. In green light the maximum polarization of Apollo 11 fines is approximately 16 percent (*3*). Since the diffusely scattered ray is only weakly polarized, the polarization is due primarily to the surface ray, which is almost completely polarized near the Brewster angle. Thus, it may be deduced that surface scattering can account for only about 16 percent of the light reflected by the fines.

The minor role of the specular component can be further illustrated by the following example: TiO_2 and H_2O ice have indexes of refraction of 2.6 and 1.3, respectively, and thus specular re-