soluble $DMPH_4$-dependent form of tryptophan hydroxylase does not manifest a change in activity in response to long-term exposure to morphine.

The presence of two measurable forms of tryptophan hydroxylase in brain, their varying regional distribution, their differing modes of regulation, and their differential response to pharmacological agents may explain some of the conflicts seen in the literature relating the action of morphine to its effects on the serotonin biosynthetic system. It is possible that some of these differences could be resolved if drug dose, time after administration, and region could be more uniform and specific. It appears that the late increases in nerve ending tryptophan hydroxylase after the short-term inhibition of enzyme activity by morphine is consonant with the findings of our program of research, which demonstrates compensatory changes in presynaptic neurotransmitter biosynthetic enzyme activity after drug-induced changes in synaptic function (*16*).

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References and Notes

1. E. W. Maynert, G. I. Klingman, H. K. Kaii, *J. Pharmacol. Exp. Ther.* **135**, 296 (1962).
2. E. L. Way, H. H. Loh, F. Shen, *Science* **162**, 1290 (1968); Y. Maruyama, G. Hayaishi, S. E. Smits, A. E. Takemori, *J. Pharmacol. Exp. Ther.* **178**, 20 (1971); G. G. Yarbrough, D. M. Buxbaum, E. Sanders-Busch, *Life Sci.* **10**, 977 (1971).
3. D. L. Cheney and A. Goldstein, *J. Pharmacol. Exp. Ther.* **177**, 309 (1971); S. Algeri and E. Costa, *Biochem. Pharmacol.* **20**, 877 (1971); D. L. Cheney, A. Goldstein, S. Algeri, E. Costa, *Science* **171**, 1169 (1971); I. Marshall and D. G. Grahame-Smith, *J. Pharmacol. Exp. Ther.* **173**, 634 (1971).
4. E. Azmitia, P. Hess, D. Reis, *Life Sci.* **9**, 633 (1970).
5. P. J. Schechter, W. Lovenberg, A. Sjoerdsma, *Biochem. Pharmacol.* **21**, 751 (1972).
6. S. Knapp and A. J. Mandell, in *Serotonin and Behavior*, J. Barchas, Ed. (U.S. Government Printing Office, Washington, D.C., in (press); *Life Sci.* **11**, 761 (1972).
7. A. Dahlstrom and K. Fuxe, *Acta Physiol. Scand.* **62** (Suppl. 232), 1 (1964).
8. E. G. Gray and V. P. Whittaker, *J. Anat.* **95**, 79 (1962).
9. A. Ichiyama, S. Nakamura, Y. Nishizuka, O. Hayaishi, *Advan. Pharmacol.* **6A**, 5 (1968); *J. Biol. Chem.* **245**, 1699 (1970).
10. J. G. Christenson, W. Dairman, S. Udenfriend, *Arch. Biochem. Biophys.* **141**, 356 (1970).
11. J. T. Scrafani, N. Williams, D. N. Clouet, *Pharmacologist* **11**, 256 (1969); S. Navon and A. Lajtha, *Brain Res.* **24**, 534 (1970).
12. E. Costa, A. Grappetti, A. Revuelta, *Brit. J. Pharmacol.* **41**, 57 (1971); E. Costa and A. Revuelta, *Neuropharmacology* **11**, 291 (1972).
13. J. A. Harvey and C. E. Lints, *J. Comp. Physiol. Psychol.* **74**, 28 (1971); R. Samanin, W. Gumulka, L. Valzelli, *Eur. J. Pharmacol.* **10**, 339 (1970).
14. J. Renson, in *Serotonin and Behavior*, J. Barchas, Ed. (U.S. Government Printing Office, Washington, D.C., in press); T. Deguchi and J. Barchas, *J. Neurochem.* **19**, 927 (1972).
15. J. Musacchio, personal communication; D. G. Grahame-Smith, *Biochem. J.* **106**, 351 (1967); H. Green and S. L. Sawyer, *Anal. Biochem.* **15**, 58 (1966); D. A. V. Peters, P. L. McGeer, E. G. McGeer, *J. Neurochem.* **15**, 1431 (1968).
16. A. J. Mandell, D. Segal, R. T. Kuczenski, S. Knapp, in *The Chemistry of Mood, Motivation and Memory*, J. L. McGaugh, Ed. (Plenum, New York, 1972), p. 105; W. Emlen, D. S. Segal, A. J. Mandell, *Science* **175**, 49 (1972); D. S. Segal, J. L. Sullivan, R. T. Kuczenski, A. J. Mandell, *ibid.* **173**, 847 (1971).
17. S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956), pp. 271–277.
18. This work was supported by NIMH grants MH-18065 and MH-23209 and Friends of Psychiatric Research of San Diego, Inc. We thank Mrs. Marion Brockett and Mrs. Arnetta McPherson for their technical assistance.

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Deficits in Feeding Behavior after Intraventricular Injection of 6-Hydroxydopamine in Rats

Abstract. *Intraventricular injections of 6-hydroxydopamine produced 95 percent depletion of telencephalic norepinephrine and 62 percent depletion of striatal dopamine in rats. Treated rats maintained body weight at subnormal levels and failed to increase food intake in response to a short-term decrease in glucose utilization. After treatment with the monoamine oxidase inhibitor pargyline, 6-hydroxydopamine produced no further norepinephrine depletion but increased the dopamine depletion to 95 percent and produced complete aphagia. These effects are comparable to events that follow bilateral electrolytic lesions of the lateral hypothalamus.*

There is increasing evidence that brain catecholamines are involved in the control of food intake in rats. For example, lateral hypothalamic lesions produce aphagia in rats (*1, 2*) and have been reported to cause widespread depletion of brain norepinephrine and dopamine (*3*). Furthermore, aphagia has been observed after extrahypothalamic electrolytic or electrothermal lesions, or knife cuts, of a nigrostriatal pathway traversing the hypothalamus (*4, 5*) that includes a large dopamine-containing fiber system (*6*). Similar observations also have been made after intracerebral injections of 6-hydroxydopamine (6-HDA) along this pathway (*5, 7*), a procedure thought to cause a relatively specific degeneration of catecholamine-containing neurons (*8, 9*).

Intraventricular injections of 6-HDA also produce long-lasting depletions of brain catecholamines and should cause considerably less nonspecific damage than any of the above treatments. However, as yet little or no apparent effect of this treatment on feeding behavior has been observed (*9*). These observations are not consistent with evidence supporting an involvement of catecholamines in the control of feeding behavior. Therefore, we have determined whether subtle effects on this behavior may have been produced by the 6-HDA treatments but eluded observation by previous investigators because they were not specifically examined. We now report that 6-HDA administered intraventricularly can produce specific deficits in the feeding behavior of rats which parallel those of rats with lateral hypothalamic damage. Furthermore, these behavioral deficits are associated with substantial decreases in the concentrations of brain catecholamines in both groups of animals.

Male albino rats of the Sprague-Dawley strain (Zivic-Miller, Pittsburgh), weighing 175 to 225 g at the beginning of the experiment, were housed and tested in individual wire-mesh cages. They were allowed free access to Purina Chow pellets and tap water unless otherwise noted. Using ether as anesthesia, we injected 20 μl of either 200 μg of 6-hydroxydopamine hydrobromide (*10*) ($n = 10$) or the vehicle (0.9 percent NaCl, 0.1 percent ascorbic acid) ($n = 6$) into the cerebrospinal fluid by way of the left lateral ventricle. Ten days later the same injection was delivered by way of the right lateral ventricle of each rat (*11*).

Food intake decreased by 27 to 46 percent during the first 24 to 36 hours after each administration of 6-HDA and then returned toward normal. Testing was begun 3 weeks after the last injection. For three successive days, food intake in the home cage (with water present) was measured every hour for 7 hours (9:30 a.m. until 4:30 p.m.). On the fourth day, after the first hour of testing, each rat was injected intraperitoneally with 750 mg of 2-deoxy-D-glucose per kilogram of body weight to induce short-term decreases in glucose utilization (glucoprivation) (*12*). 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 (Table 1). In addition to eating more food, they also ate food more often; that is, when the test session was divided into six hourly intervals for each rat, it was seen that control rats ate in more than 60 percent of the intervals whereas previously they had been eating in less than 20 percent of them ($P < .01$). In contrast, rats treated with 6-HDA neither ate significantly more food (Table 1) nor ate food more often after the injection of 2-deoxy-D-glucose. Thus, these results reveal that rats treated with 6-HDA, unlike normal rats but like rats apparently recovered from lateral hypothalamic damage (*13, 14*), do not respond to short-term glucoprivation by increasing food intakes.

In a second test of feeding behavior in response to increased regulatory demands for food, conducted 2 weeks later, rats were placed in a temperature-controlled environment for four consecutive days (ambient temperatures = 24°, 15°, 5°, and 5°C, successively), and their food intakes were measured each day. Control rats increased their daily food intakes during the 4-day period (by 19 percent, from 30.4 g on the first day to 36.1 g on the fourth; $P < .05$) as did rats treated with 6-HDA (by 31 percent, from 24.7 g to 32.4 g; $P < .01$). When these intakes were calculated again in terms of the body weight of each animal, no significant difference between the two groups of rats was observed. Similarly, the body temperatures (measured by a thermistor probe inserted 5 cm rectally) of both groups were comparable (range 35.5 to 37.0°C). Thus, these results demonstrate that rats treated with 6-HDA regulate their body temperatures normally during exposure to low environmental temperatures and, like normal rats as well as rats recovered from lateral hypothalamic damage (*13*), compensate for increased energy expenditure by increasing food intakes.

During these tests, rats treated with 6-HDA ate less food and weighed less than the control rats, although these values had been comparable when the experiments began 6 to 7 weeks previously. These observations were supplemented and confirmed by periodic monitoring of body weights and daily food intakes throughout the testing period. When rats were killed 10 weeks after the experiments had begun (for assays of brain catecholamines), the body weights of rats receiving 6-HDA treatments were considerably lower than those of the control rats (mean values, 381.5 g and 465.3 g, respectively; $P < .01$). Thus, 6-HDA administered intraventricularly in rats leads to a long-term maintenance of body weight at subnormal levels that resembles the long-term impairment seen in rats recovered from lateral hypothalamic lesions (*15*).

Table 1. Food intake during 6-hour feeding test. Control data are presented as the mean of the 3 days prior to the administration of 2-deoxy-D-glucose. Some animals received two intraventricular injections of 200 μg of 6-HDA with or without an intraperitoneal injection of pargyline (50 mg/kg) 30 minutes prior to 6-HDA. Data are presented as mean food intake ± estimated standard error of the mean. Numbers of rats are indicated in parentheses; LH, lateral hypothalamus.

Treatment	Food intake (g)	
	Control days	2-Deoxy-D-glucose
Control (6)	2.10 ± 0.41	5.94 ± 0.74*
6-HDA (10)	1.30 ± 0.22	1.70 ± 0.38
Pargyline + 6-HDA (4)	0.64 ± 0.32	1.38 ± 0.47
LH lesions (7)	1.20 ± 0.34	0.96 ± 0.34

* $P < .001$.

All of the above results have been confirmed in a second group of 12 rats.

In order to contrast the catecholamine depletions produced by the electrolytic and "biochemical" lesion procedures, a group of five rats were prepared with bilateral electrolytic lesions of the lateral hypothalamus (*16*). All of the rats with lesions showed severe aphagia and adipsia, lost more than 20 percent of their body weights, and had to be maintained by intragastric tube feeding and special diets of high palatability. These animals with lesions and several control animals of the same initial body weight were killed 1 to 2 weeks later, along with the rats given injections of 6-HDA or the vehicle. The brain of each animal was rapidly removed from the skull and dissected on ice (*17*). The diencephalon was placed in formalin. Subsequent histological examination confirmed widespread bilateral lateral hypothalamic damage at the level of the ventromedial hypothalamic nucleus, with significant damage invading the internal capsule, dorsolaterally. The striatum and remaining telencephalon were stored on dry ice and later analyzed fluorometrically for dopamine and norepinephrine, respectively, by means of a modification of previous methods (*18*).

Table 2. Effect of lateral hypothalamic lesions or 6-hydroxydopamine treatment on brain catecholamines. Data are presented as micrograms of catecholamines per gram of fresh tissue weight ± estimated standard error of the mean. Values are uncorrected for recovery (82 percent for norepinephrine, 80 percent for dopamine). Pargyline-treated animals received 50 mg/kg (intraperitoneally). 6-Hydroxydopamine-treated animals received 200 μg of 6-HDA (intraventricularly). Animals treated with pargyline plus 6-HDA received 50 mg of pargyline per kilogram of body weight 30 minutes prior to receiving 200 μg of 6-HDA. Control animals received injections of the vehicle (intraperitoneally and intraventricularly). Each animal received two identical drug or control treatments 72 hours apart. The numbers of rats are indicated in parentheses.

Group	Telencephalic norepinephrine (μg/g)	Striatal dopamine (μg/g)
Lateral hypothalamic lesions		
Control (4)	0.33 ± .01	9.88 ± 2.58
Lesion (5)	0.18 ± .05*	0.45 ± 0.16†
6-Hydroxydopamine treatment		
Control (6)	0.46 ± .01	14.33 ± 2.89
Pargyline (3)	0.48 ± .08	11.65 ± 3.50
6-HDA (10)	0.03 ± .01†	5.40 ± 1.34*
Pargyline + 6-HDA (5)	0.03 ± .01†	0.56 ± 0.20†

* $P < .05$. † $P < .01$.

Both lateral hypothalamic lesions and 6-HDA treatment produced substantial decreases in norepinephrine and dopamine concentrations. However, the pattern and absolute magnitudes of these effects were different (Table 2). The principal deficit after 6-HDA treatment was in telencephalic norepinephrine, whereas lateral hypothalamic lesions caused an almost complete disappearance of dopamine in the striatum with a smaller decrease in telencephalic norepinephrine (*19*).

The results are consistent with hypotheses that brain catecholamines are involved in the control of food intake and the regulation of body weight. Although no clear statement can be made concerning the role of individual catecholamines in specific neural control systems on the basis of present evidence, three points are evident. (i) 6-Hydroxydopamine treatment and electrolytic lateral hypothalamic lesions each produced a deficit in body weight maintenance and in the feeding response to short-term glucoprivation and were each associated with depletions of at least 40 to 60 percent of telencephalic norepinephrine and striatal dopamine. (ii) Only subtle changes in feeding behavior and body weight maintenance were observed after 6-HDA treatment despite the almost complete disappearance of telencephalic norepinephrine, suggesting that the 40 to 50 percent depletion of this monoamine after lateral hypothalamic lesions was not the cause of the observed aphagia. (iii) The striking aphagia produced by lateral hypothalamic lesions

was associated with virtually complete depletion of striatal dopamine and did not appear when the dopamine depletion was less complete after 6-HDA treatment.

To test the hypothesis that large dopamine depletions were responsible for the more dramatic feeding deficit, we treated another group of rats with 6-HDA (200 μg/kg, administered intraventricularly) 30 minutes after an intraperitoneal injection of pargyline (50 mg/kg). Pargyline, an inhibitor of monoamine oxidase, has been shown to potentiate the effects of 6-HDA on dopamine depletion but not norepinephrine depletion (*20*). During the next 24 hours the 11 rats given the pargyline plus 6-HDA treatment showed almost complete aphagia and adipsia, and lost 8 to 10 percent of their body weight. Other animals given either 6-HDA or pargyline alone showed little or none of these deficits. Within 72 hours, most of the rats given pargyline plus 6-HDA had begun to recover feeding behavior and body weight maintenance and were given a second pargyline plus 6-HDA treatment. This time they showed complete aphagia and adipsia in the first 24 hours after the combined drug treatment, whereas, of the other animals, only the rats given the second 6-HDA (alone) treatment showed even a transient hypophagia. Two of the rats given the pargyline plus 6-HDA treatment died within 3 days after the second injection, after complete aphagia and a 30 to 35 percent loss of body weight. The nine other rats showed marked hypophagia and loss of body weight. Five became moribund and were killed so that their brains might be removed for biochemical analysis; these animals showed a much larger depletion of striatal dopamine than had the animals given 6-HDA alone (Table 2), although the depletions of telencephalic norepinephrine were comparable in the two groups. The other four rats slowly recovered and were tested later.

Although aphagia accompanied the increased loss of striatal dopamine in each rat given pargyline plus 6-HDA, there are several reasons to be cautious in interpreting this association. (i) We have observed that rats may resume feeding despite an almost total depletion of this catecholamine (*21*). (ii) 6-Hydroxydopamine produces nonspecific cellular damage (*22*), some of which may be temporary unlike the more permanent destruction of catecholamine-containing terminals. By blocking deamination of 6-HDA, pargyline can be expected to increase the time during which 6-HDA can act and thus potentiate these nonspecific effects. (iii) 6-Hydroxydopa, which is converted to 6-HDA (*23*), also produces a temporary aphagia when given in doses that deplete brain norepinephrine but not brain dopamine (*24*). Thus, it is possible that other events are responsible for the aphagia and that the changes in striatal dopamine concentrations which we observed are not the primary events of significance.

The four animals given pargyline plus 6-HDA and which resumed eating Purina Chow pellets and drinking water were tested for feeding in response to short-term glucoprivation. At the same time an additional group of seven rats that had recovered from lateral hypothalamic lesions and seven control animals also were tested. Rats with electrolytic or biochemical lesions did not eat more food after the 2-deoxy-D-glucose injection than they had during the control periods (Table 1), whereas the control animals increased their food intakes significantly (from 0.80 g to 5.57 g; $P < .001$). On subsequent analysis, it was found that the brain catecholamine depletions in the rats given pargyline plus 6-HDA were not significantly different from those observed in the comparably treated animals that did not recover.

Our results demonstrate that 6-HDA administered intraventricularly can produce several aspects of the classical lateral hypothalamic syndrome (*2*), that is, the striking aphagia observed initially as well as the more subtle but unmistakable disruptions in feeding behavior and body weight maintenance that are seen after subsequent recovery. A common physiological basis for these similar effects may be the depletion of brain catecholamines produced through drug-induced degeneration of the norepinephrine- and dopamine-containing neurons (by 6-HDA treatment) or anterograde degeneration after interruption of ascending monoamine-containing fibers as they traverse the hypothalamus (by lateral hypothalamic lesions). These observations are consistent with the finding of Ungerstedt (*8*) that prolonged aphagia follows destruction of a nigrostriatal dopamine-containing pathway and with other studies suggesting a role of catecholamines in the control of feeding (*25*).

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References and Notes

1. B. K. Anand and J. R. Brobeck, *Yale J. Biol. Med.* **24**, 123 (1951); P. J. Morgane, *J. Comp. Neurol.* **117**, 1 (1961).
2. P. Teitelbaum and E. Stellar, *Science* **120**, 894 (1954); P. Teitelbaum and A. N. Epstein, *Psychol. Rev.* **69**, 74 (1962).
3. A. Heller and R. Y. Moore, *J. Pharmacol.* **150**, 1 (1965); N.-E. Andén, A. Dahlström, K. Fuxe, K. Larsson, L. Olson, U. Ungerstedt, *Acta Physiol. Scand.* **67**, 313 (1966).
4. R. M. Gold, *Physiol. Behav.* **2**, 211 (1967); D. J. Albert, L. H. Storlien, D. J. Wood, G. K. Ehman, *ibid.* **5**, 1075 (1970); G. A. Oltmans and J. A. Harvey, *ibid.* **8**, 69 (1972).
5. U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367**, 95 (1971).
6. N.-E. Andén, A. Carlsson, A. Dahlström, K. Fuxe, N.-Å. Hillarp, K. Larsson, *Life Sci.* **3**, 523 (1964); R. L. M. Faull and R. Laverty, *Exp. Neurol.* **23**, 332 (1969).
7. G. P. Smith, A. J. Strohmayer, D. H. Reis, *Nature New Biol.* **235**, 27 (1972).
8. U. Ungerstedt, *Eur. J. Pharmacol.* **5**, 107 (1968).
9. G. R. Breese and T. D. Traylor, *J. Pharmacol.* **174**, 413 (1970); F. E. Bloom, S. Algeri, A. Groppetti, A. Revuelta, E. Costa, *Science* **166**, 1284 (1969); N. J. Uretsky and L. L. Iversen, *J. Neurochem.* **17**, 269 (1970); K. D. Evetts, N. J. Uretsky, L. L. Iversen, S. D. Iversen, *Nature* **225**, 961 (1970).
10. All drug doses are reported as units of the free base.
11. E. P. Noble, R. J. Wurtman, J. Axelrod, *Life Sci.* **6**, 281 (1967). 6-Hydroxydopamine administered into the cerebrospinal fluid by way of either ventricle is distributed bilaterally throughout the brain. Bisected brains showed symmetrical depletions of catecholamines after unilateral and bilateral injections.
12. G. P. Smith and A. N. Epstein, *Amer. J. Physiol.* **217**, 1083 (1969).
13. A. N. Epstein and P. Teitelbaum, *ibid.* **213**, 1159 (1967).
14. A. N. Epstein, in *Progress in Physiological Psychology*, E. Stellar and J. M. Sprague, Eds. (Academic Press, New York, 1971), pp. 263–317.
15. T. L. Powley and R. E. Keesey, *J. Comp. Physiol. Psychol.* **70**, 25 (1970).
16. Electrolytic lesions were made with a monopolar stainless steel electrode by passing 2-ma anodal current for 15 seconds. With the skull flat, 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.
17. Dissection was performed as follows. The brain, including olfactory bulbs, was removed from the skull and placed on its dorsal surface. Transverse cuts were made through the brain at the anterior border of the olfactory tubercle and at the optic chiasm. The major portion of the striatum is found in the region between these two cuts, and it was dissected out on the basis of its distinctive cytoarchitectural appearance. The brain was then placed on its ventral surface and the telencephalon caudal to the optic chiasm was peeled from the brainstem in the rostral direction and severed from the diencephalon along the thalamic radiations. Remaining striatum was removed from this tissue and pooled with the previously dissected striatal tissue. All remaining telencephalic tissue was then combined. The average total fresh weight of striatum was 70 mg; average weight of the remaining telencephalon was 956 mg.
18. J. R. Crout, C. R. Creveling, S. Udenfriend, *J. Pharmacol.* **132**, 269 (1961); M. K. Shellenberger and J. H. Gordon, *Anal. Biochem.* **39**, 356 (1971).
19. The depletion of striatal dopamine was complete 3 to 5 days after the lesions were made. Telencephalic norepinephrine depletion was not complete until 10 to 14 days after the operation. The differential effect of lateral hypothalamic lesions on dopamine and norepinephrine concentration was apparent even when both depletions were complete.
20. G. R. Breese and T. D. Traylor, *Brit. J. Pharmacol.* **42**, 88 (1971).
21. Such recovery may be explained by increasing receptor sensitivity to transmitter still present in residual neurons. Alternatively, during recovery a new neuronal system involving the same or different receptors might assume a role once held by dopamine-containing fibers. Specific experimental evidence supporting these hypotheses is not yet available.
22. L. J. Poirier, P. Langelier, A. Roberge, R.

Boucher, A. Kitsikis, *J. Neurol. Sci.*, in press; U. Ungerstedt, in *6-Hydroxydopamine and Catecholamine Neurons*, T. Malmfors and H. Thoenen, Eds. (American Elsevier, New York, 1971), pp. 101–127; F. Bloom, in *ibid.*, pp. 135–150.
23. R. M. Kostrzewa, *Fed. Proc.* **31**, 589 (1972).
24. P. Teitelbaum, E. Satinoff, J. Marshall, R. Kostrzewa, D. Jacobowitz, *Pharmacologist* **13**, 304 (1971); J. S. Richardson and D. M. Jacobowitz, *Fed. Proc.* **31**, 529 (1972).
25. S. P. Grossman, *Science* **132**, 301 (1960); N. E. Miller, K. S. Gottesman, N. Emery, *Amer. J. Physiol.* **206**, 1384 (1964); B. D. Berger, C. D. Wise, L. Stein, *Science* **172**, 281 (1971).
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Rat Fighting Behavior: Serum Dopamine-β-Hydroxylase and Hypothalamic Tyrosine Hydroxylase

Abstract. *Male Sprague-Dawley rats were subjected to 4 weeks of daily periods of immobilization stress. One of two experimental groups was allowed 1 month of recovery. After 4 weeks of stress, there was a significant increase in shock-induced fighting, in the activity of serum dopamine-β-hydroxylase, and in the activity of hypothalamic tyrosine hydroxylase. The concentration of hypothalamic norepinephrine was not decreased. After 4 weeks of recovery, only serum dopamine-β-hydroxylase activity returned to normal; it therefore appears that long-term stress may increase central catecholamine synthesis, possibly resulting in a persistent increase in aggressive behavior.*

Norepinephrine (NE) seems to play an important role in brain mechanisms controlling behavior (*1, 2*). Sham rage, elicited by electrical stimulation of the amygdala, is associated with a fall of NE in both forebrain and brainstem (*3*). Short-term stress produced by electric shock to the footpad, by fighting, or by immobilization have been found to produce decreases in brain NE (*4*). Euphoriants such as cocaine and antidepressants such as the tricyclic agents are thought to increase the amount of NE at central NE receptors; depressant drugs such as reserpine or the lithium salts are postulated to have the opposite effect (*2, 5*). Studies in our laboratory have shown that repeated daily intervals of immobilization of rats for 7 days produces an increase in the activity of the peripheral sympathetic nervous system, as reflected by increases of dopamine-β-hydroxylase (DBH) activity in serum (*6*) and by increases of catecholamine-synthesizing enzymes in the adrenals (*7, 8*). In extending these observations, we have examined the effects of immobilization stress on aggressive behavior and the activity (in brain) of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of NE (*9*).

Male Sprague-Dawley rats (140 to 160 g) were divided into two groups (E1 and C1). One group (E1) was subjected to repeated immobilization, as described (*10*), for 2 hours daily over a 4-week interval (a total of 28 immobilization periods); the other group (C1) served as controls. A similar experiment was conducted with Sprague-Dawley rats weighing 240 to 270 g (E2 and C2).

After both sets of rats had been repeatedly immobilized for 4 weeks, blood samples were obtained (0.6 to 1.0 ml) from the lateral tail vein of each animal. Groups E2 and C2 were then killed by cervical dislocation; groups E1 and C1 were returned to their cages. Blood samples were obtained from E1 and C1 rats 4 weeks after cessation of immobilization stress; the E1 and C1 animals were then also killed by cervical dislocation. Serum activity of DBH was determined by a modification of the method described by Weinshilboum and Axelrod (*11*). Fighting and jump threshold tests were performed on days when blood samples were taken. Previous studies have shown that even repeated shock-induced aggression is not associated with altered adrenal tyrosine hydroxylase or total brain catecholamine content (*12*).

Animals were subjected, in pairs, to 50 electric footshocks (2-ma intensity, 0.4-second duration every 7.5 seconds) as described (*13*). The fighting behavior was quantified by scoring the number of attack responses as described by Eichelman (number of attacks per 50 shocks × 100) (*13*). Jump thresholds were determined with the use of a test chamber (*13*) to evaluate changes in response to the shocks. After the rats were killed, the brains were rapidly removed and dissected into brainstem, hypothalamus and the remainder of the brain frozen on Dry Ice, and kept frozen until tyrosine hydroxylase assays were performed (within 1 week). The activity of tyrosine hydroxylase remains unaltered if the assays are performed within 1 week. Brainstems and hypothalami were each homogenized in a glass homogenizer with 5 ml of 0.3*M* sucrose; the remainder of the brain was homogenized in 10 ml of 0.3*M* sucrose.

The coupled decarboxylation method of Waymire *et al.* (*14*) was used to determine tyrosine hydroxylase activity. Because of the high tissue dilution (hypothalamus), substrate with higher specific activity was necessary to increase sensitivity; but endogenous tyrosine was negligible compared to the amount of L-[1^{-14}C]tyrosine (45.5 mc/mmole) (New England Nuclear), which was used in a final concentration of $4.48 \times 10^{-5}M$. Although proportional to enzyme activity, the reaction velocity was far below V_{max} ($K_m = 2.4 \times 10^{-4}M$) (*15*); therefore the results are expressed in disintegrations per minute per gram of wet weight per hour.

In both experimental groups, repeated immobilization for 4 weeks produced a marked increase ($P < .001$)

Table 1. Effects of repeated immobilization on serum dopamine-β-hydroxylase (DBH), attack behavior, and hypothalamic tyrosine hydroxylase (TH). Rats were immobilized for 2 hours each day for 4 weeks, and serum DBH activity and attack behavior were determined. In experiment 1, these measurements were repeated for another 4 weeks before the animals were killed, and the activity of hypothalamic TH (disintegrations per milligram of wet weight per hour) was determined. In experiment 2, the animals were killed immediately. Rats were treated in pairs for the measurement of percent of attacks.

Condition	*N*	DBH in serum (nmole/ml per hour)	Attacks (%)	Hypothalamic TH (dpm mg^{-1} hr^{-1})
		Experiment 1		
Control	6	5.9 ± 0.6	32.7 ± 2.4	
Immobilized	9	11.7 ± 0.5‡	59.8 ± 5.6†	
Control	6	5.3 ± 0.6	23.0 ± 3.0	1331 ± 120
After immobilization	7	6.5 ± 0.5	60.0 ± 10.2*	2398 ± 228‡
		Experiment 2		
Control	8	7.8 ± 0.5	19.0 ± 5.0	1364 ± 144
Immobilized	11	12.2 ± 0.8‡	60.4 ± 4.2‡	1890 ± 99†

* $P < .05$. † $P < .01$. ‡ $P < .001$.