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26. We thank J. Marincic, S. Wuerthele, and J. Yen for their technical assistance. Supported by NIH grant MH-20620 and NSF grant GB-28830. Pargyline was furnished by Dr. H. G. Schoepke (Abbott Laboratories, North Chicago, Illinois). All other drugs and reagents were purchased from commercial sources.

21 March 1972; revised 30 May 1972

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†

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

in serum DBH activity (Table 1), presumably reflecting increased peripheral sympathetic nerve activity (*11*). Because increased fighting persisted in the stressed rats 4 weeks after the cessation of stress (at which time the serum DBH activity had returned to control values) (Table 1), the effect of prolonged immobilization stress on sympathetic nerve activity seems to be independent of its effect on fighting behavior. The increased fighting behavior may be related to increased hypothalamic catecholamine synthesis. The hypothalamic activity of tyrosine hydroxylase (Table 1) remained increased in immobilized rats 4 weeks after cessation of the stress. The activity of tyrosine hydroxylase was not altered in the brainstem or in the remainder of the brain in either group of experimental animals. The stressed animals exhibited no change in jump thresholds; thus, it appears unlikely that increased sensitivity to electric shock (defined by jump thresholds) was responsible for the observed increased fighting behavior.

The increase in hypothalamic tyrosine hydroxylase presumably is indicative of increased catecholamine synthesis. Previous reports have demonstrated increased conversion of [^{14}C]-tyrosine to NE in brain after short-term exposure to exercise and cold (*16*) and to electroshock (*17*). However, because both reports indicated that NE content of whole brain is decreased after exposure to stress, it appears that catecholamine release from the nerves is more rapid than its synthesis. In our study, with repeated intervals of immobilization, no decrease in hypothalamic NE was found (*18*). In the adrenal medulla of rats subjected to short-term stress, the catecholamine content decreases (*10*). After prolonged, repeated immobilization, however, the NE content of adrenals is normal or even elevated and epinephrine amounts are not diminished, although increased catecholamine release still occurs (*10*). There is, however, a striking elevation in activity of tyrosine hydroxylase in the adrenal (*8*) that is associated with an increased rate of synthesis of catecholamines; thus, concentrations of NE are maintained in spite of enhanced release. In our study, similar changes appeared to occur in the hypothalamus; tyrosine hydroxylase activity was elevated after 4 weeks of repeated immobilization, with no significant decrease in catecholamine content.

When immobilization intervals were terminated, the tyrosine hydroxylase activity in the adrenal glands returned to normal after a 2-week period (half-life, approximately 3 days). In contrast, tyrosine hydroxylase activity in the hypothalamus was still elevated 4 weeks after immobilization intervals had been stopped and the increased attack behavior in response to shock also persisted after cessation of immobilization. The persistence of the increased tyrosine hydroxylase in the central nervous system, presumably indicative of enhanced catecholamine synthesis, and the increased fighting behavior long after the cessation of stress suggest a possible mechanism whereby long-term stress can result in persistently increased aggressive behavior.

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19. We thank L. G. Carmon for his help in immobilizing the experimental animals and T. M. Cridlin for her technical assistance in performing the DBH assays. F.L. is supported by a fellowship from the Deutscher Akademischer Austauschdienst.

17 May 1972; revised 8 June 1972

(*Continued from page 1179*)

RECENT DEATHS

Edward N. Cook, 66, former professor of surgery, Graduate School of Medicine, Mayo Clinic; 26 July.

Myrtle C. Craig, 59; professor of education, Old Dominion University; 25 July.

Clifford Deutscher, 46; former assistant clinical professor of psychiatry, Albert Einstein College of Medicine; 14 April.

C. Dary Dunham, 61; former assistant clinical professor of medicine, College of Physicians and Surgeons, Columbia University; 12 April.

Clarence L. Eckel, 80; dean emeritus, College of Engineering, University of Colorado; 31 July.

Hallie G. Gantz, 62; president, Phillips University; 21 July.

Thomas Garvey, Jr., 69; associate professor of neurosurgery, New York University Medical Center; 5 April.

Charles S. Gwynne, 86; former professor of earth sciences, Iowa State University; 18 June.

Edwin S. Hammond, 78; former professor of mathematics, Bowdoin College; 22 March.

P. Arne Hansen, 69; professor of microbiology, University of Maryland; 2 June.

Richard E. Lee, 57; former assistant professor of clinical medicine, Cornell University Medical Center; 13 April.

Robert B. MacLeod, 65; professor of psychology, Cornell University; 19 June.

John N. McDonnell, 62; former president, Columbia University College of Pharmacy; 11 April.

William Menaker, 76; adjunct professor of psychology, New York University; 9 April.

David Perlman, 62; professor of chemistry, City College of New York; 6 April.

Parke H. Simer, 74; professor emeritus of anatomy, University of Illinois; 6 March.

James H. Taylor, 79; former chairman, mathematics department, George Washington University; 30 March.

Karl S. Woodcock, 76; professor emeritus of physics, Bates College; 24 March.

Conway Zirkle, 77; professor emeritus of botany, University of Pennsylvania; 28 March.