References and Notes

1. D. Malo, *Hawaiian Antiquities* (B. P. Bishop Museum Special Publication 2, ed. 2, Honolulu, 1951), p. 201.
2. Manuscript notes by Katherine Livermore on file at B. P. Bishop Museum, Honolulu.
3. Originally, the species was thought to be *Palythoa vestitus* Verrill, but reinvestigation of the Hawaiian zoanthids by G. E. Walsh and R. L. Bowers (in preparation) makes the binomial uncertain at present.
4. B. W. Halstead, *Poisonous and Venomous Marine Animals of the World* (Government Printing Office, Washington, D.C., 1965), vol. 1, pp. 297–307.
5. D. H. Attaway, thesis, University of Oklahoma (1968).
6. Y. Hashimoto, N. Fusetani, S. Kimura, *Bull. Japan Soc. Sci. Fish.* **35**, 1086 (1969).
7. We thank the Dow Chemical Co. through the efforts of Dr. K. Terada for the powdered polyethylene, designated as experimental resin QX-2187 (density 0.960, melt. index 1), which we found to be highly satisfactory for our work.
8. F. Märki and B. Witkop, *Experientia* **19**, 329 (1963). We thank Dr. Witkop for providing us with details of the micro determination: A 0.100-ml portion of each fraction was mixed with 1.00 ml of dichromate reagent (1.00 g sodium dichromate dihydrate in 20 ml of water, diluted to 1 liter with concentrated sulfuric acid) and oxidized by heating in a boiling water bath for 20 minutes. The solution was then diluted to 4.00 ml with water, and the optical density at 350 nm was determined.
9. We thank Professor C. Djerassi for determining the optical rotatory dispersion curve.
10. Analyses by Berkeley Analytical Laboratory, Berkeley, Calif.
11. Extinction coefficients are based on an assumed molecular weight of 3300 and can therefore be expressed as 15.1 M or 8.8 M, where M is the molecular weight (Fig. 4).
12. T. Tokuyama, J. Daly, B. Witkop, *J. Amer. Chem. Soc.* **91**, 3931 (1969).
13. P. Karrer, F. W. Kahnt, R. Epstein, W. Jaffe, T. Ishii, *Helv. Chim. Acta* **21**, 223 (1938).

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Norepinephrine Biosynthesis Inhibition: Effects on Memory in Mice

Abstract. *Diethyldithiocarbamate, a dopamine beta hydroxylase inhibitor, decreases biosynthesis of norepinephrine in the brain. The effects of this inhibitor coincide with alterations in memory as demonstrated in single-trial passive avoidance in C57BL/6J mice.*

Recent evidence suggests that norepinephrine may play an important role in memory. Roberts *et al.* (*1*) have demonstrated that amnesia resulting from administration of puromycin can be reversed by a variety of drugs that compete for adrenergic receptor sites. Seiden and Peterson (*2*) have shown that reserpine and α-methyl-*p*-tyrosine, both of which decrease the concentration of amines in the brain, cause a temporary failure to perform a well-learned conditioned avoidance response. Our study demonstrates that inhibition of norepinephrine synthesis in the brain at the dopamine β-hydroxylase stage is associated with early enhancement and later impairment of memory for a one-trial passive avoidance response in the C57BL/6J strain of mice.

Diethyldithiocarbamate (DDC), a dopamine β-hydroxylase inhibitor, decreases the synthesis and the brain concentration of norepinephrine, while the concentration of dopamine remains unchanged or slightly increased (*3*). Inhibition of the biosynthesis of norepinephrine in the brain was measured by two separate methods. In the first procedure, the conversion of [^{14}C]dopa to [^{14}C]dopamine and [^{14}C]norepinephrine was analyzed in the brains of control mice and those treated with DDC (*4*). The second determination assayed endogenous norepinephrine concentrations fluorometrically in both groups (*5*).

Adult male mice (70 to 100 days of age) of the C57BL/6J strain were placed in the start compartment of a two-chamber apparatus previously described by Quartermain and McEwen (*6*). After 15 seconds, the door to an adjacent larger compartment was opened with simultaneous activation of a timer and a flashing white light at the far end of the chamber. When the mouse entered the second compartment, the door was shut, and the timer stopped. For the last 2 seconds of a 20-second period after entry, a scrambled foot shock (0.16 ma for 2 seconds) was automatically delivered through the floor bars. Retention of the avoidance response was tested by replacing the mice in the start compartment and recording the latency to reenter the second compartment. The light flashed continuously during the test period. Animals failing to enter within 3 minutes were removed and given a score of 180.0 seconds.

In our first experiment, different groups of mice were injected subcutaneously with DDC [250 mg per kilogram of body weight (0.3 ml); $N = 10$] or with saline (0.3 ml; $N = 10$) 30 minutes before the foot-shock training trial and tested for retention at 1 minute, 5 minutes, 1 hour, 6 hours, or 24 hours after the training trial. Although this dose of DDC reduces spontaneous activity by approximately 30 percent (*7*), latencies to enter the compartment on the training trial were consistently shorter for the mice injected with DDC than for the controls injected with saline. Mean initial latency for all DDC injected mice was 5.6 seconds and for all saline injected mice 7.4 seconds, indicating no impairment, due to the drug, of motor ability to enter the second chamber.

The results of the retention tests are illustrated in Fig. 1. The saline injected control groups of mice show an increase in latency to reenter the compartment in which they had received foot shock after the 1-minute and 5-minute tests. The relatively poor short-term memory in controls of this strain

Table 1. The effect of DDC on [^{14}C]norepinephrine (NE) biosynthesis from [^{14}C]dopa and on endogenous NE concentrations in the brain of C57BL/6J mice. The animals were treated subcutaneously with 250 mg of DDC per kilogram, and 1 hour after administration of [^{14}C]dopa (specific activity, 3.18 mc/mole; 5 μc per mouse, given intravenously) the animals were killed. In separate groups of animals the concentrations of endogenous NE (last column) were determined. The results are the means from three experiments ± the standard error of the means. N.D., not detectable (below 200 count/min).

Time interval after administration of DDC	[^{14}C]NE (count/min per brain)	NE (nanograms per gram of tissue)
Controls (saline)	2800 ± 150	385.0 ± 11.5*
30 minutes		260.0 ± 8.5
90 minutes	N.D.	205.0 ± 6.0
4½ hours	N.D.	202.5 ± 7.0
8½ hours	1100 ± 100	215.0 ± 6.5

* There was no difference in brain NE between control mice tested 1 minute after training and those either injected with saline 30 minutes before training or those killed immediately after removal from their home cages.

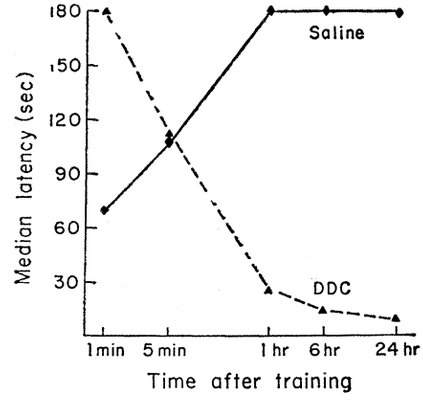

Fig. 1. Median latency to enter the large compartment on the retention test as a function of the time interval between the training trial and the retention test. Injections of DDC and saline were given 30 minutes before the training trial in all groups.

of mice in a similar test situation has been noted (*8*). Latencies of the saline injected groups tested 1 hour after training are significantly ($P = <.05$) (*9*) longer than both the 1-minute and the 5-minute groups. When mice that had been injected with DDC 30 minutes previously were tested 1 minute after training, there was a significant increase in latency ($P = <.01$), reflecting increased retention as compared with saline controls. The DDC group tested 5 minutes after training had latencies significantly shorter than the 1-minute group ($P = <.05$), indicating that amnesia was rapidly developing. When tested 1 hour after training, the DDC injected mice were clearly amnesic, as were groups tested at 6 and 24 hours. The latencies of these three groups that received DDC are all significantly ($P = <.001$) shorter than their comparable controls that received saline. These data indicate that DDC injected mice show an initial enhancement of memory, followed by the development of amnesia which reaches a maximum 6 hours after training and persists up to 24 hours.

In the second experiment, we examined the effect of injecting DDC at various times before and after foot shock, with subsequent testing for retention at 24 hours. Four groups were injected subcutaneously with DDC (250 mg/kg; $N=10$) or with saline ($N=10$) 30 minutes before, immediately after, 2 hours after, and 23.5 hours after training. Figure 2 shows the results of this experiment. Significant amnesia (compared with results from saline controls) also occurs when mice are injected immediately after the training trial ($P = <.01$), but not if the injection is delayed for 2 hours. This indicates that retention defects are not due to lack of initial registration of the stimuli. Amnesia is again produced if DDC is given 30 minutes before the retention test, 23.5 hours after training ($P = <.001$). This latter effect appears to be a clear instance of interference with retrieval and may have a different basis from amnesia observed when the drug is injected immediately after training.

The biochemical results show that DDC, in the dose administered, effectively lowers the [^{14}C]norepinephrine biosynthesis from [^{14}C]dopa and that the endogenous norepinephrine concentrations are significantly decreased in the brains of the treated mice (Table 1). The most effective inhibition of dopamine β-hydroxylase in the brain, as measured by the conversion of

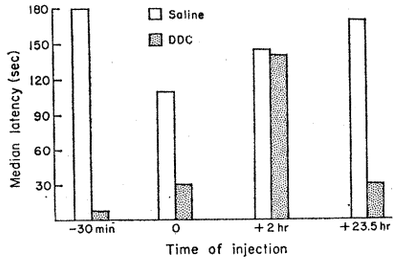

Fig. 2. Median latency to enter the large compartment on the retention test as a function of the time interval between injections of DDC and saline before and after the training trial. All groups were tested for retention 24 hours after training.

[^{14}C]dopa to [^{14}C]norepinehprine, was apparent 90 minutes and 4.5 hours after administration of DDC, with partial recovery at 8.5 hours. Endogenous brain norepinehprine was decreased at 30 minutes after administration of DDC, with a further decrease observed at 1.5, 4.5, and 8.5 hours after DDC.

The enhancement of memory, tested 31 minutes after administration of DDC in this strain of mice, is associated with a decrease in brain norepinephrine. Impairment of memory at later time intervals also is associated with a decrease in the concentration of brain norepinephrine which is more marked than that observed at 30 minutes. These results may reflect the quantitative difference in concentration or early depletion of norepinephrine from storage pools with rapid turnover. The data in this preliminary report do not explain the apparent dual effects of inhibition of norepinephrine biosynthesis, which need to be investigated further. Inasmuch as DDC inhibits other enzymes, such as aldehyde dehydrogenase, which result in formation of phenolic alcohols, the possibility remains that these results on memory might be due to factors other than decreased concentration of norepinephrine in the brain. There is, however, evidence that norepinephrine is intimately involved in affective states (*10*). Common experience attests to an influence of emotion and arousal on memory. Severe dietary restriction of amino acids essential for the biosynthesis of catecholamines in man has been associated with defects in memory (*11*). The previously noted animal studies indicate that decrease of brain amines interferes with memory. Our results more directly suggest a noradrenergic compound subserving memory in mice.

CLARK T. RANDT
DAVID QUARTERMAIN
Department of Neurology, School of Medicine, New York University, New York 10016

MENEK GOLDSTEIN
BERTA ANAGNOSTE
Department of Psychiatry, School of Medicine, New York University

References and Notes

1. R. B. Roberts, J. B. Flexner, L. B. Flexner, *Proc. Nat. Acad. Sci. U.S.* **66**, 310 (1970).
2. L. S. Seiden and D. D. Peterson, *J. Pharmacol. Exp. Ther.* **159**, 422 (1968).
3. M. Goldstein and K. Nakajima, *ibid.* **157**, 96 (1967).
4. M. Goldstein, A. J. Friedhoff, S. Pomerantz, J. F. Contrera, *J. Biol. Chem.* **236**, 1816 (1961).
5. A. Anton and D. F. Sayre, *J. Pharmacol. Exp. Ther.* **138**, 360 (1962).
6. D. Quartermain and B. S. McEwen, *Nature* **228**, 677 (1970).
7. Unpublished data on activity measured in a photoactometer at various dosages of DDC.
8. D. Quartermain, B. S. McEwen, E. C. Azmitia, Jr., *Science* **169**, 683 (1970); C. T. Randt, B. Barnett, B. S. McEwen, D. Quartermain, in preparation.
9. Mann-Whitney U tests were used for all statistical comparisons.
10. J. J. Schildkraut and S. S. Kety, *Science* **156**, 21 (1967).
11. B. K. Lester, R. E. Chanes, P. T. Condit, *Amer. J. Psychiat.* **126**, 71 (1969).
12. Supported in part by the Grant Foundation, NIH grant MH 02717-13, and NSF grant GB 4465.

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Language Production: Electroencephalographic Localization in the Normal Human Brain

Abstract. *Slow negative potentials, which are at a maximum over Broca's area in the left hemisphere, were recorded when normal subjects spontaneously produced polysyllabic words. Bilaterally symmetrical potentials were seen with analogous, nonspeech control gestures. These potentials began up to 1 second before word or gesture articulation. These results are the first demonstration of localization of language production in normal human brain.*

In man's cerebral cortex the foot of the third frontal convolution in the left hemisphere is called Broca's area, named after Paul Broca who first suggested that it plays a major role in the expression of speech (*1*). Current neurological theory on the organization of language in the brain (*2*) is in basic