temperature fell precipitously, too closely related (in time) to the period of drinking to be accounted for by diencephalic circulation of blood cooled (by the ingested fluid) within the main body mass. Further, rectal temperature was seen to follow, rather than lead, these temperature changes in the hypothalamus. It would appear, therefore, that the reduced diencephalic temperature under these conditions was due to a loss of heat along conductive, rather than convective avenues.

The lowered hypothalamic temperature is accompanied by a period of peripheral vasodilation as seen in Fig. 2 (top). Only those body areas shown demonstrated these influences; there were no changes in the temperature levels of the tail, back, or distal portion of the hind leg. That this peripheral vascular response is more related to the reduced hypothalamic temperature and not to the dependent head position during drinking, or to the act of drinking, itself, is shown in Fig. 2 (bottom), which shows the result of ingesting milk at approximately body temperature. No predictable change in rectal or hypothalamic temperature and only a slight response in one extremity was relatable to the period of drinking in the latter test.

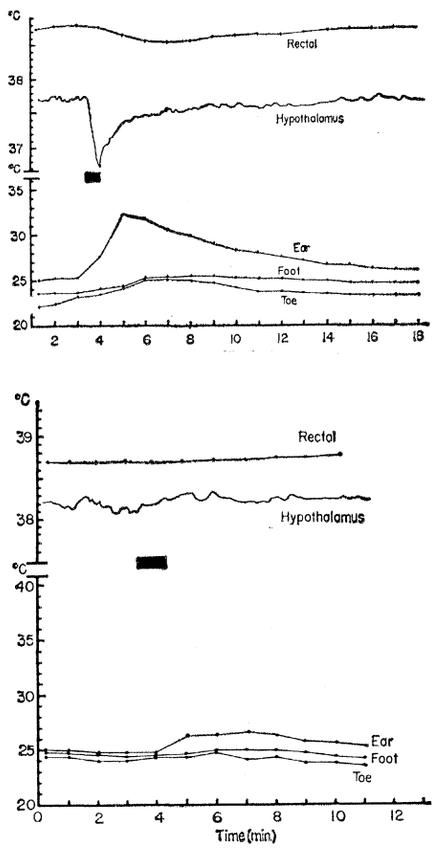


Fig. 2. Rectal, anterior hypothalamic, and extremity temperatures are shown during the ingestion of milk at 5°C (top) or at body temperature (bottom). The period of drinking is indicated by the shaded block under the hypothalamic temperature tracing.

The observation that peripheral vasodilation was consequent to lowering hypothalamic temperature in this manner is particularly surprising, since lowered anterior hypothalamic temperature by the use of thermodes in the cat and other animals has been reported to result in the more appropriate thermoregulatory peripheral vascular response of vasoconstriction (*5*).

The results of changing diencephalic temperature with water-perfused thermodes in the same animals used in the present study confirm the more predictable thermoregulatory responses of peripheral vasoconstriction with hypothalamic cooling (*7*); peripheral vasodilation only followed local hypothalamic heating with this technique. It should be noted, however, that the manner of inducing these thermal effects in the hypothalamus, either locally with thermodes or by feeding cold liquids, is quite different, and certainly more neural tissue is brought under the experimental thermal influence with the latter technique. Nonetheless, these two methods of inducing central nervous system temperature changes would not appear to be comparable physiologic testing procedures (*8*).


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## Alteration in Learning Ability Caused by Changes in Cerebral Serotonin and Catechol Amines


Abstract. *Excess of cerebral serotonin decreased maze-learning ability of adult mice; deficiency of serotonin and catechol amines increased it slightly.*


Although there is now a substantial body of evidence to show that malfunctioning of serotonin in the brain can lead to profound changes in behavior of men and laboratory animals (*1*), very little information exists on the relation of this hormone to learning ability. The situation is similar with respect to the catechol amines. The behavioral effects have been found during the exploration of the relationship of serotonin and catechol amines to mental diseases such as schizophrenia, as first described by Woolley and Shaw (*2*). We have wanted to find out whether serotonin likewise might be causally related to some of the inherited idiocies. Consequently, we have wanted to determine whether it has a connection with various aspects of intelligence, such as learning ability, in addition to its relation to behavior.

To measure learning ability of mice, a simple maze was used, consisting of a T-shaped brass channel 3.3 cm wide, with a transparent top so that the mouse always saw the observer. An adult mouse was placed in the maze at the bottom of the stem of the T. As it moved up to the union of the stem with the crossarms it reached the point of decision. If it turned one way and ran along the arm, it received a reward in the serif of the arm of the T. If it turned the other way and ran along that arm, it found no reward. The reward was to escape from the view of the experimenter into a dark cubicle in the serif. In the other arm of the T there also was such a dark hiding hole, but the mouse was prevented from entering it by a screen not visible from the point of decision. The animal was allowed exactly 2 minutes to explore the maze, and to learn that the reward was to be obtained only by making the correct turn. The mouse so trained was then tested in ten tries to determine how well it had learned its lesson. If it had learned nothing in the 2 minutes, chance alone would dictate that it would make the correct turn five times in ten tries. If it had learned perfectly, it would make a score

of 10. Actually when a group of ten adult mice was tested in this way, the average score was 7.4.

Although the test was so simple as to invite scorn of its accuracy, trials with large numbers of animals demonstrated that the results were surprisingly reproducible. Thus, the average scores for six groups of ten mice each were 7.5, 7.1, 7.8, 7.3, 7.1, 7.5; standard deviation ± 0.2; standard error, ± 0.1 This example illustrates the variation found in other experiments of this series. A variety of experiments were performed to check the reliability of the test and to make sure that it was relatively free of the errors which are sometimes present in maze assays. Thus, the relative positions of the hiding hole and the screened-off hiding hole were reversed with every alternate mouse, but without change in the average score. Furthermore, suitable experiments also showed that the animals were not receiving clues from the relative positions of objects in the room, from variations in the light source, or from the position of the experimenter. The maze was thoroughly washed after each experiment so clues could not be obtained from odors from previous inhabitants.

The maze-learning test was used in preference to a "Skinner box" with operant conditioning. Skinner boxes may not give answers as inclusive as a maze test, and they have other disadvantages for the purpose at hand. In the maze test, lengthening of the learning interval allowed one also to distinguish certain additional facts, as, for example, whether a change in score of treated mice represented a change in incentive rather than a change in learning ability. Such experiments indicated that the change induced by serotonin was in learning ability rather than in incentive. For example, if the decrease in learning ability caused by specific increase of cerebral serotonin had been due to the mice becoming less eager to hide (change in incentive), an increase of the time allowed for learning the correct turn should not have changed the score. Actually, however, it was found that a 5-minute period did increase the scores both of controls and of treated mice. The type of reward was chosen because it was the one most alluring to mice, and one for which the appetite was never satiated. Food rewards were not effective.

Specific increases in serotonin in the brain alone were induced with 5-hydroxytryptophan (HTP) plus 1-benzyl-2-methyl-5-methoxytryptamine (BAS) according to the method of Woolley *et al.* (*3*). Increases in serotonin throughout the body were accomplished with HTP alone, without BAS (*4*). Catechol amines were increased with dihydroxyphenylalanine (DOPA). Increases in both serotonin and catechol amines were caused by injection of iproniazid to inhibit monoamine oxidase (*5*). The injections were made intraperitoneally 15 to 30 minutes before the testing, and the optimal doses (as shown in Table 1) were determined in preliminary trials with each of the substances used. The increases in cerebral serotonin and catchol amines were demonstrated by suitable chemical analyses (see *3–5*). These changes were measured in parallel series of mice treated with the chemical substances in the same way and for the same time but not used in the maze test. The increase in cerebral serotonin caused by HTP (60 mg/kg) plus BAS (15 mg/kg) was from 2.3 μg per gram of brain (controls) to 3.4 μg (see *3*).

Decreases in serotonin and catechol amines were induced by feeding DL-phenylalanine plus L-tyrosine from the time of weaning (35 gm each per kilogram of ration). This has been shown by Auerbach *et al.* (*6*) in rats to cause urinary excretion of phenylpyruvic aci → and by Huang *et al.* (*7*) and Wang *et al.* (*8*) consequently to cause decreases in serotonin and catechol amines in the tissues. Similar changes have now been found in mice. Reserpine (injected 1 day before the testing) also was used to reduce serotonin and catechol amines.

The changes in learning ability are summarized in Table 1. It is clear that increases in cerebral serotonin, or serotonin plus catechol amines, resulted in decreases in learning ability. This was true regardless of the method used for inducement of the increase, although a specific increase in serotonin (by use of HTP plus BAS) was the more effective. Increases in catechol amines (caused by DOPA), however, were ineffective. By contrast, decreases in serotonin plus catechol amines brought about an increase in learning ability. Such mice were slightly superior to normal mice in this respect. The changes in learning ability were not permanent. When the mice were retested several days after cessation of the treatments they always exhibited normal scores.

These findings do not conflict with the suggestion (*1*) that the idiocy of phenylketonuria may arise from a deficiency of serotonin or serotonin plus catechol amines. It is well known that the crucial period in phenylketonuria is early infancy, whereas the animals used in this study were adults. It is the deficiency induced early in life which damages the intellect, as will be shown in a subsequent paper. By contrast, deficiency induced in adulthood somewhat enhanced learning ability (*9*).

Table 1. Effects of changes in brain serotonin (S) and catechol amines (CA) on maze-learning ability of adult mice. The changes in S and CA were produced by the methods indicated. S.D., standard deviation.

| Treatment | Dose (mg/kg) | Effect on brain S | Effect on brain CA | No. of animals | Score in maze | S.D. |
|---|---|---|---|---|---|---|
| None | | None | None | 60 | 7.4 | 0.2 |
| BAS | 15 | None | None | 20 | 7.0 | 0.1 |
| HTP + BAS | 60 + 15 | Increase | None | 20 | 6.1 | 0.3 |
| HTP + BAS | 300 + 15 | Increase | None | 20 | 5.1 | 0.3 |
| Iproniazid | 3 | Increase | Increase | 30 | 6.1 | 0.2 |
| DL-Phenylalanine + L-tyrosine | fed | Decrease | Decrease | 46 | 8.3 | 0.3 |
| Reserpine | 1.2 | Decrease | Decrease | 17 | 8.1 | 0.2 |
| DOPA | 15 | None | Increase | 29 | 7.4 | 0.5 |

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