higher forms of learning. An understanding of the neural processes underlying long-term habituation in *Aplysia* may therefore provide insights into neural mechanisms of more complex long-term memory.

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6. The performance of independent groups was compared by means of Mann-Whitney U tests (intergroup comparisons) and of related groups by means of Wilcoxon matched-pairs signed-ranks tests (intragroup comparisons). In cases where more than two groups were involved, a preliminary analysis of variance (Kruskal-Wallis for intergroup and Friedman two-way for intragroup) was performed to establish an overall significant difference.
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9. Gill withdrawal for experimental and control animals was compared by expressing each gill withdrawal elicited by siphon stimulation as a percentage of a "complete" gill withdrawal, which previously had been produced by vigorous stimulation of the anterior mantle region. Both 24-hour and 7-day control animals exhibited an initial gill withdrawal on trial 1 (produced by siphon stimulation) that was comparable to their "complete" response, whereas both experimental groups exhibited a significantly decremented initial gill withdrawal compared to controls. Twenty-four-hour groups: experimental, 37 percent; control, 93.5 percent ($P < .001$). Seven-day groups: experimental, 43 percent; control, 91 percent ($P < .001$). Furthermore, as in the previous siphon habituation studies, the net response tendency of gill withdrawal (sum of percent full contraction for trials one to ten) of the experimental animals was significantly lower than controls ($P < .001$ at both 24 hours and 7 days).
10. We thank B. Jahan-Parwar for his suggesting that handling may confound behavioral studies; K. Hilten for her assistance in preparing the illustrations; V. Castellucci, I. Kupfermann, and W. Alden Spencer for their thoughtful criticism of the manuscript; and W. Hening for his help in the independent replication of some of the experiments. Supported by PHS grants MH 19795 and NS09361. Additional support was provided to T.J.C. by fellowship from postdoctral program in biological psychiatry to New York University School of Medicine and by career scientist award MH18,558 to E.R.K.

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## Operant Behavior Changes Norepinephrine Metabolism in Rat Brain

Abstract. *Rats performing a lever-pressing response for water reward in an operant situation, when compared with control groups, showed an increase in brain norepinephrine metabolism. One control group included rats which were handled and deprived of water in the same way as the experimental group but were not trained to perform the operant task. We conclude that performance in an operant situation affects norepinephrine metabolism.*

We now report that performance in an operant situation increases the metabolism (*1*) of norepinephrine (NE) in the brain of the rat. Previous experiments (*2*, *3*) with drugs that affected both norepinephrine metabolism and behavior have demonstrated the opposite relationship, expressing changes in behavior as a function of NE metabolism. We have demonstrated that behavior itself can modify NE metabolism.

Previous experiments on changes in NE metabolism have tested the effects of aversive stimulation rather than the behavior of the animal. In general, acute aversive stimulation causes a reduction of the endogenous concentration of brain NE (*4*, *5*), whereas long-term aversive stimulation causes a rise (*6*). Increased "turnover" of NE is associated with aversive stimulation and increased motor activity (*5*, *7–9*). These experiments demonstrated the effects of aversive stimulation on NE metabolism, but they did not show that the change in NE metabolism is a function of the behavior (performance) of the animal.

Rats performing in an operant situation showed a greater decrease in total brain dopamine (DA) and NE than control rats, after both groups of animals were treated with $\alpha$-methyltyrosine, an inhibitor of synthesis (*3*). That experiment suggested a relation between operant behavior and NE metabolism; however, interpretation of the relation was confounded by the drug. We now describe the effects of performance in an operant situation on NE metabolism in the rat brain; a tracer amount of tritium-labeled NE was used in order to avoid the effects of drugs or other variables.

Male, albino, Sprague-Dawley (Holtzman) rats, approximately 60 days old and weighing 250 g, were used. With the animals under ether anesthesia, we placed an indwelling cannula in the right lateral ventricle, a modification of the method described by Hayden *et al.* (*10*). Animals were housed in pairs and were randomly divided into three groups: (i) an ad lib (food and water) control group, (ii) a water-deprived control group, and (iii) a group trained to press a lever for water reward. The water-deprived group was included in order to test the effect of water-deprivation on NE metabolism; this condition was present in the trained group since a state of water-deprivation is necessary to maintain lever-pressing behavior reinforced with water. Animals were trained as in the experiment with $\alpha$-methyltyrosine (*3*); a variable interval, 30-second (VI-30) schedule was used. On this schedule lever-pressing behavior is reinforced intermittently with an average interval between reinforcements of 30 seconds.

At the end of a 2-hour session, the trained rats were taken from the chambers and returned to their home cages; 15 minutes later they were watered for 5 minutes. The animals in the water-deprived control group were handled in the same way as the animals in the trained group; they were removed to empty cages for 2 hours each day, returned to their home cages, and 15 minutes later were watered for 8 minutes. By watering the animals in the water-deprived group for 3 minutes longer than the animals in the trained group, we were able to make sure that the amount of water and food consumption was the same in both groups, as evidenced by the fact that their body weights did not differ.

On the 15th day of the VI-30 schedule all rats were injected with 1.0 $\mu$c (10 $\mu$l) each of tritiated NE (New England Nuclear; specific activity 10.8 curie/mmole), dissolved in Merle's solution (*11*), with a 50-$\mu$l Hamilton syringe.

Table 1. Effect of operant behavior and water-deprivation on the concentration of NE, tritiated NE, and the specific activity of NE in the rat brainstem-diencephalon. Each rat was injected with 1 $\mu$c of $^3$H-NE and killed 2 hours later; C is the control group (untrained, with free access to water); WD is the water-deprived control group (untrained); VI is the water-deprived group which was lever-pressing for water reinforcement on a VI–30 second schedule. Values represent the mean and standard error of the mean for five animals.

| Group | NE ($\mu$g/g) | $^3$H-NE (nc) | Specific activity of NE (nc/$\mu$g) |
|---|---|---|---|
| C | 0.555 ± .022 | 92.7 ± 5.3 | 168 ± 13 |
| WD | .599 ± .036 | 102.2 ± 3.8 | 175 ± 17 |
| VI | .620 ± .039 | 77.1 ± 4.1 | 127 ± 11 |

Immediately after injection, the trained rats were placed in operant chambers, the water-deprived control rats were placed in empty cages, and the ad lib control rats were returned to their home cages. Two hours after injection all rats were decapitated, and their brains were dissected (*12*), weighed, and stored in liquid nitrogen. The brainstem-diencephalon preparations were assayed for tritiated and endogenous NE (*13, 14*). In order to validate the assay, NE and $^3$H-NE were identified by thin-layer chromatography (*15*).

In comparison with the two control groups, rats lever-pressing for water (the trained group) had approximately 25 percent lower specific activity of NE 2 hours after injection of $^3$H-NE. The difference in specific activity was due entirely to the difference in the amount of $^3$H-NE, since there was no difference in the total concentration of endogenous NE between these groups (Tables 1 and 2). There was no significant difference in the brainstem-diencephalon weights among the three groups, nor did the two control groups differ from each other significantly with respect to the concentration of $^3$H-NE or specific activity of NE (Table 2). The ad lib control rats maintained the daytime quiescent behavior typical of rats, but the water-deprived control rats were active during the 2-hour period which preceded their daily access to water. Hence neither water-deprivation nor interruption of the daytime quiescent behavior affected the metabolism of NE.

The two most likely possible hypotheses which could explain the lower specific activity in the trained group are (i) increased synthesis of NE or increased preferential release of $^3$H-NE, or both, and (ii) decreased initial accumulation of $^3$H-NE. Measurement of the specific activity of NE at a time point shortly after injection should provide evidence favoring one or the other of these hypotheses.

Tritiated norepinephrine disappears from the brain in a multiphasic manner, first rapidly and then slowly; at the 2-hour time point $^3$H-NE was leaving the brain in the initial rapid phase (*16*). The initial rapid phase is important to study because acute grid shock depletes $^3$H-NE after the intraventricular injection of $^3$H-tyrosine only if the grid shock follows the injection of tritiated tyrosine within a short interval of time (*8*). We were able to see the effect of operant behavior on NE metabolism shortly after injection of $^3$H-NE because the indwelling cannula eliminates surgical trauma and the need for an anesthetic agent which would incapacitate the rat after an acute injection, and thus the rat is able to perform in an operant situation immediately after injection.

Although we avoided use of a drug, such as $\alpha$-methyltyrosine, which would interfere with NE metabolism and with behavior, one might argue that the injection of $^3$H-NE causes such interference. But NE metabolism is probably not affected by this procedure since the tracer quantity of NE injected (15.6 ng) was too small to affect the NE pool. Neither was the behavior of the animals affected by the injection procedure. No statistically significant difference was seen in either the number of reinforcements received or in the number of responses made, when the experimental day was compared with the previous day.

Since the injection of $^3$H-NE had no measurable effect on the behavior of the animals, the independent variable in this experiment was the behavior of the animal and the dependent variable was the metabolism of NE. Although the converse of this relationship is alluded to by most previous experiments (*2, 3, 17*), experiments which related drug-induced changes in behavior to drug-induced changes in NE metabolism, it seems that in the light of evidence we have presented, behavior and NE metabolism mutually interact.

We propose that some aspect of the rat's behavior in the operant situation is responsible for the observed increase in NE metabolism. Lever-pressing and water consumption are the two most obvious behaviors in this experiment; in addition, one must consider the contingency relationship between lever-pressing and water reward. However, behavior in an operant situation is a function of several variables which also may account for the changes in NE metabolism. Certain stimuli, such as the click of the lever, the periodic click of the water dipper, or the presentation of the water, might contribute to the effect. The possibility remains that the effect is caused by afferent stimulation unrelated to the motor component of behavior. This does not seem likely, since changes in NE metabolism associated with afferent stimulation have been demonstrated only under intense aversive stimulation (*4–9*), which was not present in our experiment. The exact factor or factors present in the operant situation which account for the increase in NE metabolism is an appropriate area for further research.

Table 2. Percentage ratios of NE, tritiated NE, and specific activity of NE. The values given were calculated on the basis of the absolute values of endogenous NE, $^3$H-NE, and specific activity shown in Table 1. The *P* values were calculated from Student's *t*-test.

| Groups | NE | $^3$H-NE | Specific activity of NE |
|---|---|---|---|
| VI/WD | 104* | 75.5 ($P < .005$) | 72.4 ($P < .05$) |
| VI/C | 112* | 83.2 ($P < .05$) | 75.3 ($P < .05$) |
| WD/C | 108* | 110* | 104* |

* Not significant

The implications of the relation between behavior and NE metabolism apply not only to physiology and behavior but also to psychopharmacology. A technique is now available with which to further investigate the studies of Dews (*18*), which have shown that a drug, such as amphetamine, will have different effects on behavior, depending on the specific type of behavior. A possible biochemical explanation for the phenomenon described by Dews is suggested by our study, since we have demonstrated that behavior itself can affect brain chemistry, thereby changing the chemical substrate on which the drug acts.

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13. The neurochemical assay is the following: the brain tissue is homogenized in 82 percent (by volume) ethanol, adjusted to *p*H 6.8, and centrifuged. An equal volume of water containing ethylenediaminetetraacetate (EDTA) (0.2 percent) and $Na_2S_2O_5$ (0.2 percent) is added to the supernatant which is then passed through an Amberlite CG 50 column buffered to *p*H 6.1. Catechols are eluted with 5 ml of 0.2*N* acetic acid. To the eluate is added 5 ml of water containing $Na_2S_2O_5$ (0.2 percent) and EDTA (4 percent). The mixture is adjusted to *p*H 8.4 with 2*N* tris buffer and passed through an alumina column buffered to *p*H 8.4 with 0.2*N* sodium acetate. The column is washed with 5 ml of 0.2*N* sodium acetate and then with 5 ml of $H_2O$. $^3$H-NE and NE are eluted by passing through 5 ml of 0.2*N* HCl. A portion of the eluate is added to Bray's solution [*Anal. Biochem.* **1**, 279 (1960)] and $^3$H-NE is counted with a Packard Tri-Carb liquid scintillation spectrometer. Another portion of the eluate is oxidized and measured fluorimetrically.
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# Variations of the Visual Responses of the Superior Colliculus in Relation to Body Roll

Abstract. *A large percentage of the directional units of the superior colliculus of the curarized cat modify their response to a particular moving visual stimulus as a function of the position of rotation of the animal about its longitudinal axis.*

The message from a receptor can be modified by influences stemming from other receptors. For example, modifications in the judgment of size, shape, or orientation of an object occur after exposure to other visual or vestibular stimulation (*1*). Moreover, electrophysiological recordings have shown that some cells of the visual cortex of the cat modify their responses in relation to body roll (*2*).

We studied the effect of body roll on the visual responses of cells in the superficial layers of the superior colliculus of the cat. This structure shows convergence of many sensory modalities and therefore seems a suitable place for interaction between vestibular and visual messages (*3*).

Cells in the superior colliculus superficial layers can be subdivided into two classes, directional cells and nondirectional cells, depending on whether or not they respond equally to the various directions of a moving visual stimulus (*4*). We will show that a large percentage of directional units alter their responses as a function of body roll.

Two or three days before the experiment, adult cats ($n = 10$) were anesthetized with sodium pentobarbital, and a craniotomy was made over the projections of both superior colliculi. A metallic chamber was positioned stereotaxically around the opening and was fixed to the bone with dental cement. On the day of the experiment the animal was briefly anesthetized with halothane, and tracheal and venous cannulas were inserted. The wounds were carefully infiltrated with a local anesthetic. After the removal of the dura, the anesthesia was interrupted, curare was injected, and artificial ventilation was used.

Pupils were dilated with atropine. The refraction of the eyes of the cat was determined by means of retinoscopy and was corrected with suitable contact lenses. The animal was fixed by clamping the metallic chamber cemented on his head on a table that could be rotated up to 70 degrees about the longitudinal axis of the animal. Also, the body of the animal was fixed to the table. The optic stimulator and a tangent screen were both attached to the tilting table. The optic stimulating system projected the image of a slide on the tangent screen by reflection on a

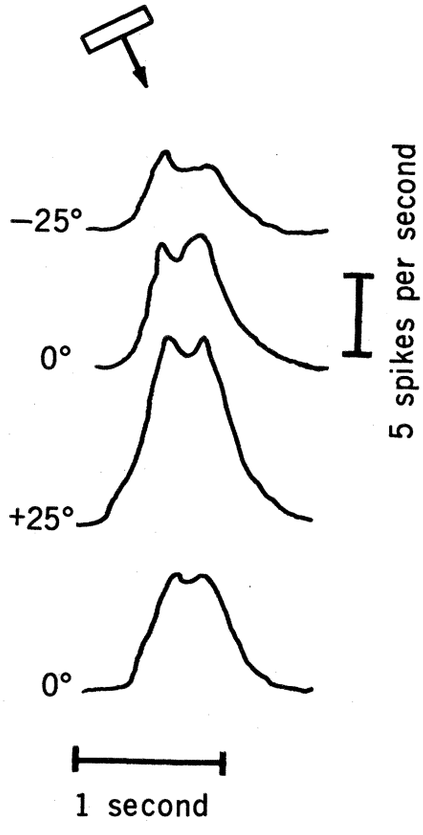


Fig. 1. Responses of an orientation sensitive unit of the left superior colliculus for three different positions of the tilting table. The stimulus was a luminous bar (15 degrees by 2 degrees; luminance, 10 cd/m$^2$ superimposed on a dimmer background) moving in the direction indicated by the arrow at a constant speed (18 degrees per second). This was the preferred direction of the cell when the table was horizontal and remained fixed with respect to the retina at the various positions of the table. Each record is the average of ten responses. The numbers on the left of each record indicate the degrees of roll (negative body roll means that the side where the electrode is placed is set downward). The bottom record is a control of the cell response for the 0-degree position of the table. The calibration (vertical line, spikes per second) on the right of the figure refers to a regular train of pulses. The average discharge of the cell did not change with the rotation of the table and was of the order of four to five impulses per second.