The successful transfusions of blood to induce hibernation includes working in a cold room, so that the donor animal and the instruments used for dissection are refrigerated during the blood withdrawal. The animal must remain in hibernation, so speed is important to avoid a possibility of collecting any "arousal substance" that might be produced during the dissection. It is possible that such a substance could vitiate activity of a "trigger substance." Therefore, total time for dissection and blood withdrawal was never more than 45 seconds.

These results, although based on a very few experiments (Fig. 1), indicate that a "trigger" for natural mammalian hibernation in the ground squirrel is carried in the blood of the hibernating squirrel and can be transferred by transfusion to nonhibernating summer animals from the warm room, causing them to hibernate after their introduction into the cold.

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## Facilitation of Brain Self-Stimulation by Central Administration of Norepinephrine

Abstract. *Rats with electrodes implanted in the medial forebrain bundle stimulated their own brains at sharply reduced rates after systemic administration of disulfiram or intraventricular administration of diethyldithiocarbamate. Both drugs inhibit dopamine-β-hydroxylase, the enzyme responsible for the final step in the biosynthesis of norepinephrine. The suppressed behavior was reinstated by intraventricular injections of* l-*norepinephrine, but not by injection of its biologically inactive isomer,* d-*norepinephrine. Intraventricular administration of dopamine and serotonin did not restore self-stimulation. The rewarding effect of medial forebrain bundle stimulation may depend on the availability of norepinephrine as a transmitter, but not on dopamine or serotonin.*

Several lines of evidence support the idea that catecholamines in the central nervous system mediate rewarding or positively reinforcing effects on behavior. This idea was suggested initially by studies of the effects of drugs on self-stimulation of reward areas in the brain (*1*). Drugs that facilitate self-stimulation (for example, amphetamine) release catecholamines rapidly from physiologically active sites. Conversely, drugs that inhibit self-stimulation deplete the brain of catecholamines (reserpine, α-methyl-*p*-tyrosine) or block adrenergic transmission (chlorpromazine). Furthermore, if catecholamines are protected from destruction by inhibitors of monoamine oxidase, or, if the reuptake of catecholamines is retarded by drugs similar to imipramine, the facilitatory action of amphetamine on self-stimulation is increased. On the other hand, if the stores of catecholamines in the brain are depleted by reserpine or α-methyl-*p*-tyrosine, the facilitating effect of amphetamine is decreased.

An anatomical basis for this relation between positive reinforcement and catecholamines is provided by the coincidence of behavioral and histochemical experiments. The behavioral work shows that the most intensely rewarding points in the brain are distributed along the medial forebrain bundle (*2*); the histochemical work (*3*) shows that the medial forebrain bundle is the principal diencephalic pathway of ascending noradrenergic fibers. Recently, these relations were verified by experiments which demonstrate in vivo that rewarding electrical stimulation of the medial forebrain bundle releases norepinephrine and its metabolites into solutions perfused through the hypothalamus and amygdala (*4*).

Taken together, these data suggest that self-stimulation depends on the release of norepinephrine at synapses of the medial forebrain bundle. However, it has not been possible to demonstrate that the rate of self-stimulation may be increased by central administration of norepinephrine. Indeed, such administration suppresses rather than facilitates self-stimulation behavior reinforced by minute injections of chemicals into the hypothalamus (*5*), and generally causes inactivity, sedation, and stupor with increasing doses (*6*).

Our experiments show that self-stimulation can be facilitated by central administration of norepinephrine under certain conditions and that self-stimulation depends more critically on the availability of norepinephrine than it does on that of dopamine or serotonin.

Bipolar platinum electrodes were stereotaxically implanted in the medial forebrain bundles (at the level of the mamillary bodies) of 29 rats anesthetized with pentobarbital; cannulas for injection of solutions were implanted in the lateral ventricle on the opposite side. The design of electrodes and cannulas has been described (*4*). Accuracy of placements was verified histologically at the end of the experiment. The animals were trained to stimulate their own brains by pressing a lever in a Skinner box, according to the technique of Olds and Milner (*7*). Each lever press delivered a 0.15-second train of rectangular pulses 0.2 msec long at 100 pulses per second through an isolation transformer. The current varied between 0.1 and 0.4 ma, and was adjusted in each case to the lowest intensity that maintained a stable rate of self-stimulation.

After several sessions of preliminary training, animals were injected with disulfiram or diethyldithiocarbamate (DEDTC). These inhibitors of dopamine-β-hydroxylase (E.C. 1.14.2.1) block the final step in the biosynthesis of norepinephrine, and thus selectively deplete it (*8*). Animals that were tested with disulfiram were kept on the regular reinforcement schedule. A dose of 200 mg per kilogram of body weight, suspended in 0.3 percent Tween 80 in saline, was injected intraperitoneally 1 hour after the start of the test session. Animals that were given DEDTC were trained to respond on a schedule in which reinforcements were programmed at variable intervals (on the average, once every 10 seconds). Two milligrams of DEDTC were dissolved in 25 μl of Ringer-Locke solution (adjusted to *p*H 7.5) and injected intraventricularly ½ hour after the start of the test. One to three hours after injection of disulfiram, or 15 minutes after injection of DEDTC, various neurohormones as hydrochlorides (*l*-norepinephrine, *dl*-norepinephrine, *d*-norepinephrine, dopamine, and serotonin) were injected into

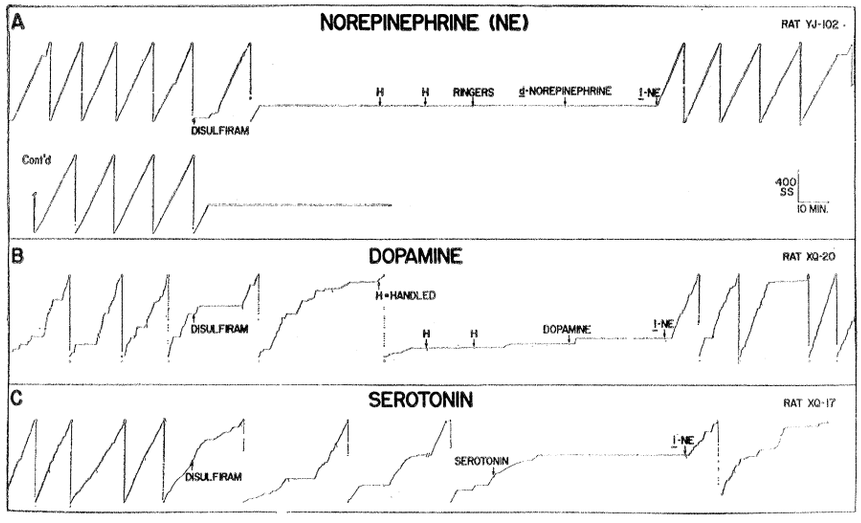


Fig. 1. Suppression of self-stimulation (*SS*) by disulfiram (200 mg/kg, intraperitoneal) and reversal of behavioral suppression by intraventricular injection of *l*-norepinephrine [5 $\mu$g in (A) and (B), 20 $\mu$g in (C)]. Equivalent doses of *d*-norepinephrine, dopamine, or serotonin do not restore self-stimulation. The curves are drawn by cumulating self-stimulations over time; the pen resets automatically after 100 self-stimulations.

the lateral ventricle in different experiments. The neurohormones (4.8 to 20 $\mu$g) were dissolved in 25 $\mu$l of Ringer-Locke solution (*p*H 7.5). Animals in the disulfiram group received an injection of *l*-norepinephrine 30 to 60 minutes after the injection of *d*-norepinephrine, dopamine, or serotonin.

Disulfiram decreased the rate of self-stimulation to 20 percent or less of the control value within 1 to 3 hours (Fig. 1 and Table 1). On the other hand, a much smaller dose of DEDTC suppressed self-stimulation rates within a few minutes. Possibly, DEDTC is the active metabolite of disulfiram (*9*); if so, the introduction of DEDTC directly into the brain would explain its high potency and the rapid onset of action. The suppressive effect of disulfiram persisted for many hours, whereas the effect of DEDTC, possibly because of the low absolute dose, wore off in 30 to 45 minutes. In a number of cases, some tolerance to DEDTC developed upon repeated injections.

Intraventricular injections of *l* or *dl*-norepinephrine largely reversed the behavior-suppressant effects of both inhibitors within a few minutes (Fig. 1 and Table 1). Such a reversal was not obtained with the biologically inactive isomer, *d*-norepinephrine. This negative result excludes nonspecific physicochemical factors and any effects attributable to the injection procedure as explanations of the *l*-norepinephrine reversals. In addition, peripheral effects of *l*-norepinephrine can be ruled out because systemic administration of this substance did not produce a reversal. Finally, neurohormonal specificity was suggested by the finding that intraventricular administration of dopamine and serotonin also failed to restore self-stimulation. None of these negative findings could be attributed to a lack of responsiveness of the animals, because, in every case, self-stimulation was immediately reinstated upon injection of intraventricular *l*-norepinephrine in the same test session (Fig. 1 and Table 1).

Observations of the appearance of the animals coincided with the behavioral data. After injection of the inhibitors, the animals were sedated and appeared "disinterested" in the electrical reinforcement. [Sedation per se does not necessarily interfere with self-stimulation; after large doses of barbiturates, for example, rats may respond at normal or even supernormal rates (*1*).] Injections of *l*-norepinephrine, but not of the other substances, rapidly produced a state of arousal and alertness.

In supplementary experiments, 5 $\mu$g of *l*-norepinephrine hydrochloride or serotonin hydrochloride was administered intraventricularly to untreated rats to determine the behavioral effects of these substances under conditions of normal norepinephrine metabolism. Mean rates of self-stimulation during the first 15 minutes after injection were reduced 19 percent by norepinephrine ($t = 1.88$, d.f. $= 5$, $P > .1$) and 48 percent by

Table 1. Suppression of self-stimulation after inhibition of norepinephrine biosynthesis by the dopamine-$\beta$-hydroxylase inhibitors disulfiram (200 mg/kg, intraperitoneal) and diethyldithiocarbamate (2 mg, intraventricularly). Neurohormones were administered intraventricularly 1 to 3 hours after injection of disulfiram or 15 minutes after injection of diethyldithiocarbamate in an attempt to reinstate the suppressed self-stimulation behavior. In the disulfiram experiments, a second reversal test was made with 5 $\mu$g of *l*-norepinephrine after initial tests with other neurohormones.

| Neurohormone | Dose ($\mu$g) | Experiments (No.) | Control rate of self-stimulation* (response/min) | Percent of control self-stimulation rate* | | |
|---|---|---|---|---|---|---|
| | | | | Inhibitor drug (15 min before neurohormone) | Neurohormone (first 15 min) | *l*-Norepinephrine (first 15 min) |
| | | | *Disulfiram* | | | |
| *l*-Norepinephrine | 5.0 | 6 | 72.0 ± 9.7 | 7.0 ± 3.0 | 62.5† ± 11.0 | |
| *dl*-Norepinephrine | 10.0 | 7 | 81.6 ± 11.2 | 6.7 ± 2.5 | 61.9† ± 8.8 | |
| *d*-Norepinephrine | 5.0 | 5 | 78.2 ± 9.5 | 10.4 ± 8.0 | 9.6 ± 8.3 | 58.2† ± 12.3 |
| Dopamine | 5.5 | 6 | 40.9 ± 5.6 | 6.5 ± 4.6 | 15.1 ± 12.2 | 66.7† ± 14.9 |
| Serotonin | 4.8–20 | 5 | 51.4 ± 12.6 | 20.8 ± 5.3 | 7.6 ± 3.8 | 45.5† ± 8.2 |
| *l*-Norepinephrine (injected i.p.) | 5.0 | 3 | 50.4 ± 16.6 | 14.6 ± 11.5 | 0.3 ± 0.2 | 56.6† ± 14.6 |
| | | | *Diethyldithiocarbamate* | | | |
| *l*-Norepinephrine | 5.0 | 9 | 35.8 ± 5.4 | 9.7 ± 4.2 | 66.1† ± 15.8 | |
| *d*-Norepinephrine | 5.0 | 10 | 52.2 ± 4.5 | 23.9 ± 7.2 | 33.3 ± 8.9 | |
| Dopamine | 5.5 | 4 | 42.1 ± 6.2 | 11.8 ± 5.0 | 16,8 ± 8.4 | |
| Serotonin | 4.8 | 4 | 48.8 ± 5.3 | 24.8 ± 5.4 | 8.3 ± 3.2 | |

* Results are expressed as the mean ± standard error. † Indicates significant increase over mean in adjacent column at $P \leq .05$.

serotonin ($t = 4.51$, d.f. $= 4$, $P < .02$). Serotonin was also strongly inhibitory in animals first treated with disulfiram and DEDTC (Table 1; serotonin mean compared with inhibitor drug mean: $t = 2.96$, d.f. $= 8$, $P < .02$).

These experiments show that inhibition of norepinephrine biosynthesis by disulfiram and DEDTC suppresses self-stimulation. Because central administration of norepinephrine selectively reverses the suppression, we conclude that disulfiram and DEDTC produce this effect by their inhibitory action on dopamine-$\beta$-hydroxylase and the consequent depletion of norepinephrine, and not by some other action unrelated to the metabolism of norepinephrine (*10*). Furthermore, we can rule out an important role for serotonin and dopamine in our experiments. Neither substance is depleted after disulfiram or DEDTC (*11*), and neither is capable of reversing the effects of the drugs.

According to recent models of noradrenergic function (*12*), norepinephrine in the nerve ending is contained in two pools—a small functional pool and a larger, essentially nonfunctional, reserve pool. Because the norepinephrine in the reserve pool does not transfer readily to the functional pool, noradrenergic transmission probably depends primarily on the synthesis *de novo* of norepinephrine in the functional pool. If so, inhibition of norepinephrine biosynthesis would cause noradrenergic transmission to fail after the small reserve in the functional pool was exhausted. The rapid action of centrally administered DEDTC in our experiments suggests that, in the case of self-stimulation, the reserve in the functional pool can be exhausted in a few minutes.

In animals treated with disulfiram and DEDTC, the rapid reinstatement of suppressed behavior after intraventricular administration of norepinephrine is probably due to replenishment of depleted functional pools, and not to other possible actions, such as direct combination with noradrenergic receptors. These other actions in fact appear to suppress, rather than to facilitate, self-stimulation. This conclusion is based on our observation that, in untreated rats, the 5-$\mu$g dose of norepinephrine caused mild suppression of self-stimulation. Since the functional pools are intact in untreated animals, the exogenous norepinephrine cannot act by replenishment, and therefore must suppress self-stimulation by some other means (*13*). Mild suppression similarly must be exerted on the behavior of the disulfiram-and DEDTC-treated animals, but presumably it is obscured by the strong facilitating effect of replenishment (*14*).

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13. Possibly, self-stimulation is influenced by more than one system of noradrenergic synapses in the brain; that is, in addition to the behaviorally facilitatory system, there also may be systems that inhibit behavior. The suppressive effects of hypothalamic administration of norepinephrine in the self-injection experiments of Olds *et al.* (*5*) suggest that these hypothetical, behaviorally suppressive synapses have a diencephalic location. On the other hand, the noradrenergic synapses that facilitate behavior probably are located mainly in the forebrain [L. Stein, in *Reinforcement*, J. Tapp, Ed. (Academic Press, New York, in press)].
14. Further support for the replenishment idea is provided by the observation that intraventricular norepinephrine fails to reverse the behavior-suppressant effects of reserpine [E. R. John, B. M. Wenzel, R. D. Tschirgi, *J. Pharmacol. Exp. Therap.* **123**, 193 (1958)]. Such failure may be due to the fact that reserpine impairs the capacity of the noradrenergic neuron to take up and bind norepinephrine [M. Holzbauer and M. Vogt, *J. Neurochem.* **1**, 8 (1956); J. Glowinski and J. Axelrod, *J. Pharmacol. Exp. Therap.* **149**, 43 (1965)], and thereby prevents the replenishment of the functional pools.
15. We thank A. T. Shropshire for excellent technical assistance and Ayerst Laboratories, Inc. for donating the disulfiram.

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## Steady Potential Correlates of Positive Reinforcement: Reward Contingent Positive Variation

Abstract. *A positive reinforcement with food produced high-voltage bursts of alpha activity over the posterior marginal gyrus in a cat deprived of food and water. This synchronization was always associated with a large (180 to 300 microvolt), positive steady potential shift comparable to that occurring during the onset of sleep. Since this shift was contingent upon the relative appropriateness and desirability of food reward, it was termed reward contingent positive variation.*

In cats deprived of food, click or flash stimuli reinforced with food produce either negative or positive epicortical steady potential shifts with reference to the skull. Their magnitude diminishes as a function of the volume of food eaten. Hence, it was suggested that they reflect the degree of drive and motivation (*1*). A negative steady potential shift maximum at the vertex occurs in normal human subjects whenever a conditional stimulus is followed by an unconditional one that is expected to involve an action or decision. Such steady potential shifts return to the base line at the instant that the response is performed. Since the appearance of this steady potential is contingent on the significance of an association of unconditional stimulus, it was termed "contingent negative variation." Later it was termed "expectancy wave" since its magnitude is a function of the probability of occurrence of the unconditional stimulus (*2*). In cats deprived of food which were trained to press a lever for milk rewards, the electrocorticogram (ECoG) over the parietooccipital region shows dramatic fluctuations from desynchronized to highly synchronized patterns