24 hours later. It should, according to the hypothesis, resemble a group that has partially forgotten. There should therefore be a smaller amnesic effect than in the 14-day group trained to criterion (group 1), or even an enhancement of memory, as in the 28-day group (group 3). The results (Table 2) show that on retest this group required a mean of 17 trials to criterion, making the total number of trials to criterion 47, while the average of initial training for the other five groups is 46.4. The result, showing no evidence of amnesia in undertrained rats, is in good agreement with the hypothesis.

A further prediction from the hypothesis is that the amnesia caused by DFP should not be permanent, but that memory should return. Some evidence in favor of this has already been reported (*1*). Group 6, trained and treated in the same way as group 1, was retested 5 days later, instead of 1 day later. Return of memory was almost complete (Table 2). Besides supporting the view that the substrate of memory is a change in synaptic conductance, mediated by increasing amounts of transmitter, the present report suggests that forgetting lies in a reversal of this change.

Whether the observed effects on memory are related to the hippocampal locus of injection is at present undetermined. That there is considerable spread of the drug can be seen because the intrahippocampal injection of the drug in the experimental animals causes pupillary constriction. It seems likely that the effects on memory are due to anticholinesterase action of DFP. Injections of the anticholinergic scopolamine (*5*) produce the greatest amnesia 1 to 3 days after initial learning and a minimum of effect on memory at 14 days, the reverse of the case with DFP (*1*). This corroborates the notion that cholinergic synapses are being affected and that the quantity of transmitter emitted at these synapses soon after learning is low and gradually increases with time after learning. From the results reported here it seems the amount of transmitter at synapses modified by learning finally declines, rendering transmission across such synapses progressively less efficient and so producing behavioral forgetting.

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## 6-Hydroxylation: Effect on the Psychotropic Potency of Tryptamines

Abstract. *6-Hydroxy-5-methoxy*-N,N-*dimethyltryptamine and 5-methoxy*-N,N-*dimethyltryptamine were synthesized and their psychotropic effects compared on trained rats in a Skinner box. The nonhydroxylated form was the more potent. The metabolism of 5-methoxytryptophol acetate ester was also studied to determine whether hydroxylation might occur in other than the six position with exogenous indoles. One metabolite was formed, with properties of a hydroxy-5-methoxyindole-3-acetic acid, which proved on chromatography not to be the 6-hydroxy structural isomer. Pharmacologic and metabolic studies suggest that psychotropic activity of tryptamines may result from metabolites other than the 6-hydroxylated forms.*

It has been generally held (*1*) that in mammals, when most exogenous indoles that are substituted in the three position are metabolized by hydroxylation, this occurs at the six position. Szara and Hearst (*2*) postulated that the behavioral effects of *N,N*-dimethyltryptamine in rats are due to a metabolite because a substance having high potency was isolated from urine of rats receiving this compound. They suggested, on the basis of chromatography, that the 6-hydroxyl metabolite was the active form (*3, 4*). This is an important premise since it suggests that other indoles might also have their biological actions enhanced by 6-hydroxylation.

We have synthesized by an unequivocal chemical route the 6-hydroxylated analog of 5-methoxy-*N,N*-dimethyltryptamine, a potent psychotropic agent (*5*). It was prepared from 6-benzyloxy-5-methoxyindole by treatment with oxalyl chloride and dimethylamine to give 6-benzyloxy-*N,N*-dimethyl-5-methoxyindole-3-glyoxylamide. The amide was reduced with lithium aluminum hydride and the benzyl group was removed from the resulting amine by hydrogenation (*6*). The structure of the parent compound (I), synthesized according to an established procedure (*7*), and that of the hydroxylated form (II) are shown in Fig. 1.

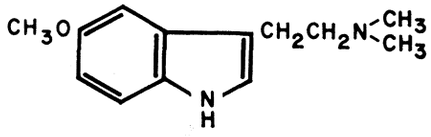
The two compounds were compared by measuring their interference with standardized behavior in rats trained to press a bar for food reward in a Skinner box (*8*). White male rats (average age 14 weeks), weighing between 320 and 395 g, were used. They were fed a diet of 12 to 16 g of Rockland rat and mouse chow each evening and trained 1 hour daily for a minimum of 8 weeks in a Skinner box on a positive-reinforcement, variable-interval schedule. Animals were not fed each day prior to their stay in the chamber. Intervals between opportunities for reward were variably spaced so that the rats were unable to remember their duration. For the rats to obtain maximum profit from the situation they continued to press the bar at a steady rate for the hour in the chamber. Their efforts expressed as bar presses per hour were automatically recorded. Each animal had an individual mean work rate ranging from 26 to 68 bar presses per minute. Normal performance of each animal served as its own control against work rates when under test.

Standard deviation based on data for 12 typical control days ranged from ± 7.9 percent to ± 21.4 percent. Dosages required for known psychotomimetic agents to alter the work rates of rats were higher than those which produced mental changes in human beings. Psilocybin produced marked reductions of work rates at 3 mg/kg, and 150 μg/kg of LSD-25 were required in these studies (intraperitoneal injections).

The animals were divided into four groups according to the compound and dose they received (see Table 1). At doses of 6-hydroxy-5-methoxy-*N,N*-dimethyltryptamine ranging from 6.7 to 7.5 mg/kg (15.9 to 17.7 μmole/kg) (group 1) no significant changes in rates occurred in any of the six experiments (Table 1). However, in the same dose range, the nonhydroxylated analog (group 2) caused significant changes of performance in all six experiments, with values approaching complete extinction of work rates. Three animals (group 3) that received lower doses (5.6 to 6.2 mg/kg) of the nonhydroxylated tryptamine all exhibited signifi-

cant changes in performance. At doses of 2.6 to 3.0 mg/kg the nonhydroxylated form still caused significant changes in two of five experiments. This indicates that the 6-hydroxylated analog is less active than the parent compound. These results are congruous with our previous studies (*9*) in which we compared 5-methoxytryptamine with 6-hydroxy-5-methoxytryptamine. Using the same type of behavioral study, we found that 5-methoxytryptamine, injected intraperitoneally at a dose of 3.3 mg/kg, consistently caused significant reductions of work rates, whereas the 6-hydroxylated compound had no significant effect at 18 mg/kg. Recent investigations with human subjects by Rosenberg *et al.* (*10*), who used several criteria of behavior including the occurrence of hallucinations, showed the parent *N,N*-dimethyltryptamine to be active while its 6-hydroxylated analog at similar doses was not.

These observations on three different tryptamines do not support the generalization that 6-hydroxylation confers greater psychotropic potency on tryptamines; but instead they indicate that it reduces activity. Since most of the pharmacological observations (*2*) which indicate that 6-hydroxylation confers greater potency were made on indoles isolated from urine, our findings suggest that some metabolite of *N,N*-dimethyltryptamine other than the 6-hydroxyl compound may be the active agent. Structural isomers in which hydroxylation occurs in a position other than six might be difficult to distinguish from each other on chromatography. This has recently been shown to be true in the case of skatole where the 6-hydroxylated metabolite was previously thought to be the major one excreted in man (*11*). Development of new chromatographic procedures, new availability of synthetic standards, and study of the color reactions of hydroxylated skatoles (*12*) has shown a mixture of the 5-, 6-, and 7-hydroxylated skatole isomers to occur in man (*13*).



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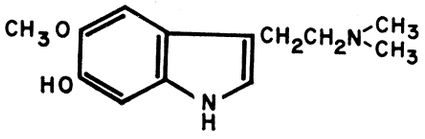

II

Fig. 1. Formulas for 5-methoxy-*N,N*-dimethyltryptamine (I) and 6-hydroxy-5-methoxy-*N,N*-dimethyltryptamine (II).

Table 1. Comparison of performance rates, on a variable-interval positive-reinforcement task, of rats that received 5-methoxy-*N,N*-dimethyltryptamine and 6-hydroxy-5-methoxy-*N,N*-dimethyltryptamine. Monday, Tuesday, Thursday, and Friday were control days on which the animals received no compounds. On Wednesday an aqueous solution of the experimental compound was injected intraperitoneally immediately before the animal was placed in the Skinner box. Values are bar presses for 1 hour. Means are of 12 normal days clustered around and including the week of study. Deviations from means on the day the drug was administered are expressed as bar presses (A) and percent deviation (B) equal to: [*CBP* (means) − *DBP*]/*CBP* × 100, where *CBP* is control bar presses and *DBP* drug bar presses. Standard deviations (S.D.) were obtained for the same 12 days as means and are expressed as bar presses per hour (A) and S.D./means × 100 (B). The last column of the table gives significant deviations from means at the 1 percent probability level. Animals in group 1 received 6-hydroxy-5-methoxy-*N,N*-dimethyltryptamine (oxalate) at 2.5 mg per rat (15.9–17.7 μmole/kg); group 2, 5-methoxy-*N,N*-dimethyltryptamine (oxalate) at 2.5 mg per rat (16.9–19.1 μmole/kg); group 3, the latter compound at 2.0 mg per rat (13.6–15.2 μmole/kg); and group 4, the latter compound at 1.0 mg per rat (6.2–7.3 μmole/kg).

| Group | Dose (μmole/kg) | Mean | Deviation from mean | | Standard deviation | | Significant deviation from mean (%) |
|---|---|---|---|---|---|---|---|
| | | | A | B | A | B | |
| 1 | 15.9 | 4120 | 554 | 13.4 | 509 | 12.4 | 37.9 |
| | 15.7 | 2825 | 416 | 14.7 | 225 | 8.0 | 24.5 |
| | 17.7 | 3207 | 1091 | 34.0 | 381 | 11.9 | 36.4 |
| | 16.9 | 2041 | 480 | 23.5 | 177 | 8.7 | 26.6 |
| | 17.1 | 3016 | 149 | 4.9 | 466 | 15.5 | 47.4 |
| | 17.7 | 3158 | 442 | 14.0 | 551 | 17.4 | 53.2 |
| 2 | 16.9 | 3736 | 3107 | 83.2 | 441 | 11.8 | 36.1 |
| | 17.5 | 2781 | 1720 | 61.8 | 363 | 13.1 | 40.0 |
| | 17.5 | 4012 | 3690 | 92.0 | 859 | 21.4 | 65.5 |
| | 17.4 | 2557 | 2277 | 89.0 | 201 | 7.9 | 24.2 |
| | 19.1 | 1574 | 1145 | 72.7 | 269 | 17.1 | 52.3 |
| | 17.9 | 4099 | 3631 | 88.6 | 531 | 13.0 | 40.1 |
| 3 | 13.7 | 2585 | 1936 | 74.9 | 225 | 8.7 | 26.6 |
| | 13.6 | 2042 | 1178 | 57.7 | 177 | 8.7 | 26.6 |
| | 15.2 | 3016 | 2879 | 95.5 | 466 | 15.5 | 47.4 |
| 4 | 6.4 | 2585 | 517 | 20.0 | 225 | 8.7 | 26.6 |
| | 6.2 | 2781 | 1122 | 40.3 | 363 | 13.1 | 40.0 |
| | 7.3 | 3207 | 559 | 17.4 | 381 | 11.9 | 36.4 |
| | 6.3 | 4012 | 1648 | 41.1 | 859 | 21.4 | 65.5 |
| | 6.3 | 2042 | 1162 | 56.9 | 177 | 8.7 | 26.6 |

We have examined the metabolism of 5-methoxytryptophol acetate ester to determine whether hydroxylation might occur with exogenous indoles in other than the six position. Injections of radioactive 5-methoxytryptophol acetate into rats resulted in excretion of a single radioactive metabolite. It was not extracted by ethyl acetate at *p*H 7 but was removed quantitatively on acidification and gave positive reactions for a hydroxyl group, which indicates that it was a hydroxy-5-methoxyindole-3-acetic acid. 6-Hydroxy-5-methoxyindole-3-acetic acid was synthesized (*14*) to serve as a chromatographic standard. That compound had an $R_F$ value of 0.23 on a mixture of chloroform and acetic acid (95:5) on silica thin-layer chromatography while the metabolite's was 0.41. Thus hydroxylation occurred in a position other than six. The specific site remains to be determined.

These observations indicate that hydroxylation of exogenous indoles is not generally in the six position, but may occur elsewhere. It is concluded that tryptamines affecting behavior do not function through a 6-hydroxylated metabolite. If a metabolite is involved in the function of these substances, our data suggest that hydroxylated metabolites on positions other than six must be considered as possibilities.

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## Control of Somatosensory Input by Cerebral Cortex

Abstract. *Direct stimulation of the pyramidal tract increases the size of the excitatory receptive fields of neurons in the somatosensory cortex of the cat. This effect reflects greater transmission of cutaneous information through the dorsal column nuclei as a result of the facilitation of cells in these nuclei by pyramidal tract fibers.*

In the cat, each cell of the dorsal column nuclei—the primary somatosensory relay nuclei located at the caudal end of the brain stem—is either inhibited or facilitated, but not both, by stimulating the anterior cerebral hemisphere (*1*). The facilitation comes exclusively by way of pyramidal tract fibers, while the inhibition depends upon activation of bulbar reticular neurons through various cortical efferent routes (*2*). Anderson and his co-workers (*3*) have proposed a model of the synaptic organization within these nuclei in which essentially all cells facilitated by cortical stimulation are interneurons. By this model, primary afferent fibers ending on cuneothalamic relay neurons undergo a presynaptic inhibition which is mediated by the cortically (pyramidally) excited interneurons that are found deep in the nuclei. Thus, cortical or pyramidal stimulation should depress the transmission of cutaneous information through these nuclei—an effect not easily reconciled with the observations presented here.

The primary datum in this report is whether or not stimulation of a particular patch of skin causes an individual neuron in the somatosensory cortex to discharge. The aggregate of all the patches of skin which, when stimulated, cause the neuron to discharge constitutes the neuron's excitatory receptive field. The field may include only a few square millimeters of skin or it may occupy a large fraction of the body surface, usually as a continuous area. Because the pattern of evoked discharge of a neuron varies with the site (as well as with the intensity) of stimulation, a region of maximal effectiveness may be identified; this region is designated as the "center" of the receptive field, whether or not it is the centroid of the area. Cells that form a vertical column through the cerebral cortex have receptive fields of widely different sizes, but the fields overlap extensively and tend to have the same "center" region.

In cats anesthetized with $\alpha$-chloralose and paralyzed with decamethonium bromide, one anterior cerebral hemisphere and the medullary pyramids were surgically exposed; occasionally the dorsal column nuclei were also exposed. The evoked activity of single neurons in the primary somatosensory cortex that is associated with the contralateral forepaw was recorded extracellularly. This region was located just medial to the caudal edge of the coronal sulcus, at the site of maximum amplitude of the *b*-wave (*4*) and about 8 mm caudolateral to the main origin of the pyramidal tract (*5*). Studies in our laboratory show that this coronal recording site contains few pyramidal tract neurons (3 percent of a sample of 570 neurons), the number increasing sharply 4 mm rostromedially, so it is highly unlikely that recurrent collaterals from such neurons penetrate in any number, if at all, into the coronal recording site. A large recording electrode was placed at the lateral tip of the cruciate sulcus to monitor the pyramidal antidromic response produced by stimulating the medullary pyramids. For such stimulation, advantage was taken of the fact that the corticospinal and corticobulbar fibers that comprise the pyramidal tract assemble into a compact and exclusive bundle on the ventral surface of the medulla (caudal brain stem). When bipolar stimulating electrodes are placed over the exposed surface of this bundle, they can either activate the pyramidal tract fibers exclusively or, by increasing the stimulus intensity, activate adjacent fibers of the medial lemniscus as well. Since the latter comprise one leg of the main route to the somatosensory neurons in the cerebral cortex, care was taken to activate only pyramidal tract fibers (stimulus intensity was so low that only an *a*-wave was evoked in the cortex).

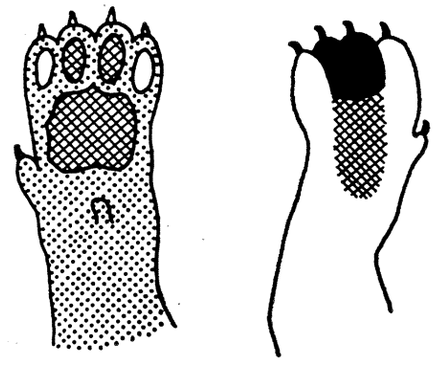

Fig. 1. Cutaneous receptive field of touch neuron isolated 424 $\mu$ deep in coronal cortex. Black area shows extent of "normal" field; black, hatched, and dotted areas show size of field immediately after a 15-second train of shocks at 312 per second applied to bulbar pyramids. Black and hatched areas are touch-sensitive; dotted area is hair-sensitive. Neuron's field included black area, touch only, 4 minutes later.

The experimental procedure was to isolate a single neuron in the somatosensory cortex, find its natural stimulus (for example, hair deflection, skin tap, and so forth), carefully define the nature and extent of its excitatory receptive field, and then determine how this field is altered by activating pyramidal tract fibers. Since pyramidal fibers have a brief and weak effect on neurons of the dorsal column nuclei (pyramidal neurons usually discharge with a burst of spikes about 3 msec apart), long stimulus trains (312 per second) were applied to the pyramidal tract to exaggerate their effect. Monitoring electrodes on the cuneate nucleus did not reveal "convulsive" activity. Receptive field measurements were attempted during the pyramidal conditioning stimulation. Additional measurements were then made immediately after and were continued until the receptive field had returned to its original state. The long train of high-frequency stimulation probably hyperpolarized the synaptic terminals of the pyramidal tract fibers, yielding the phenomenon of posttetanic potentiation (evidenced by potentiation of a small, surface-negative wave dependent upon pyramidal stimulation and confined to the dorsal column nuclei). If this were true, when the pyramidal tract was intact each testing stimulus from the skin would reflexly