THE EFFECTS OF CERTAIN DRUGS ON CEREBRAL SYNAPSES

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As Edward Evarts has so clearly indicated in his contribution to this volume, we are all interested in determining the neurophysiological correlates of mental disturbance in the hope of thereby gaining an inkling of its underlying mechanisms and developing a rational therapy for it. Humphry Osmond has drawn a dramatic picture of the opportunity presented by the situation made possible by the psychotomimetic drugs, which afford us the means of inducing at will a reversible model psychosis. This model psychosis, even though it bears only a fragmentary resemblance to schizophrenia, nevertheless simulates certain aspects of mental disturbance by perhaps similar mechanisms. Furthermore, the so-called model psychosis also can be shortened and terminated at will by the tranquilizers for which clinical effectiveness in schizophrenia is claimed. The use of drugs as tools thus creates favorable conditions for studies of mental illness.

Our efforts, as investigators, are directed more toward an intelligent application of the hypotheses of mechanism rather than toward simple clinical evaluation. The conditions that we wish to interpret are fully and truly exhibited in man but, before we can take full advantage of controlled conditions induced in humans, it is necessary to perform some prototype experiments in animals since, in such experiments, more procedures are permissible and in them those experiments intended for man can be constructed and rehearsed. This purpose, the needed groundwork before work with humans can be done, is my justification for presenting some data on animals and making comparisons with clinical conditions and experimentally induced conditions in man.

Figure 1 summarizes the data that led my co-workers and me to a hypothesis that served as the point of departure for studies in this field. It shows that in our survey of a variety of sites in the nervous system we find, as far as we have gone, that a consistent reciprocal relationship exists between excitation or enhancement by acetylcholine and acetylcholinelike substances, including anticholinesterases, on synaptic-transmission phenomena and inhibition by epinephrine, norepinephrine, all sympathomimetic amines in varying degrees, and related substances. It seemed plausible that any perversion of metabolism that would distort the balance of endogenous chemical or neurohumoral control of synaptic-transmission processes could lead to abnormal cerebral performance or mental disturbance, and that chemicals or drugs could alter the equilibrium of transmission and thereby alter cerebral and mental function in the direction of health or disease.

The limitations of communication with animals make it exceedingly difficult, though not impossible, to relate the behavioral disturbances that can be produced in them with mental disturbance in man. Since our basic premise, however, is that all cerebral function, including both behavior and mental processes, is made up of functional units accumulated in series and in parallel...
combinations to form patterns, I believe it of value to study such units, that is, the synapses.

The transparent model of the brain of the cat (FIGURE 2) illustrates a relatively simple synaptic* preparation that we have found convenient for study. I must emphasize, at the outset, that we consider the experiment pertinent to the extent that it deals with visual pathways, since the powerful psychotomimetic drugs exhibit an important visual component in the hallucinations, dramatically so with mescaline. More important than that, our findings impress us with the similarities rather than the differences between synaptic performance and susceptibility to chemicals, either endogenous or exogenous (drugs). Therefore, we are really using the transcallosally activated cerebral synapses in the visual area of the cat merely as representative of cerebral synapses in general, all of these synapses having qualitative similarities and varying principally by differences of threshold. We do not intend to suggest that an alteration in this specific pathway is necessarily responsible for mental disturbance.† A little later I shall outline a general working hypothesis based

* "Synapse" is used throughout in the sense of designating the total complex involved at the functional articulation of 2 neurons, that is, presynaptic nerve ends, transmission process, postsynaptic dendrites, and soma.

† Chronic interruption in a system such as the transcallosal, as mentioned by Edward Evarts, should not necessarily be expected to produce the same changes as an acute interruption by drugs unaccompanied by surgical trauma and subsequent degenerative processes.
Figure 2. Transparent model of the brain, showing 2-neuron intercortical (transcallosal) pathway. The schematic arterial blood supply is the pathway for the injected material.
on a disruption of normal patterns that results from alteration in amounts of synaptic regulators or in the thresholds of the neurons upon which they act. Since Edward Evarts has already outlined our technique I can be very brief in pointing out certain features. Because the brain is a communication system it seems most appropriate to measure function by recording the handling of a test message. The test message is supplied in the form of a submaximal electrical stimulus applied to 1 optic cortex in a cat that has received a light dosage of pentobarbital sodium. This stimulus initiates a conducted response in the association or transcallosal tract that connects the stimulated point to a symmetrical point in the contralateral cortex where, after synaptic transmission, the stimulation evokes a cortical potential, as first described by Curtis and Bard. To help distinguish between peripheral effects that would contribute to the afferent drive constituting the background against which the impulses are elicited and the strictly central effects, we take advantage of the fact that an intracarotid injection will achieve a transient, higher concentration of drug on the ipsilateral or recording side but, when diluted by the blood in the general circulation, the concentration of the drug is brought down to levels that are below the threshold for the peripheral effects. Under the conditions of our experiment, the amounts of the drug passing through the circle of Willis to the other cortex are unimportant.

In this way it becomes possible to demonstrate (FIGURE 3) that epinephrine, a chemical natural to the body, one known to produce anxiety when accumulated in sufficient amounts, either endogenously or exogenously, also produces cerebral synaptic inhibition, as indicated by the reduction in the signal (surface negative wave) corresponding to outflow, while the inflow (surface positive wave) is essentially unaltered. The same type of synaptic inhibition is shown for another cerebral neurohumor, norepinephrine, in the next line of the same

![Figure 3](image-url)

**Figure 3.** The cerebral synaptic action of epinephrine and norepinephrine in a 2-neuron intercortical (transcallosal) system. Potentials are evoked in the optic cortex by the electrical stimulation of a symmetrical point in the contralateral cortex. Epinephrine (10 μg./kg.) was injected into the ipsilateral carotid artery after A, and norepinephrine (150 μg./kg.) was injected after D. A and D are controls, B and E represent inhibition, and C and F show recovery.
Figure 4. Types of phenyl-ethyl amines producing mental effects.

Figure, but this action is evidently weaker than the other, requiring a larger dose to produce approximately the same degree of inhibition.

In Figures 4 and 5 are shown some structural chemical similarities of compounds with which other contributors to this volume have already dealt. Attention is called to the close structural similarity (Figure 4) of epinephrine to amphetamine, which is also capable of producing anxiety, and mescaline, which does so regularly and with dramatic intensity, producing a full-blown "model psychosis." These drugs in turn are related to the group shown in Figure 5, in which epinephrine is once more presented alongside a first-oxidation product, adrenochrome, which is an indole. Below these are pictured d-lysergic acid diethylamide (LSD-25), the very highly potent psychotogen, which can be considered to be built on an indole nucleus, and 5-hydroxytryptamine, or serotonin. The epinephrine-like psychotogens thus can be chemically related to the indole-like ones, including established drugs such as LSD-25, reputed drugs such as adrenochrome, described at the beginning of this monograph by Humphry Osmond, and by myself elsewhere, and the naturally occurring indole found in the brain, which is postulated by Woolley and Shaw to be sufficiently related to LSD-25 possibly either to compete with or to add to its action. We now looked to see whether there was any functional parallelism or neurophysiological correlate of this relationship by using the objective test of cerebral performance afforded by the evoked-potential technique in the cat. Figure 6 presents the data showing that there is an actual correspondence in structure, and that all the compounds produce synaptic effects.
inhibition identical in kind to that produced by epinephrine, but vary in degree of effectiveness, so that for the approximately equivalent effects shown it required milligram amounts of mescaline, but only microgram quantities of LSD-25, which duplicates the relative potency of these compounds as found in clinical experience. The dosages used throughout our experiments are intentionally of a size selected to produce incomplete actions, so that recovery back to the control level can be secured more readily.

Very interesting is the finding with serotonin, which turns out to be the most effective cerebral synaptic inhibitor of all, being effective in as little as 1-µg. doses. Accordingly, rather than being an antagonist, this indole, or something like it, may represent the type of endogenous substance that is instrumental in bringing about some forms of spontaneously occurring mental disturbance.

Furthermore, since serotonin is naturally present in the brain and is so highly potent (about 20 to 25 times as potent as epinephrine in the same experiment), serotonin becomes, as we pointed out over a year ago, an even better candidate than either epinephrine or norepinephrine, which are also found in the brain, for the role of inhibitory neurohumor. This finding would

* Serotonin must penetrate the blood-brain barrier at least in the small amounts required to exercise the cerebral action described.
point even more closely to a derangement of neurohumoral balance at synapses as a potential mechanism of cerebral or mental derangement.

Unfortunately, except for the intraventricular injections described by Sherwood, there have been, thus far, no documented reports of serotonin-induced mental disturbance* in man that are clearly separable from the natural anxiety initiated by the profound peripheral effects such as circulatory disturbance, other autonomic effects, and emesis. There are, however, such reports for a close analogue of serotonin, dimethyl-serotonin, or bufotenin, which is used for its mental effects by some primitive peoples and has been observed by Fabing to produce such disturbances in man experimentally. These 2 substances, as well as adrenochrome, a presumed metabolite of epinephrine that

* The fact that patients with carcinoid have large amounts of circulating serotonin without showing marked symptoms of mental derangement could represent an adaptation to very high levels of serotonin that has developed and accumulated gradually. This suggestion would account for the relative immunity of such patients to the possible central effects of high doses of serotonin injected intravenously.
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**Figure 7.** Cerebral synaptic inhibition by indoles in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex every 2 seconds. The injections were given in the ipsilateral common carotid artery.

Hoffer, Osmond, and Smythies\(^9\) report as reproducing some aspects of the clinical syndrome of schizophrenia when injected intravenously in man, are compared in the cat in **Figure 7**. Again, all these compounds have the identical qualitative effect, namely, synaptic inhibition, but bufotenin, tested in the same animal, exhibits twice as much effectiveness as does serotonin, which required 10 µg. for its effect on this occasion. Adrenochrome, though it does induce synaptic inhibition, requires so large a dose, 2 mg., that it seems an unlikely candidate for the role of endogenous psychotogen responsible for a form of mental illness, although a substance somewhat like it might be responsible.

The great effectiveness of serotonin not only suggests that this is the type of chemical structure implicated, with the reservations already noted, but that it constitutes 1 link, another being its natural occurrence in the brain, in the chain of evidence identifying it as a cerebral neurohumor. A required piece of information to round out this evidence would be the measurement of the actual liberation of serotonin during, or prior to, the recorded synaptic activity. This is a tedious and difficult type of experiment, and it is attended by special handicaps in work on the brain. Another approach leading to a similar conclusion, however, is quite readily followed. This approach is the accumulation of what must be naturally occurring serotonin, strategically located at the synapses, by the poisoning of the enzymes that normally lead to the destruction of serotonin and account for the ready reversibility and short duration of the action of serotonin. This is the technique that has been used so successfully in the study of the function of acetylcholine in the brain, and it is in this manner, by the use of a powerful anticholinesterase, that we demonstrated the presence and operation of acetylcholine at cerebral synapses.\(^1\) Serotonin is known to be very susceptible to destruction by monoamine oxidase, which is abundantly
present in the brain. We therefore attempted to inhibit this enzyme by iproniazid (Marsilid). Figure 8 shows the result of a preliminary experiment in which we injected iproniazid into the common carotid artery of the cat in the same way that we had done previously with serotonin. The effect produced duplicated the serotonin effect as if, indeed, the serotonin at the synapse had been preserved by the inhibition of monoamine oxidase by the iproniazid. I believe this finding offers another piece of important evidence that serotonin is present naturally, not only in the brain, but at strategic sites where it is capable of influencing synaptic transmission. We have not as yet measured, as we need to do, how much this dose of iproniazid, given in this way, inhibits cerebral monoamine oxidase in the cat.

We believe that the somewhat discouraging attitude of some investigators toward basing clinical prediction on animal experimentation is not entirely justified, since this procedure is a natural result of the comparison of objective criteria such as we have just described with clinical evaluation based upon questionnaires and much undoubtedly shrewd clinical observation, both of these types of data being very difficult, indeed impossible, to quantitate. Accordingly, we are more impressed by the degree of correspondence obtainable rather than by the discrepancies that are to be found. Thus our evoked-potential experiments in the cat rank the psychotogens and psychotomimetic substances studied so far, in general, in the order of clinical effectiveness, and they suggest that at least part of the mechanism responsible for mental disturbance is to be found in an imbalance in the regulation of synaptic transmission. One such imbalance we have already described.

If this hypothesis is truly useful, and if the animal preparation used bears other than a merely empirical relation to the clinical data, we should expect that the various tranquilizers for which varying degrees of clinical success have been claimed would have some action here also. We proceeded to test this extension of our thinking, and we found that all of the several types of tranquilizers are capable, when administered prophylactically to cats, of partially preventing, in the doses used, the cerebral synaptic inhibition of a test dose of mescaline.

Figure 9 shows this reaction, using chlorpromazine (Thorazine). The figures now read from top to bottom instead of from left to right, as in the previous
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FIGURE 9. The prevention of the mescaline effect by chlorpromazine in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex every 2 seconds. The injections were made in the ipsilateral common carotid artery.

The first column shows the control, the mescaline inhibition at B, and the recovery at C. After this, chlorpromazine is given in doses which, per se, have no apparent effect on synaptic transmission, as shown by the new control D in the second column, but now when mescaline is given again, the synaptic inhibition E is much reduced when compared to B. Without the tranquilizers, the same degree of inhibition of mescaline can be repeated several times in succession, provided that complete recovery is allowed between injections. Records G and H show again that this dose of chlorpromazine did not impede synaptic transmission despite the ability of the drug to protect against mescaline. If the dose is increased twentyfold it does have a depressant

FIGURE 10. The prevention of the mescaline effect by reserpine in a 2-neuron intercortical (transcallosal) system. The potentials evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex every 2 seconds. The injections were given in the ipsilateral common carotid artery.
action on synaptic transmission. The same prophylactic action is obtained with reserpine (Serpasil), as shown in Figure 10, and with azacyclonol (Frenquel), as shown in Figure 11. Another point of correspondence with clinical findings is that the margin of safety, in this case the range between the prophylactic and the synaptic-depressant dose, is large, the depressant dose being 15 to 20 times the prophylactic dose with both chlorpromazine and azacyclonol, but the factor is only 2 with reserpine. The latter drug approximates the action of the barbiturates, which can reduce the degree of demonstrable inhibition from mescaline by reducing synaptic transmission in the first place.

I feel justified in saying, then, that the preparation described is pertinent to the clinical situation in that it ranks the psychotomimetic substances in the order of their clinical effectiveness, and that the action of mescaline, the only drug that we have tried so far, is prevented by the tranquilizers.

By use of the evoked-potential technique, we have demonstrated that:

1. There exists an equilibrium of neurohumoral control of transmission at cerebral synapses and throughout the nervous system, as far as I have surveyed it, that is susceptible to distortion and imbalance by disturbance in the amounts of chemical regulator or the susceptibility of neurons.

2. The psychotogens and psychotomimetic substances discussed, structurally and functionally resemble the actions of the fairly well-established inhibitory synaptic neurohumors, epinephrine and norepinephrine, and of serotonin, the new one that we have described.

3. Serotonin or its dimethyl derivative, bufotenin, comes close, even closer than does LSD-25, to representing the type of endogenous psychotogen that might be a natural cause of some forms of mental disturbance.

We speculate that such disturbance can be produced by direct perversions of normal patterns of neuronal activity by the undue influence of synaptic inhibitors or, indirectly, by such inhibitors impeding the flow of impulses from higher controlling centers and releasing the more primitive, simpler, and less well-adapted patterns of activity that we call abnormal.
References

THE EFFECT OF THE HALLUCINOGENIC DRUGS LSD-25 AND MESCALINE ON THE ELECTRORETINOGRAM

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There is a controversy in the literature concerning the precise manner in which d-lysergic acid-dl-hydroxybutylamide-2 (LSD-25) and mescaline induce visual hallucinations in normal human subjects. It is essential that this problem be clarified since the designation of these 2 drugs as "psychotomimetic" is influenced by the concept that the central nervous system participates in the production of these hallucinations.

Our present knowledge of this problem has arisen from clinical and experimental studies on humans and on cats. Klüver has described in great detail the visual hallucinations induced in human subjects. These hallucinations take 2 forms: early appearing geometric patterns in many colors, and later appearing panoramas. A publication by Stoll depicts hallucinations produced by LSD-25 with line drawings of honeycombs, lattices, filigrees, and ruffles. These patterns appear to the subject in luminescent yellow, green, and red colors. These colors are similar to the bright phosphores that appear in the visual field after the mechanical stimulation of the eyeball. These hallucinations do not appear to subjects unless their optic nerves are intact. Physiologists have studied the effect of LSD-25, in both nontoxic and toxic doses, on the visual pathways of cats. Purpura and Rovetta has deduced that this drug facilitates synaptic transmission in the central nervous system, and Rovetta finds spikes on the occipital cortex that are similar to the spikes caused by strychnine.

A perusal of these clinical and experimental data indicates that the source of the visual hallucinations might be in the retina itself rather than in the central nervous system. The present study investigates the extent to which the retina is involved in the production of visual hallucinations induced by LSD-25. The study is based on the principle that an electrical potential accompanies activity in any nervous element.

Action potentials may be detected anywhere on the surface of the eyeball following marked changes in the illumination of the retina. These potentials indicate activity in the retina, and they occur only when retinal elements are transmitting electrical impulses. It follows, therefore, that such an electroretinogram would be induced by the hallucinogens when the illumination of the eye remained constant only if the retina were participating in the production of spontaneous visual experiences. It seemed worthwhile, therefore, to investigate the effects of hallucinogenic drugs on the retinas of cats.

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