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EFFECTS OF PSYCHOSOMIMETIC DRUGS IN ANIMALS AND MAN

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I SHOULD LIKE TO USE my topic merely as a framework to discuss the possibilities, and above all, the limitations of a pharmacologist's intrusion into the phenomena of mental life and of behavior. To be sure, this is by no means a new approach. The ancient, well known, and by now richly documented effects of the so-called phantastica wind their way in and out of the fabric of legend, lore, and literature, making their spurious and surreptitious, almost illegitimate appearances in medicine and psychology until, through a combination of empiricism and systematic enquiry, confused and confounded by massive exogenous pressures, they burst upon us in their present florid and bewildering variety. The classical accounts are familiar, and have indeed been covered more than adequately at a previous meeting of this group. With these I will not burden you. And I will not take it for granted that we are not aware of the silent and effective revolution wielded in the management of our mental hospital wards by the newer ataractic compounds. It is to the challenge implicit in these facts that one must look. Drugs can induce mental derangement and help the mentally deranged. This raises high hopes, but I hope not too high. For the briefest reflection will bring home the limitations of the approach as well as its conceivable promise. The lasting contribution of pharmacology to psychiatry cannot, and will not, depend upon empirical leaps but rather on its steady contribution to our understanding of the brain as an integrating, feeling, information-storing, predicting, and computing organ. Of environment, forever playing upon the brain detector, and so often disrupting and distorting it, pharmacology will tell us nothing. Nor does the limitation end there, for two other aspects of the subject must be clearly borne in mind: Firstly, that modern pharmacology is itself inseparable from biochemistry and

biophysics; and secondly, that the animal experiment, which forms so large and vital link in pharmacologic studies, can but be a necessary preliminary and intermediate in the study of what are essentially and uniquely human attributes and functions. Experiment in psychiatry, to be fully valid, is clinical, or it is nothing. Therefore, pharmacology must approach psychiatry at at least three levels: the tissue, the animal, and man. And it is of these three levels that, in very brief outline, I should like to speak, indicating principally approach and method, and illustrating it here and there with some results from our own laboratories.

If I may, I should like to start with some simple considerations concerning chemical transmission within the central nervous system; to indicate the scope and limitations of electrophysiologic and behavioral studies in the conscious unanesthetized animal; and to mention finally a few, but by no means new, clinical data which will perhaps emphasize the care which has to be exercised in drawing conclusions in this field.

I choose to consider the process of chemical transmission within the central nervous system because of the ambiguous, unsatisfactory, yet by no means unpromising, state of our knowledge in this area; for the abundant data available on humoral transmission at such peripheral sites as autonomic ganglia, secretory cells, smooth and skeletal muscle help one only up to a point. To be sure, the mode of action of various drugs at these sites has been extensively studied. Yet the relevance or irrelevance of these findings to the central nervous system can only be determined in the light of data coming from within the central nervous system itself; and it is only lately that such data have been coming forward.

We owe it to Feldberg's (1) clear and concrete thinking that much confusion in this field has been dispelled. In keeping with some of his ideas, I would like to start by asking you to agree to at least a minimum of four simple *desiderata* (Table X), which one would like to see fulfilled by a substance claimed to have a transmitter role in the central nervous system: The first of these is that the substance should be present within the central nervous system. It should also vary in quantity with the functional state of the central nervous system and should be identifiable by sensitive and unequivocal tests. Secondly, there should be enzymes present both for the synthesis and the destruction of the substance in question. Thirdly, there should be clear effects if either the enzyme for synthesis, or that for destruction is specifically inhibited. In the case of the latter enzyme, inhibition should lead to effects attributable to the endogenous accumulation of the substance

TABLE X

A Neurohumoral Transmitter Substance in the Central Nervous System: Some *Desiderata*

1. Presence in the central nervous system.
   Variation in local concentration with function.
2. Presence of specific enzyme systems for
   (a) synthesis
   (b) destruction
   of substance in question.
3. Demonstrable effects of specific enzyme inhibition.
4. Demonstrable effects of addition of hypothetical agent, applied either topically, or by vascular route.

in question. Fourthly, there should be clearly demonstrable effects of the application of the hypothetical agent to the central nervous system, either topically or by a selected vascular route.

Abramson: Couldn't excretion be just as suitable a term as destruction? Must it be destruction?

Marrazzi: Yes. That is my point, also.

Elkes: That is a good point. However, the effect of a neurohumoral transmitter at a neuron surface must surely depend upon the turnover, and turnover depends upon the rate of synthesis, the holding, i.e., temporary anchoring, and the destruction of the transmitter. Excretion would be an additional factor which no doubt would play a role in the ultimate fate of the substance in question. I am, however, thinking mainly in terms of the local action of the neurohumoral transmitter at the effector site.

Abramson: But aren't you limiting yourself, then, to certain reaction velocities which are categorized to rapid reaction velocities? Suppose you had a slow reaction velocity; wouldn't you then have to change that?

Elkes: Reaction velocities at the receptor sites are notoriously fast. It is important to distinguish between destruction and excretion.

Abramson: Is your title, then, a neurohumoral transmitter in the central nervous system which produces rapid reaction velocities?

Elkes: I certainly would have nothing against considering rate of excretion, provided it were clearly understood that factors outside the central nervous system will play a dominant part. Here we are considering mainly events inside the central nervous system.

Abramson: It seemed to me that that scheme was limited by high reaction velocities.

Elkes: Yes, but these are the conditions which presumably obtain in neurohumoral transmission.

Brodie: What do you mean by the third item?

Elkes: Demonstrable effects of highly specific enzyme inhibition in terms of either biochemical, electrophysiologic, or behavioral criteria.

Mirsky: But, then, the third is actually part of the second.

Elkes: I do not think that presence and inhibition are the same. Presence implies demonstration of the existence and of the specificity of one enzyme by the use of specific substrates. The inhibition of an enzyme cannot be proven unless its presence is first established. The third follows out of the second; they are by no means identical.

Kety: Dr. Elkes, I should like to question your use of the term, *desiderata.*

Elkes: I referred to some, that is, minimal, *desiderata.*

Kety: That will not prove that a particular substance is acting as a neurohumoral transmitter. But acetylcholine satisfies most of them, does it not?

Elkes: Only with certain important reservations. I am grateful to Dr. Kety for introducing acetylcholine at this stage, with which, I, too, was about to begin.

To be sure acetylcholine is present in the central nervous system (2), and its concentration does vary with the functional state. Richter and Crossland (3) Elliot, Swank, and Henderson (4), Tobias, Lipton, and Lepinat (5), and Wajda (6) have all studied this aspect. Relatively high acetylcholine values have been obtained during sleep (3) and barbitone and ether anesthesia (4,5,6) and low values in wakefulness (3). Richter and Crossland (3) have also shown a peculiar phasic relationship of acetylcholine content to convulsive activity, electrical stimulation being followed first by a drop of acetylcholine content, and convulsions apparently only supervening after the return of the values to nearly normal level. Since the time intervals in their experiments were approximately from 10 to 20 seconds, a massive synthesis or more probably a massive release of acetylcholine must be suspected. The factors of storage and release have been further brought out by

Tower and Elliott (7) who have drawn attention to the importance of distinguishing free from bound acetylcholine, and have emphasized the probable role played by ionic forces in regulating the release of acetylcholine from its unknown anchoring surface.

Brodie: Do you think that these criteria for a central transmitting agent might equally fit serotonin and norepinephrine, both of which are present in the central nervous system?

Elkes: I shall be dealing with these agents. However, I should like to carry on with a consideration of acetylcholine first. We now come to the second criterion, as defined, namely the presence of enzyme systems for the synthesis and destruction of the hypothetical agent. With certain very important reservations these requirements, too, appear to be satisfied for acetylcholine. The acetylcholine synthesizing system was described by Nachmansohn (8) and was studied in the central nervous system in detail by Feldberg and Vogt (9), and Feldberg, Harris, and Lin (10), and Wajda (6) in our laboratory. These workers used a sensitive technique of their own. The findings broadly agree, and indicate a curiously uneven distribution of acetylcholine synthesizing power throughout the central nervous system. For example, in Feldberg and Vogt's study, the sensory roots were found to be poor, the gracile and cuneate nuclei rich in acetylcholine synthesizing power, or, again, the pyramidal tracts low, and the anterior roots high. And in studying the distribution of acetylcholine synthesizing power along the optic pathway, Feldberg, Harris, and Lin (10) again showed a curious alternation in this power between various portions of the pathway.

What is important in these findings is not so much that acetylcholine synthesizing power exists in certain parts, but that some areas of the central nervous system are comparatively deficient in this power. Furthermore, Dr. Wajda in our laboratory showed phylogenetic differences in the distribution of the choline-acetylase system in various areas of the brain of pigeon, rat, cat, and man, though no attempt could be made in these studies to relate them to cell population. This, incidentally, is an essential prerequisite, and unless and until enzyme constitution is related to density and types of neuronal and nonneuronal elements in a particular area, no valid conclusions can be drawn. The methods of Pope (11) and Lowry (12) have not, to the best of my knowledge, been applied to the choline-acetylase system.

With regard to the so-called cholinesterases little need be said, because so much is common knowledge. Two enzyme systems, the so-called acetylcholinesterase and the nonspecific cholinesterase (pseudocholinesterase) must, however, be clearly distinguished. If, for example, as has been done by Dr. Todrick in our laboratory (13), the develop-

ment of acetylcholinesterase and pseudocholinesterase in the growing rat brain is studied using specific inhibitors for the detection of these enzymes, it is found that while the acetylcholinesterase increases steadily from the 2nd to the 22nd day, the pseudocholinesterase shows relatively little change for the first 10 days, then increases for the next 10 days, and levels off after that. If now the development of these two enzymes in various areas of the brain is studied, it is found (13) that whereas, for example, the thalamus shows a marked relative increase in the nonspecific (pseudo) cholinesterase between the 8th and the 22nd day, the relative increase in the acetylcholinesterase density is less marked. The obverse applies to a structure like the olfactory bulb, where the relative increase of enzyme activity is mainly owing to an increase in the acetylcholinesterase. There are thus admittedly tenuous indications that even in terms of development these two enzymes may be distinct, and that the nonspecific cholinesterase in the central nervous system may be much more than a mere epiphenomenon of the acetylcholinesterase. Until more details as to the morphology are available, particularly in terms of neuron and glial population, and vascularization, it will not be possible to judge the meaning and function of the pseudocholinesterase in the central nervous system. Of its existence, however, there can be little doubt. Burgen and Chipman (14) and Ord and Thompson (15) have demonstrated this quite conclusively, and the histochemical evidence coming from Koelle's (16) laboratory is not out of keeping with a possible autonomous and independent existence of the nonspecific cholinesterase within the nervous system. Some time ago (13) we thought that, possibly in keeping with Koelle's (16) findings, the pseudocholinesterase might be related to vascular elements in the brain and the unknown substrate upon which it acts concerned with the tone of the smaller cerebral vessels. This, however, must await further evidence.

That these two enzyme systems must, in fact, be distinguished, is borne out by a third set of observations related to the third criterion which was postulated at the outset. The highly specific susceptibility of these enzymes to specific inhibitors is very real. We have studied a number of these in our laboratory (17). Some of them are listed in Table XI. No. 62C47, for example, will inhibit acetylcholinesterase selectively, whereas ethopropazine methosulfate will leave this enzyme system almost untouched, and yet be a highly specific inhibitor of the pseudocholinesterase. What is more, this specificity will still operate in mixtures of the two enzymes.

Bain: What is the structure of the acetylcholinesterase inhibitor C^{47}?

Elkes: These inhibitors can and have been used to map the distribu-

TABLE XI

Inhibition of Rat Acetylcholinesterase and Pseudocholinesterase by Selective Inhibitors

Inhibitors	Concentration (M)	Percentage Inhibition	
		Acetyl-cholinesterase	Pseudo-cholinesterase
	Selective Acetylcholinesterase Inhibitors		
Nu 1250	5×10^{-7}	81	1
	2×10^{-6}	98	8
62C47	5×10^{-6}	94	1
	2×10^{-5}	101	3
284C51	3×10^{-5}	100	2
	1×10^{-4}	102	5
	Selective Pseudocholinesterase Inhibitors		
Ethopropazine methosulfate (Lysivane)	3×10^{-5}	−1	90
	8×10^{-5}	9	95
Tetraisopropyl-pyrophosphoramide (iso-OMPA)	1×10^{-5}	0,1	90
	3×10^{-5}	6,11	92
	1×10^{-4}	24	-

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tion not merely of the acetyl, but also the nonspecific cholinesterase in various areas of the brain and they determine relatively small concentrations of one enzyme in the presence of the other. Our own results and those of others add up, in my own mind, to the strong impression

that the nonspecific cholinesterase in the central nervous system, is an entity in its own right, and that presumably a physiologic substrate other than acetylcholine must exist in the central nervous system and be related to elements, the nature of which is as yet undetermined.

To add to the difficulty, however, there is a further set of observations: If the cholinesterases are inhibited from birth by a powerful and irreversible cholinesterase inhibitor (18), and the emergence of automatic innate behavior patterns is studied (such as, for example, the eye opening reaction, the body righting reaction, the placing reaction, the startle reaction, and reflex suspension), the rate of the emergence of these patterns is found not to be materially affected, despite a high and sustained inhibition of the enzyme.

Marrazzi: Before we leave our discussion of distribution of enzymes, I would like to mention one difficulty. The importance of richness or paucity of enzymes in any particular part of the brain might conceivably be of only a second order of magnitude, because it might reflect a safety margin. A richness may only say there is a large safety margin at that site, and a small concentration may be perfectly adequate for function but have a safety margin.

Elkes: You say "safety margin," but the odd thing is that there is a differential distribution in the safety margin. Why, for example, should there be so much cholinesterase into the caudate nucleus?

Marrazzi: My point is that a minute concentration would satisfy your minimum desiderata.

Elkes: Quite possibly. But what is perhaps worth noting is that although we went on inhibiting cholinesterase from birth to a degree which gave us a mortality in our animals of up to 30 per cent, the animals which survived showed an emergence of the innate behavior responses at a rate which did not seem to be significantly different from the group of animals receiving arachis oil, the vehicle in which the inhibitor was dissolved.

Fremont-Smith: But Dr. Marrazzi's point might be that even though it is kept well down, there may still be plenty of margin, and the mortality may be a result of other causes than the complete inhibition of this.

Elkes: I think this is perfectly proper.

Fremont-Smith: So it still leaves this margin. On the other hand, your emphasis on the uneven distribution is obviously vital, and eventually must be explained.

Elkes: It has to be explained not only in terms of, shall we say, the broad geography of the distribution of the enzyme systems in relation

to large cell masses in the brain, but much more important, in terms (if one may use the metaphor) of the geology of the brain, that is, the fine layer-to-layer structure. The indications are that even in terms of finest histologic and chemical organization, there may be an uneven distribution of different types of neurons within the central nervous system.

Fremont-Smith: An uneven distribution of neurons?

Elkes: Yes, a limited and probably quite small number of types of neurons may be unevenly distributed throughout the central nervous system. The chemical differentiation which we see grossly and crudely, in terms, for example, the striking differences between the caudate nucleus and the rhinencephalon must be carried to a much finer level of organization and may lead us to conclude that neurons of slightly different constitution may have differentially populated various areas and built themselves into these areas by virtue of possessing slight but definite differences which meet the excitatory or inhibitory requirements of particular functional patterns of a particular neural net.

Thus, even at this early stage there is the question of what the universal function of acetylcholine in the central nervous system is. On the one hand, there are areas which appear relatively deficient in acetylcholine synthesizing power; then again, there is an enzyme system which appears to prefer a substrate other than acetylcholine to acetylcholine itself.

Brodie: I was wondering whether it might not be more important to consider the relative distribution of the enzyme which is responsible for the synthesis of acetylcholine rather than for its destruction?

Elkes: I think so, yes.

Brodie: For example, the concentration of serotonin in brain bears a much closer relationship to the distribution of 5-hydroxytryptophan decarboxylase, the enzyme responsible for its synthesis, than to the distribution of monoamine oxidase, the enzyme responsible for its destruction. For my own information, what is the relative distribution in brain of choline-acetylase, cholinesterase, and acetylcholine?

Elkes: I have already referred to the work of Feldberg and Vogt (9), Feldberg, Harris, and Lin (10) and the Montreal group (7).

Cantoni: I would like to pick up Dr. Brodie's point, but I do not agree with you. I do not think it is possible to say whether the enzymes which are involved in the synthesis or the destruction are the ones which are more important, or how easy they are to demonstrate, etc. Both are important, and I do not think we can rig experiments in favor of either one substance or another by selecting the enzymes which are

involved in destruction. I think that both are important and at the present time we cannot be particular about which one is more so.

Elkes: As we said earlier, there is presumably a balance between synthesis, binding, release, and destruction. This has been worked out with special reference to acetylcholine, and the reason for my mentioning acetylcholine is the suspicion that has been entertained by some that an ester other than acetylcholine may be quite active in the central nervous system.

Kety: When you mentioned this other esterase, did you imply that the pseudoesterase may be important?

Elkes: Yes.

Bain: Isn't it true or reasonably well demonstrated that the neuron is principally inhibited by the true esterase, and that pseudoesterase occurs in glia and other structures?

Elkes: Yes, though the evidence which also comes to mind in this context is that of Desmedt and La Grutta (19), who showed that the intracarotid injection of small doses of a pseudocholinesterase inhibitor desynchronizes spontaneous electrocortical activity, whereas relatively larger doses of the true cholinesterase inhibitor had no such effect. This observation illustrates the care which must be exercised in accepting acetylcholinesterase as the only cholinergic agent in the central nervous system. It may even be possible, as I mentioned earlier, that the pseudocholinesterase plays a part in regulating the tone of the smaller cerebral vessels. The point I am arguing is that an ester other than acetylcholine may form a natural physiologic substrate for the nonspecific cholinesterase.

Marrazzi: On the other hand, the evoked potentials in the cortex of the cat are sensitive to minute amounts of true cholinesterase inhibitors, by intercarotid injection.

Elkes: Spontaneous activity?

Marrazzi: We have more data on the evoked potentials.

Elkes: This was spontaneous activity. The difference might be interesting.

Brodie: Though I am generally in favor of Dr. Elkes's view that there is probably more than one cholinergic agent, I would still like to express some slight reservation in regard to the possible role of pseudocholinesterase in nervous function. Pseudocholinesterase is nonspecific and apparently catalyzes the hydrolysis of a number of esters of quite varied structure. This suggests the possibility that the role of pseudocholinesterase in brain is to protect it from the harmful effects of foreign esters that might penetrate the blood-brain barrier.

Hoagland: What about monoamine oxidase, which has a low degree

of specificity, because so many amines are affected by it, and yet it is clearly quite important in this connection?

Brodie: The point is well taken.

Quastel: Dr. Elkes, are you making a point of the different distribution of the enzyme responsible for the synthesis of acetylcholine in different parts of the brain? Is that an important point of your argument?

Elkes: I think it is a point which cannot be lost sight of, yes.

Quastel: Are you referring to the work of Feldberg and his colleague (9) for the demonstration?

Elkes: Yes.

Quastel: You may recall that in that work, the demonstration was carried out by making various homogenates of these various parts of the nervous system and then showing synthesis of acetylcholine, using ATP.

Elkes: Yes.

Quastel: They obtained different rates of synthesis with the different homogenates. But so far as synthesis of acetylcholine in the intact brain is concerned, one must consider the activities of several enzymes, one concerned with the formation of acetyl-CoA, one producing the condensation between acetyl-CoA and choline, and the enzymes responsible for the formation and breakdown of ATP. There is here a complex system and it is uncertain which of these reactions is controlling the actual rate of acetylcholine synthesis in the intact brain. Thus, the synthesis of acetylcholine in any part of the brain must depend on a variety of factors besides that responsible for the choline condensation. Therefore, that the figures representing acetylcholine synthesis in various parts of the brain do not necessarily give us a fair understanding of the true distribution of the rates of acetylcholine synthesis in the living or intact brain.

Elkes: I am grateful to Dr. Qaustel for raising this point. You will recall, Dr. Quastel, that I carefully said that although in the case of the cholinesterases we are dealing with highly specific enzymes (the specific inhibition studies bear this out), in the case of the choline-acetylase we may be dealing with a system which, used *in toto,* and in identical conditions, is capable of acetylcholine synthesis. Would you view the differences in terms of a differential distribution of the ATP activating system?

Quastel: That is for investigation to show. There may be a true deficiency in the choline condensing system, but there may have been some other deficiency resulting in a lack of acetylcholine synthesizing ability.

Elkes: The differences observed in Feldberg and Vogt's carefully controlled *in vitro* studies may reflect a real difference *in vivo.* The differences may be a result of a deficiency in one component of the system, and it would perhaps be wiser to talk in terms of acetylcholine synthesizing power than choline-acetylase activity. Nevertheless, the fact remains that there are striking differences between various areas, however these differences may be accounted for.

Bain: At the time Dr. Feldberg did those experiments, was he aware of the acetyl-coenzyme A story?

Quastel: He used ATP as the drive acetylcholine synthesis in his homogenates.

Cantoni: Yes, but the point that Dr. Quastel made is well taken, because if the ATP acetate-activating system is lacking in these particular areas, that would affect the experiments of Feldberg, as they were done at a time when the exact nature of the enzyme and coenzymes involved was not known. These results might merely reflect the activity of the ATP-acetate system.

Elkes: I would accept that.

Cantoni: There have been recent experiments by Stotz (20) in which phosphorylcholine seems to be even better in some areas, in some tissues, than acetyl-CoA, for the synthesis of acetylcholine. This is a different type of reaction. It might play a role in some areas of the brain, although not in other parts of the body. But it becomes difficult to say, and all that one can say from the experiments of Feldberg is that in certain areas, the ATP does, whereas in others it does not form acetylcholine.

Quastel: Just one more point, maybe, to emphasize this. The distribution of vitamin B_1 may play a dominant role. Many years ago, I was able to show that if the brain were deprived of B_1, the whole acetylcholine synthesis was diminished, so if there is a relatively different distribution of thiamine pyrophosphate in different parts of the brain, it will be seen that this is reflected in various rates of acetylcholine synthesis in these parts of the brain (21).

Elkes: This is the point I am making, the uneven distribution of acetylcholine synthesizing power in various areas of the brain.

Quastel: But my point is, do we know that?

Elkes: It may be affected, and likely is affected, by the factors which you brought out so clearly.

Quastel: Yes, but have we the evidence yet to say that that is the case? We have the *in vitro* evidence.

Elkes: How do we get it?

Quastel: Do we know that it is actually the case in the intact brain?

Elkes: How do we propose to get such evidence?

Quastel: I do not know.

Brodie: Do you think that perhaps the most important factor to consider is the concentration of total (bound plus free) acetylcholine in various parts of the brain? Is this known?

Elkes: Yes. Richter and Crossland (3) have figures.

Brodie: It seems to me that the comparative concentration of neurohumoral agent in the various brain areas should be a more important consideration than the activity of the enzyme that produces or destroys it. In the final analysis is it not the asymmetric distribution of acetylcholine that must be explained?

Elkes: That is correct, but the factors governing the storage and release of acetylcholine are complicated, and as I said earlier a careful distinction should be drawn between bound and free acetylcholine.

Quastel: There is an argument that there is no free acetylcholine in the brain at all.

Hoagland: The histochemical procedures of Pope and others (22) do not really answer this question either.

Elkes: I do not think so, because they reflect mainly the distribution of the specific and nonspecific cholinesterases. I hope that you will agree that acetylcholine satisfies the four *desiderata* we set out at the beginning, only with important reservations; and that it would therefore be appropriate to question the universal transmitter role of acetylcholine. In this regard, I personally have accepted Dr. Feldberg's views.

Purpura: May I amplify your general point? Recently Desmedt and La Grutta (23) found that inhibition of butyrocholine esterase, an enzyme chiefly associated with glial elements, was capable of producing arousal reactions in *encéphale isolé* cats. They suggested that neuroglial elements might be capable of participating in the control of humoral agents bathing neurons. It is quite possible that our consideration of the role of glial elements in central nervous activity is due for revision.

Elkes: I am interested to learn of these experiments and am also glad that Dr. Purpura introduced the glia into this discussion. Sooner or later the nonneuronal elements, namely the glia, the interneuronal, and the vascular elements must be considered. Yet little is known of the chemistry of these elements in terms of enzyme distribution and specificity. Dr. Glees may have some information for us.

Marrazzi: So long as the specificity is not entirely absolute, then there may still be an action on true cholinesterase?

Elkes: Yes. There is an overlap, though the rate of hydrolysis of different substrates by the two enzymes is different.

Marrazzi: I mean with reference to Dr. Purpura's point; when something that has a preponderant action on pseudocholinesterase is used, unless it is completely specific for that and has no action on true cholinesterase, it still is not possible to arrive at any more definite conclusion than before.

Elkes: Yes, but it depends entirely on the local concentration at a particular time.

Marrazzi: That is what I mean. The pattern may be to some extent irrelevant.

Bain: May I just say, too, that this problem of the specificity of the inhibitor depends upon *in vitro* experiments in which the level of enzyme, the level of substrate, and the level of inhibitor are rather artificially manipulated. The amount of inhibition *in vivo* is quite different. The only approach to that is a histochemical one, which is going to be difficult to develop.

Elkes: I agree, particularly in relation to this problem. One admires the scepticism of the work of Couteaux (24) and Koelle (16), and the care which they exercised in setting up their controls; but the precise location of the enzyme in relation to the receptor site, or precise configuration of the receptor in relation to specific enzymatic activity must still remain an open question. Everyone here is presumably aware of Bergmann's (25) views on the subject.

We now come to the possible existence of a noncholinergic neurohumoral transmitter, and here the evidence is suggestive and provocative, but by no means conclusive. The sensory roots which are notoriously low in acetylcholine synthesizing power have been particularly studied for this hypothetical agent. Holton and Holton (26) suggested that adenosine triphosphate may act as a transmitter along this afferent pathway; Lembeck (27) claims to have identified substance P, a depressor substance in the posterior roots; and Florey and McLennan (28), whose work was mentioned earlier in the discussion, have recently identified an inhibitory substance in the central nervous system and have studied its properties in some detail; their work is of great importance. As pointed out by Feldberg (1), the suggestion that cholinergic neurons should alternate with noncholinergic ones brings to mind the peripheral sympathetic nervous system where acetylcholine acts as a transmitter at ganglia and epinephrine and norepinephrine is liberated at peripheral effector sites. In this connection, the work of the Edinburgh school (29,30,31) and of Dr. Brodie's laboratory (32,33) is particularly relevant. I hope that Dr. Brodie will give us

the benefit of some of his knowledge of the subject during the discussion. As is well known, the Edinburgh findings indicate quite clearly an uneven distribution of both norepinephrine, 5-hydroxytryptamine, and substance P in the brain. The areas rich in these agents include, among others, the hypothalamus, the area postrema, the mesencephalon, and the medial thalamic nuclei. The lateral thalamic nuclei (which differ functionally from the medial nuclei), the cortex, and rhinencephalon were relatively low in norepinephrine content. Furthermore, in the same paper (29) Dr. Vogt showed that whereas some drugs such as caffeine, leptazol and ergometrine did not significantly change the hypothalamic norepinephrine level, other drugs like apomorphine and morphine caused a distinct fall. Moreover, significantly, this fall was accompanied by a corresponding decline of adrenal medullary amines. This brings to mind several other points. Folkow and von Euler (34) found that according to the precise local stimulation of discrete areas of the hypothalamus, the adrenal medulla could be made to yield either epinephrine or norepinephrine, thus once again emphasizing the specific interplay between the hypothalamus and the adrenal medulla. You will recall, also, Dr. Weil-Malherbe's (35) observations on the relationship of the level of circulating blood sympathins and wakefulness, and Dr. Funkenstein's (36) observations on the central sympathetic reactivity patterns best accounted for, in his view, by a differential release of either epinephrine or norepinephrine. To mention a wider range, there are Bülbring and Burn's (37) and Bülbring and Burn's (38) older experiments which showed that epinephrine added to perfusate of the superior cervical ganglion or the spinal cord will modulate the response to acetylcholine, and Dr. Marrazzi (39) has described the central inhibitory effect of epinephrine in the transcallosal monosynaptic pathway. The central effects of intraventricular injections of epinephrine and norepinephrine and serotonin have also been fully described by Feldberg and Sherwood (40).

Thus, in terms of our first and fourth *desiderata*, the requirements are partly fulfilled. However, some startling gaps remain. We know, despite the work of Blaschko (41), relatively little of the local synthesis or destruction of epinephrine and norepinephrine in the brain. Gaddum and Giarman (42) have shown that 5-hydroxytryptophan decarboxylase is present in the brain. The enzyme has roughly the same distribution as 5-hydroxytryptamine. It is, therefore, probably concerned with the local synthesis of serotonin. We know of the presence of monoamine oxidase in the brain and its probable role in the destruction of naturally occurring catecholamines. Yet it is difficult to claim a high specificity for this enzyme. We know, also, of the powerful serotonin release

brought about by reserpine, as observed by Dr. Brodie and his colleagues (32,33), which leads us back to the distinction made by Dr. Brodie between free and combined serotonin, a condition already encountered in connection with acetylcholine. In any event, I think you will agree that, when seen against the four criteria postulated at the beginning of the discussion, the gaps far exceed the body of knowledge.

I have said elsewhere (43,44) that thinking in terms of the selection by chemical evolution of small families of related compounds which by mutual interplay would govern the phenomena of excitation and inhibition instead of thinking in purely unitary terms would be more to the point. Acetylcholine, norepinephrine, serotonin, and histamine may be parent molecules of this sort. But a comparison of the effects of acetylcholine with succinylcholine or norepinephrine with its methylated congener brings the realization of how profound can be the effects of even slight changes of molecular configuration. The astonishing use which chemical evolution has made of the steroids is but another example of the same economy. It is possible that the inhibitory substance in the central nervous system may turn out to be the chemical analogue of some of the neuropharmacologist's oldest friends. It is also likely that neurons possessing slight but definite differences in chemical constitution are unevenly distributed throughout the central nervous system, and that phylogenetically older parts will, in terms of fine detail of chemical constitution, be significantly different from the parts characteristic of human evolution. I hope to have occasion to return to this theme a little later.

Purpura: May I emphasize one point, Dr. Elkes? Instead of postulating many transmitters one may consider that the same transmitter is acting rather selectively on different receptors of the postsynaptic membrane. Thus, the same transmitter may produce opposite effects depending on the type of synaptic activity which it induces. This has been demonstrable for acetylcholine and epinephrine. Both activate synapses and both are capable of producing excitation or inhibition if the postsynaptic membrane depolarizes or hyperpolarizes.

Elkes: I am glad this point has come up. For one thing, I am not postulating many transmitters; I postulate only a few (an hierarchy as it were) substances from which variants have evolved. Whether acetylcholine excites or inhibits will surely depend on local conditions.

Purpura: Not at all, no. The important thing is the specific response of the postsynaptic patch whether it is responding in a hyperpolarizing or depolarizing manner; in other words the same transmitter may

activate different receptors which are selectively geared to different ionic mechanisms.

Elkes: Would you then postulate a differential distribution of receptor sites, that is, of receptor configuration at the ultrastructure level?

Purpura: I am afraid that I cannot comment on that.

Elkes: I know nothing about the structure of receptor sites. Is there any reason to suppose a similarity between the receptor site and the active patch of the enzyme, specific for the neurohumoral transmitter?

Mirsky: No, as I understand it, the point that is being made is that the receptor varies in responsiveness to different agents or to a single agent. And as can be demonstrated with peripheral responsiveness, the state of the receptor will determine the response to any specific agent. There are all kinds of combinations and permutations with very few agents, if changing receptor responsiveness is postulated continuously.

Elkes: Continually changing? A statistical distribution is presumably what you mean.

Mirsky: A normal distribution of responsiveness.

Elkes: I am trying to force this point a little further, because there are people in this room who know more about it than I do, and I would like them to commit themselves. If I may put the question again, is it likely that the configuration of the receptor bears any relation to the configuration of the active portion of the enzyme itself?

Purpura: I am trying to re-emphasize for the purposes of the present discussion what has been more lucidly pointed out by others.

Evarts: Axelrod (45) has done some interesting work recently on the activity of the enzymes which *N*-demethylate narcotic drugs during tolerance to narcotic drugs. He finds that during the tolerant state, there is a decrease in the activity of the enzymes which *N*-demethylate morphine. He has recently put forward the idea that in this system of the enzymes and receptors for narcotic drugs, the enzyme activity may serve as a model for the affinity of the drug for the receptor.

Cantoni: We have been interested in this, particularly as far as drugs or physiologic agents in general are concerned, with regard to the mode of action of enzymes; in other words, one question as we see it is whether the active centers of enzymes which act on the same substrate are similar. Is the substrate always bound by the same forces or by the same groups to the enzyme molecule? Of course, we do not have the answer as yet, and furthermore by examining enzymes from different tissue and trying to think about this problem, I am afraid we cannot reach even a first approximation. There is, in fact, no evidence.

It is quite possible that the enzyme surface, the active center, varies, depending on the reaction which is catalyzed; in other words, depending on which part of the molecule is being strained or broken, there would be binding to a specific site.

I think it is not reasonable that the binding of acetylcholine to cholinesterase should be the same as the binding of acetylcholine to choline-acetylase, because the situation is somewhat different.

Glees: I am not a chemist, but I think this is like a key which can lock and unlock the same door, meaning locking inhibition and opening excitation. That would be a possible comparison.

Cantoni: You might have a key which locks and unlocks many doors, after all. There are some.

Glees: A master key.

Cantoni: Yes, like a master key. There are many substances upon which similar enzymes act. Acetylcholine is one that we are talking about, glyceraldehyde is another, and there is a variety of the other substances. They might be the master keys. How the enzyme surface fits them, which is the lock, somewhat depends on the door, which might be the reaction.

Glees: And also how far you push the key in, which might have various markings. This may be too naive a way of putting it.

Bain: Don't you think, Dr. Cantoni, that the logic of this is a little bit this way? The enzyme is doing something to the substrate, when it acts upon it. The substrate, on the other hand, is doing something to the receptor when it acts on it. It would almost seem as if the two sites of action would need to be different. Furthermore, consider a molecule like acetylcholine, for example, which has two ends, one positive charge and one negative charge, and Nachmansohn's and Wilson's (46) model. One could conceive of several different types of chemical groups which will attract a positively charged one. They do not have to be the same. So I think it is very possible to jump from receptor site to receptor site structures.

Brodie: The problem is still more confusing when it is considered that there are some drugs that do not undergo measurable transformation in the body, yet exert marked pharmacologic effects.

Quastel: There are many examples of substances which have differential affinities for different types of proteins, benzedrine, for example. Benzedrine has a high affinity for aminoxidase, as is well known now, but it also has a high affinity for cholinoxidase. Presumably, the approach of benzedrine to these two proteins is different, because the other types of substrates are different, too.

Let us consider a molecule such as fumaric acid, which is one of

best known metabolites of the body. It may be affected by different enzymes. It is capable of combination and activation by aspartase (which converts it to aspartic acid), or by fumarase (which converts it to *L*-malic acid) or by succinic dehydrogenase (which converts it to succinic acid). The combining groups in the different enzymes for fumaric acid are different as shown by the different effects of inhibitors. This is merely to illustrate that any given molecule may affix itself to a protein in quite different ways, according to the type of protein being dealt with.

Glees: Are these drugs which are not broken down but which have, as you say, a profound action, enzymes, by definition, or how must we imagine they work?

Brodie: This is a question that the organic chemist is constantly asking. How can drugs possess pharmacologic action if they are chemically inert *in vivo*? However, a compound may have a high affinity for an enzyme or receptor site and thus inhibit it without undergoing chemical change itself.

Masserman: The key does not have to break in the lock.

Glees: Yes, that is true.

Cantoni: I was interested in what Dr. Purpura said, and I wondered if he would elaborate on it somewhat. Has he really any evidence that when acetylcholine reacts on the peripheral receptor, which responds with inhibition, or one which has found this stimulation, that there is a change? Can it be shown that the hyperpolarization of the membrane is affected in this way? I am not aware of it. I would be much interested to hear it.

Purpura: To begin with it may be profitable to recall the work of Lundberg (47). Using the microelectrode technique, Lundberg found that the various cells in the salivary gland responded with hyperpolarization or depolarization to sympathetic or parasympathetic stimulation. In his Type I responses, gland cells were hyperpolarized following stimulation of both sympathetic or parasympathetic nerves. Type II responses were obtained from cells which responded with hyperpolarization to parasympathetic stimulation and depolarization to sympathetic stimulation. Type III responses are obtained from other cells which depolarize following sympathetic or parasympathetic stimulation. Of particular interest for the present discussion is the fact that Lundberg found that Type I responses could be obtained with the intracarotid injections of acetylcholine, pilocarpine, or epinephrine. In other words, Type I cells responded to these agents with hyperpolarization.

Secondly, there is the work of Del Castillo and Katz (48). These investigators utilizing the microelectrode technique examined the

response of the pacemaker cells to vagal stimulation and iontophoretic application of acetylcholine. In both instances hyperpolarization is produced at the cardiac receptor sites the effect of which is to slow or stop the rhythmic depolarizations present in this special cardiac tissue. There is now a growing body of evidence on this whole subject which has been summarized by Dr. Grundfest (49).

Marrazzi: Some of those examples, however, are not as clear-cut as they seem, because in the case of the cardiac example, there are said to be intracardiac ganglia which are stimulated by acetylcholine, and, in truth, act by liberating epinephrine or something like it; so that the final action in that case, whether or not it is depolarization, is not attributable to the acetylcholine but to the final chemical substance, epinephrine.

Purpura: In Del Castillo and Katz's work the iontophoretic application of acetylcholine was to the receptor site with intracellular electrodes, not to the intracardiac ganglion cells. At the intracardiac ganglion, the presynaptic vagal fibers presumably liberate acetylcholine which depolarizes the postganglionic cell and is therefore excitatory in action. The postganglionic cell also liberates acetylcholine, but the effect of the neurohumor on the cardiac receptors is hyperpolarizing or inhibitory. So acetylcholine can produce depolarization or hyperpolarization depending on the receptor response to activation by the transmitter.

Elkes: I think that answers my question. As I understand it, a possible similarity at the ultrastructure level between the configuration of the receptor and the configuration of those portions of the enzyme molecule which are specifically concerned with either the synthesis or the destruction of the transmitter substance can by no means be discounted.

Whatever the value of purely biochemical studies, they cannot, unaided, materially contribute to our knowledge of the time relationships between various cell groups; and it is here that electrophysiologic methods come into their own. From the point of view of one interested primarily in the relation of electrical activity to behavior (and the effects of drugs on both) work on the conscious preparation is important for three reasons: First, the vitiating effect of anesthetics is avoided; second, effects on behavior can be studied at the same time as effects on the electrical activity, and the relation or the lack of relation between electrical activity and behavior can be determined; and third, it is wiser in such pharmacologic studies to work with the same animals over long periods than with different animals over short

periods. Individual variation from animal to animal is considerable, and it is safer to vary the drug rather than to vary the animal.

Evarts: This can be questioned. If one cat is studied repeatedly, a great deal is learned about that particular cat.

Elkes: I still think it is wiser to use the same animal as his own control, and to use as many animals as possible to establish the validity of a finding.

Fremont-Smith: When this issue comes up, as it does again and again in every discussion, and the generalization, it is more important to do this or it is more important to do that, is made, if the goal *with respect to what it is more important,* is specified, there is no longer any issue. Both are more important. Both are the most important. But each is the most important *with respect to a different purpose.*

Evarts: If it is not the goal to obtain data on the particular characteristics of an individual cat, a great many cats should be used rather than doing prolonged studies on the individual cat.

Masserman: There is one other difficulty I might point out that is even more specific. There is no procedure that can be used with any animal that does not modify its reactions to later experiences. That, I can show during this discussion, if you like. You are not using the same cat time after time. It is partially a new cat modified by intervening experiences.

Fremont-Smith: But you are using a better known cat than when you are using a lot of cats about which you know nothing as to their past experiences, because you have not caused those past experiences.

Elkes: In all we have used 22 cats with long-term implanted electrodes.

Brodie: Even a worse objection is that the experimentalist is not the same every day.

Elkes: I agree with Dr. Masserman; as I hope to show, the same drug in the same animal and the same drug in different animals produce consistent effects.

However, to proceed, chronic preparations with implanted electrodes which could be kept for months rather than weeks became essential, and Dr. P. B. Bradley in our laboratory has devoted some time to developing a suitable technique (50). The cortical electrodes consisted of small silver spheres resting gently on the pia and held in place by stainless steel screws inserted into the skull. There screws merely served as carriers of the electrodes, and did not form part of the recording circuit.

Fremont-Smith: Are they in the cerebrospinal fluid, or below it?

Elkes: No, they rest on the pia.

Fremont-Smith: Essentially in the spinal fluid?

Elkes: The subcortical electrodes were made up of electrolytically reduced stainless steel needles inserted into selected places by the Horsley-Clarke technique according to a previously prepared map, and cemented to a plastic flange inserted into the skull during an earlier part of the operation. The leads were brought out in the interscapular area, and attached to a small 12-point miniature socket which the animal carried on its back in a small harness. These animals were allowed to live in colonies of from three to six, and were found to interfere little with each other. Fur growth and healing were normal (Figure 76), and they could be kept from 6 to 12 months in good condition, although usually they were killed after 3 months to check electrode placement. An additional refinement to the above technique was the insertion of a Feldberg-Sherwood (40) intraventricular cannula in electrode-carrying animals (Figure 77), thus making possible injection by the intraventricular, as well as the systemic route. Recording was carried out in a simple observation chamber which consisted of a soundproofed, insulated, DC heated and lit box. The front wall of this was made of plate glass which could, if necessary, be covered by a hinged partition which contained an opening for observation and filming. An electronic stroboscope lamp was let into the wall. The electrode leads, plugged into the socket and counter-balanced so to minimize drag and intereference with the animal's spontaneous movements, were led out to an EEG machine. A remote trigger unit controlled the rate of the stroboscope. Continuous observation of the unrestrained animal from a distance was thus made possible while the electrical activity was being recorded (Figure 78). More recently, the soundproofed box was transferred to a soundproofed room. The behavior of the animal was regularly protocolled on a proforma sheet of suitable design.

In addition to these chronic preparations, a series of acute preparations of the type described by Bremer (51) was used. These were the so-called *encéphale isolé,* sectioned at the level of the first cervical vertebra, and the *cerveau isolé* sectioned at midbrain level. Though the operation itself was carried out under ether anesthesia, an hour was always allowed to elapse for the anesthetic to wear off before the actual experiment was begun. These preparations provided complementary information, and their particular usefulness rested in the fact that they made possible the control of such factors as blood pressure and respiration, and also the administration of drugs by close arterial (carotid) or intraventricular injection.

Magoun: I would like to mention a preparation which we have called the *Naquet isolé.* This is a modification of Bremer's *cerveau*

isolé, which Dr. Robert Naquet of the University of Marseilles, France, employed when he was in Los Angeles. It resembles the pyramidal cat of Giuseppe Moruzzi; Professor Moruzzi is Director of the Institute of Physiology at the University of Pisa, Italy.

Under ether, two little burr holes are made in the cranium and,

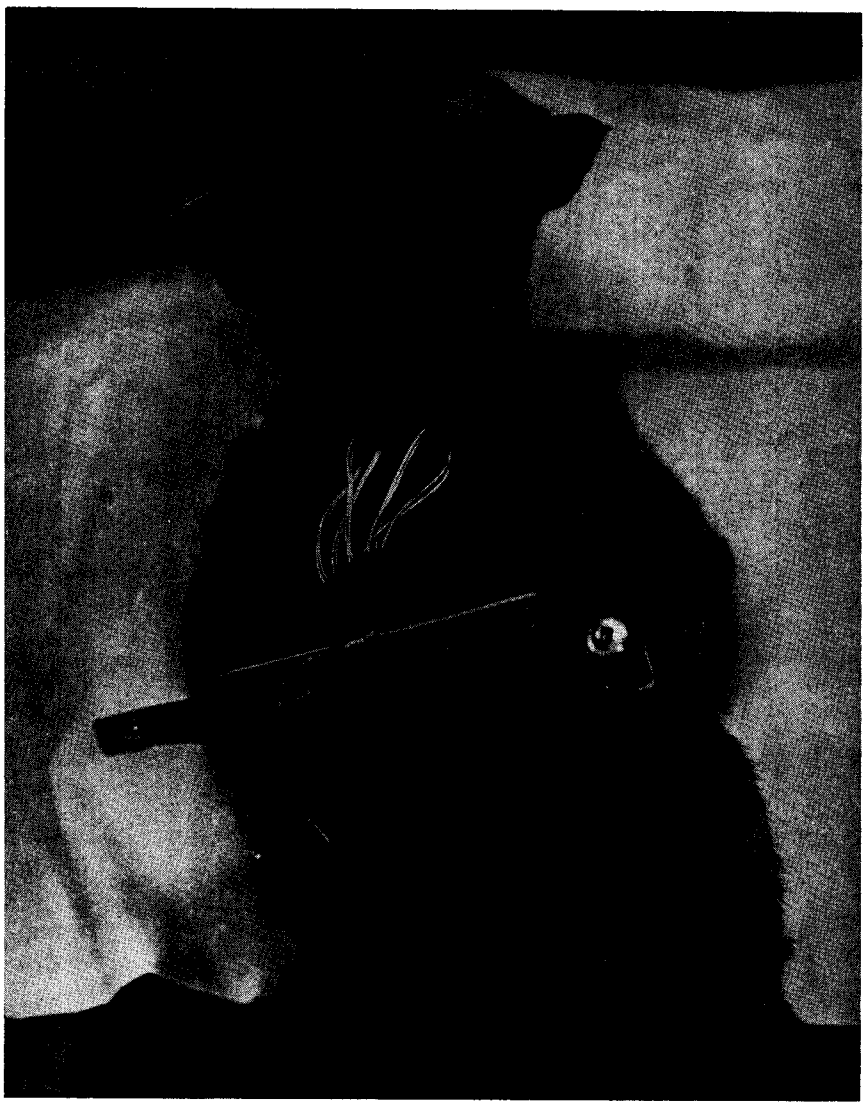

FIGURE 76. To show fur growth and healing around electrode leads. (Photograph taken 4 months after original operation.)

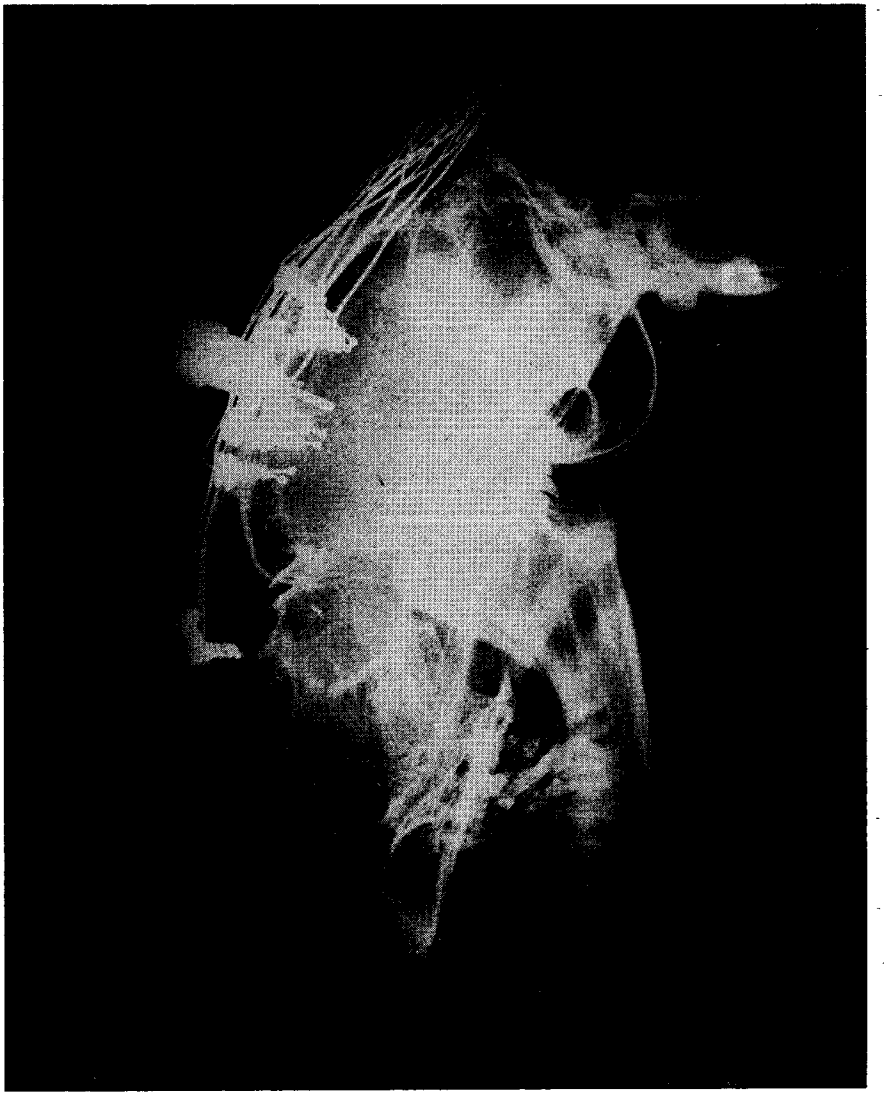

FIGURE 77. Roentgenogram showing implanted cortical electrodes and intraventricular cannula (marked by arrow).

stereotaxically, two wires, the tips of which are bared a couple of millimeters, are lowered into the midbrain tegmentum on either side of the midline. Then a high-frequency current is passed between the tips, and, by varying the time and intensity of the current, one produces a focal coagulated lesion of the cephalic tegmentum. Upon withdrawing

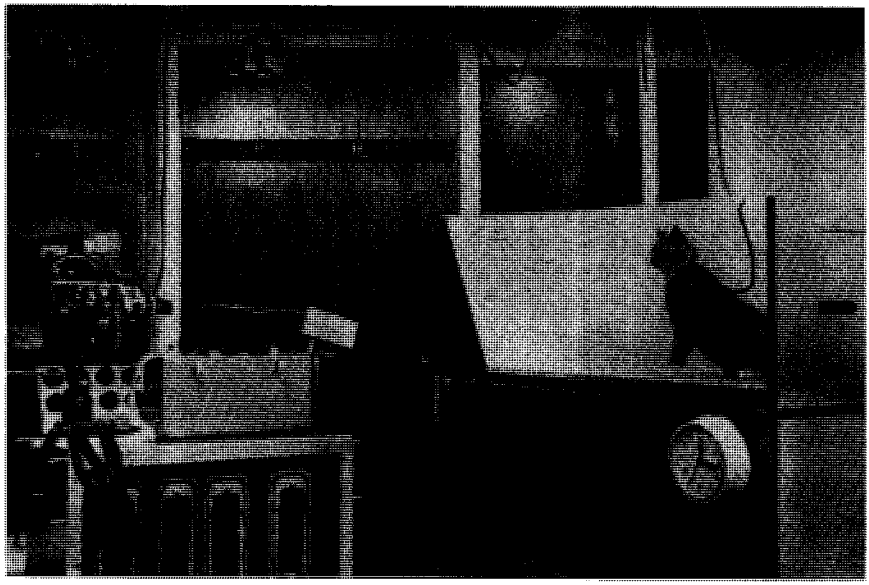

FIGURE 78. To show the general arrangement for simultaneous recording of electrical activity and behavior in the conscious preparation.

the electrodes and letting the ether blow off, one proceeds as with a *cerveau isolé.*

The lesion should not be made so large as to close the cerebral aqueduct, either directly or by succeeding edema, for then herniation of the hemisphere results.

Brodie: I wonder if you would define these terms.

Magoun: These are French terms introduced by Bremer in 1935 (51), and commonly used by neurophysiologists since that time. The *cerveau isolé* refers to the isolated cerebral hemisphere, separated from the rest of the central nervous system by a transection through the midbrain. The *encéphale isolé* refers to the isolated brain, separated from the spinal cord by a transection at C1 on Figure 90 (p. 250). These preparations are comparable, respectively, to the late Dr. Charles Sherrington's* decerebrate and spinal animals, but call attention, as did Bremer's classical studies, to the parts lying *in situ* ahead of, rather than behind, the level of transection. The *Naquet isolé* is a synonym which we have liked to use, both for its euphony and as a means of poking a little Gallic fun at Dr. Robert Naquet.

Elkes: The drugs used in our early experiments are summarized in Table XII. You will note that they follow a certain order, and fall into

*Formerly Professor of Physiology at the University of Oxford, England.

TABLE XII

Drugs Used in Electrophysiologic Studies in the Conscious Animal and in Acute Preparations

Physostigmine	5-hydroxytryptamine
Neostigmine	LSD-25
Diisopropylfluorophosphate	DL-amphetamine
Acetylcholine	D-amphetamine
Atropine	L-amphetamine
D-hyoscyamine	Chlorpromazine
L-hyoscyamine	

four broad groups: those whose action is related to either acetylcholine accumulation or acetylcholine block; those related to catechol amines (the amphetamines); serotonin, and a drug related to serotonin, LSD-25; and a so-called tranquilizing agent, chlorpromazine. This order was intentional. For although highly tempted to examine the effects of LSD-25 first, it was felt advisable to clarify the effects of familiar drugs first, and, having laid down such reference points, to study further the interaction between the less familiar and the more familiar. Since these experiments were completed, a number of other drugs have been studied by Dr. Bradley, Mr. Hance, and Mr. Key (52,53). The effects of some of these agents on electrical and behavioral arousal thresholds have also been examined (54).

A typical resting electrocorticogram in the cat (Figure 79), made while the animal is sitting quietly or lying down, is characterized by bursts of rhythmic activity at from 5 to 8 cycles per sec. These normally have an amplitude of about 200 μv. and are not uniformly distributed over the whole cortex, being mainly seen in the cortical areas which are not primarily sensory or motor. Sometimes they are preceded by bursts at higher frequencies; if the animal lapses into light sleep, slow waves at from 1 to 3 cycles per sec. and up to 500 μv. voltage may supervene. It is important to stress that only through long familiarity can the slight individual variations from animal to animal be given their due

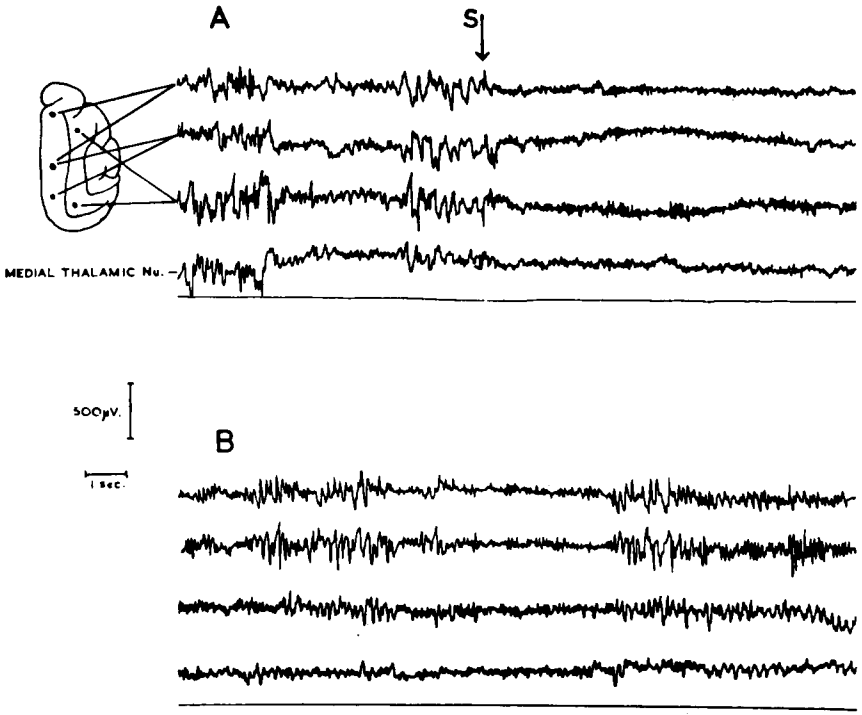

FIGURE 79. To show normal record obtained in unrestrained preparation and the effect of arousal: (*A*) Left—drowsy, Right—arousal and (*B*) quiet. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

weight. This, coupled with an adequate acclimatization period in a constant environment (of about one hour's duration) prior to the administration of any drug, ensured that the pharmacologic agent remained the principal variable in these experiments.

The so-called alerting response can also be seen in Figure 79. Here, in response to a sensory stimulus (of light, or sound, or touch), there is an "alerting" of electrical activity showing itself in the appearance of low-voltage fast activity in all leads. This corresponds with behavioral arousal. The phenomenon is familiar, and was first described by Rhineberger and Jasper (55).

The intraperitoneal administration of physostigmine (Figure 80) in doses of from 0.1 to 0.3 mg./kg. resulted in the appearance of low-amplitude fast activity in all regions, similar to that seen in the fully alert animal. However, in the dose used, the animal did not show corresponding behavioral alerting. Larger doses led to the appearance

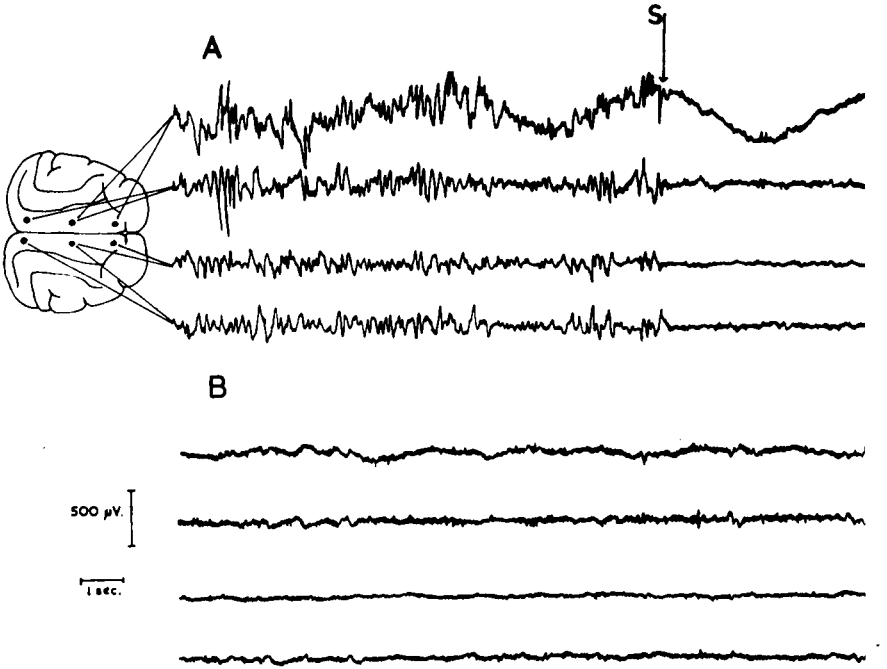

FIGURE 80. To show the effect of physostigmine (0.08 mg. intraperitoneally) on the electrocorticogram of the conscious cat: (*A*) normal conscious cat-arousal; (*B*) 10 min. after 0.08 mg./kg. physostigmine I.P. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

of parasympathomimetic symptoms such as lacrimation and salivation. Neostigmine, even in high doses sufficient to produce marked peripheral effects, had little effect on the electrical activity of the brain. Diisopropylfluorophosphate (DFP), because of its irreversible nature, was administered to only a few animals. Though different in some respects, the effects bore strong resemblance to physostigmine (56).

The effects of atropine are shown in Figure 81. This drug, administered intraperitoneally in doses of from 2 to 3 mg./kg., leads to the appearance of high-voltage slow activity in all leads, which, in general form, is not unlike the activity seen in deep sleep, though the amplitude as a rule is somewhat larger. The animal, however, remains fully awake and in some instances, may even be excited. Thus, here to, there is no correspondence between electrical activity and behavior.

Unna: Can the atropine effect be interrupted by the arousal response?

Elkes: No, Dr. Unna, it cannot. This is very important. The behavioral arousal response to a sensory stimulus comes through clearly.

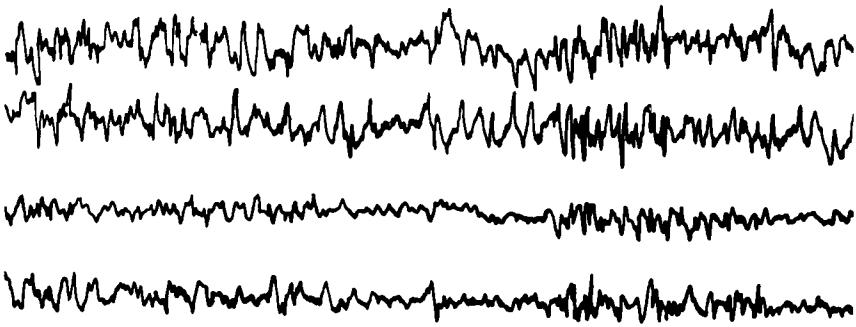

FIGURE 81. To show the effects of 3 mg./kg. atropine I.P. on the electrocorticogram of the conscious cat. Record made 20 min. after administration. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

The arousal response in terms of electrical activity, however, does not appear unless (as referred to below) physostigmine is given following atropine.

The importance of route of administration, however, is clearly shown in Figure 82. Here atropine in a dose of 300 μg. was administered intraventricularly. This record, taken 17 minutes afterward, shows low-voltage fast activity in all leads and there is also behavioral change. Thus, if atropine is administered intraperitoneally there is a loss of correspondence between electrical activity and behavior. Intraventricularly administered atropine, however, leads to the behavioral phenomena observed by Feldberg and Sherwood (40) and a correspondence between electrical activity and behavior.

The effects of the hyoscyamines have also been examined: *d*-hyoscyamine, even in very high doses of up to 10 mg./kg., was found to have relatively little effect; *l*-hyoscyamine, on the other hand, in doses roughly one third of the effective dose of atropine, produced effects similar to those of atropine, there being, again, no correspondence between electrical activity and behavior. The interplay between atropine and physostigmine in the fully conscious animal is shown in Figure 83 and here, too, is contained the reply to Dr. Unna's question. Within 20 minutes of the administration of 2 mg. atropine intraperitoneally the typical atropine activity appears, and as previously mentioned is not related to the behavioral state. If this is followed by physostigmine in doses of 0.5 mg./kg., there is a change in the electrical activity and also a resultant state in which, 10 minutes later, the electrical alerting response begins to appear. If the physostigmine dose is increased (0.9 mg./kg.) and 15 minutes were allowed to elapse, the

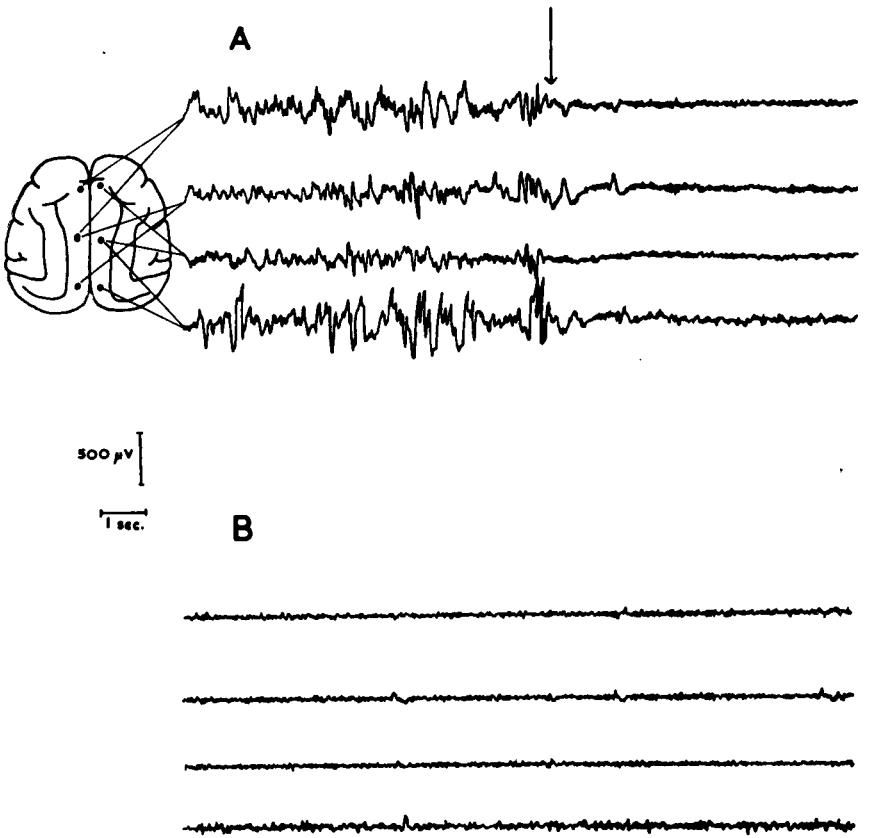

FIGURE 82. To show the effect of an intraventricular injection of 300 μg. atropine on the electrocorticogram of the conscious cat. The difference between this effect and the effect of an intraperitoneal injection of the same drug is evident: (*A*) normal conscious cat arousal; (*B*) 17 min. after 300 μg. atropine was given intraventricularly. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effect of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

low-voltage fast activity is seen to appear and this completely supplants the atropine-induced activity. The animal here remains quiet but not unduly retarded. The effects of physostigmine can then be abolished by a further dose of atropine. Thus, the central interaction between atropine and physostigmine appears to be much more readily and reciprocally reversible than the coresponding phenomena at peripheral effector sites.

Brazier: What is your criterion for alerting?

Elkes: Usually a pricking up of the ears, raising of the head, and dilation of the palpebral fissure.

Glees: Do you have a film record made at the same time?

Elkes: Yes, we have in selected experiments, though to make one has not been the universal rule so far.

Glees: I wonder if Dr. Magoun or Dr. Brazier would remark on this startling finding, that there is behavior dissociated from electrical changes. Is this a common experience? I am surprised that no one has made any reply to this.

Magoun: I gather that it is possible by means of these drugs to change activities in the outer layers of the cortex and so modify the EEG, and, yet, changes here are not always tied in with the animal's conscious, alert activity. I should like to hear from Dr. Purpura about this.

Hoagland: There is an example following electroshock of mental patients in which a marked dissociation between the electrical activity and behavior can be observed. Some patients show a very marked slowing of the EEG, which may be of the order of a drop from 10 dominant cycles to 5 or 6 per second and these may speed up to normal over a period of weeks after cessation of treatments. Others will show little change and behaviorally we have observed no correlation between this marked slowing of the EEG or its lack and the daily behavior of the patients. There are also some cases of drug action in man, in which marked slowing may occur, without correlating behavior differences.

Glees: I find this a very serious challenge.

Hoagland: It is.

Elkes: An interpretation will be attempted in the light of the data given below.

Beecher: May I ask why you use such large doses? Did you have to use them to get the effect?

Elkes: Presumably you refer to atropine.

Beecher: Yes. They are tremendous doses.

Elkes: With atropine the doses must be very large, considerably larger, in fact, than those used to obtain peripheral effects. I do not think that species difference alone would account for this large dose. The obverse, however, applies to physostigmine where quite small doses of the order of from 0.1 to 0.2 mg./kg. are effective.

Beecher: You do not get peripheral effects with smaller doses, in other words?

Kety: I should like to add, before we come to an explanation of this dissociation, that Dr. Wikler (57) has also noticed in man that with certain drugs there is a dissociation between the electrical activity of the EEG and the behavior of the individual. He has had sleep records

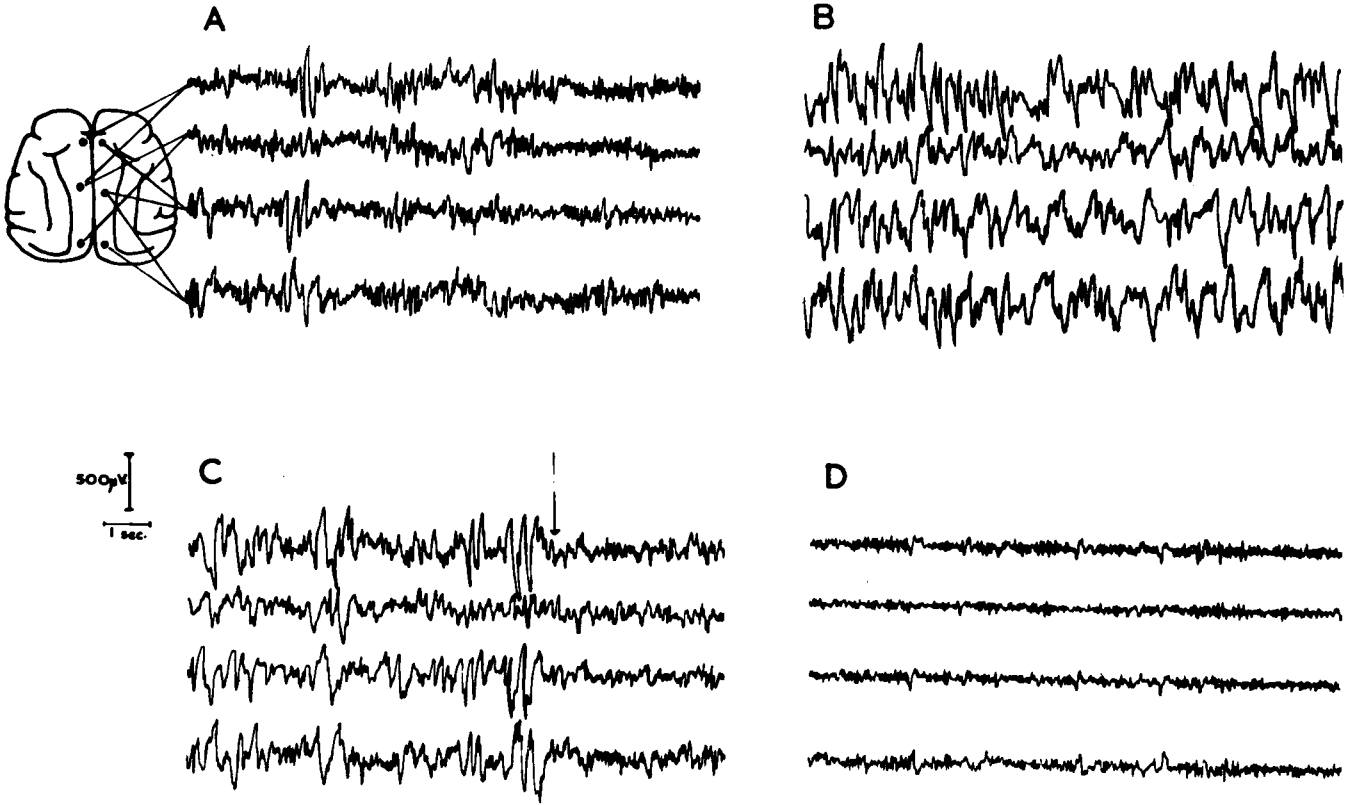

FIGURE 83. To show the interaction between physostigmine and atropine on the electrocorticogram in the conscious cat: (*A*) normal; (*B*) 20 min. after 2 mg./kg. atropine I.P.; (*C*) 10 min. after 0.5 mg./kg. physostigmine I.P.; and (*D*) 15 min. after 0.9 mg./kg. physostigmine I.P. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

when the individual was not asleep, and I am interested in what the explanation may be.

Hoagland: When persons hyperventilate and blow off CO_2 one may see marked slowing of the EEG without behavioral changes.

Olds: Are you going to tell us something about these electrode recordings?

Elkes: The changes from the deep areas from which we recorded (the caudate nucleus, the several medial thalamic nuclei, and the hypothalamic nuclei) were so similar to those obtained from the cortex that, in our later experiments, we abandoned subcortical leads. We did not, however, record from the amygdala, the hippocampus.

Olds: I missed that. Perhaps you would give us some answer to Dr. Magoun's suggestion, by telling us what you picked up from your deeper electrodes. I noticed that you used them.

Fremont-Smith: This disclosure does not surprise me. It seems exactly what should be expected because of the fact that the EEG is a summation rather than an idiosyncratic response of a particular cell or cell layer.

Elkes: Yes, at this point in the discussion I wish to stress that we have so far been considering only one group of drugs. In these there is a lack of correspondence between electrical activity and behavior. A further group of drugs, namely, the amphetamines and LSD-25, which will be considered presently, shows a striking correspondence between electrical activity and behavior. It would perhaps be well to defer the discussion until then.

Bain: May I ask Dr. Elkes, just for information, what is the evidence in normal cats or individuals that the response in EEG is necessarily associated with slow behavior?

Elkes: I do not recall using the term slow. A clear distinction should be made between the quiescent, drowsy, and sleepy animal which is readily alerted, and the retarded animal such as is seen following an injection of chlorpromazine. The gentle gliding from one functional state into another is characteristic of the normal animal; an adequate, congenial and constant environment is a prime condition for an adequate experiment.

Purpura: I do not know if this will clarify things but I am sure that everyone here would agree that in the unanesthetized, intact, undrugged, untraumatized animal the electrographic picture which is obtained in transition from sleep to wakefulness is characterized by the appearance of a low-voltage fast activity in place of a previously synchronous type of slower activity. This is what characterized the so-called "arousal" or activation pattern. If anyone has any objection

to this we ought to hear it right now. If this is established, then there is no need for further discussion.

Elkes: With regard to Dr. Purpura's question I am extremely glad Dr. Kety brought in Dr. Wikler's name. Dr. Wikler (58) was the first to have quite independently observed in dogs what Dr. Bradley and I observed in cats. He pointed out the dissociation between electrical activity and behavior using atropine in rather large doses.

Purpura: When we do something to the animal we do not have to use drugs, trauma, freezing or anything else that in some way disorganizes the basic records, and I think Dr. Brazier will confirm this that in some instances in cases of coma caused by hypoglycemia or anesthetic accidents as reported by Gastaut (59) the EEG may not be much different from that found in normal records and indeed may be indistinguishable from an alert pattern. I think many clinical encephalographers have seen such cases. In addition it was reported (60) that some patients with a pathologic condition of the cortex do not respond to alerting stimuli in the classical manner but may develop hypersynchronous slow activity. The dissociation between the electrographic picture and the behavioral picture resulting from trauma or drugs represents a disorganization of the normal physiologic mechanisms interacting to produce the "normal EEG." With so little knowledge at hand relating to the physiologic mechanisms I do not see how one can reasonably argue either about the site of action of various agents or the presence or lack of behavioral and EEG correlations.

Glees: Does this dissociation occur after the cat has been given atropine for a long time, or is it the first time atropine or any other drug has been given, and do you find this dissociation between behavior and EEG?

Elkes: The change is seen within 20 to 25 minutes of the administration of an intraperitoneal dose of atropine in the doses stated. It is one of the most consistent results we have obtained.

Brazier: This discussion is hampered a little by this kind of global comment about the EEG, and this occurs in nearly all discussions of this kind. Some, especially those not close to the field, say, "Does the EEG change or doesn't the EEG change?" This is, as I say, a global way of looking at the subject. What is this activity that you are recording as the EEG? The electricity that is activating your amplifiers is indubitably coming from cells in the cortex, but the characteristics of the electrical currents those cells release are under control of centers far deeper in the brain, whose own electrical activities are out of reach of surface electrodes.

I am really speaking out of turn with Dr. Magoun on my right, but

clearly, there could be differences in arousal according to whether the system in the brain stem was being primarily acted upon by the drug or whether the cortical neurons onto which it plays are the primary site of the drug action.

Elkes: Dr. Brazier, you have anticipated what I was about to say. I wonder whether you will support the conclusions which I wish to draw in the light of the evidence I am about to put forward.

Brodie: Perhaps an analogy can be drawn from biochemistry by saying it is probably as difficult to interpret changes in EEG in terms of what transpires in a particular part of the brain as it is to interpret the oxygen consumption of a tissue slice in terms of a particular biochemical reaction.

Elkes: I too regard this as a first crude approximation which, however, has one advantage, namely, that in the conscious animal an attempt can be made to relate simple variations in electrical activity to variations in the behavioral state.

We now come to our second and third group of drugs. The effects of amphetamine are shown in Figure 84. Amphetamine in doses of 2.3 mg./kg. intraperitoneally produces low-voltage fast activity in all regions. There is a very striking, definite and sustained correspondence between electrical activity and behavior. The animal is easily aroused, alert, and sometimes it may be excited. This is particularly so with high doses. *l*-Amphetamine appeared slightly less active than *d*-amphetamine in this regard.

The effect of LSD-25 in doses of 15 μg/kg. is shown in Figure 85. Though bearing some resemblance to the effects of amphetamine, this resemblance is superficial and misleading; for we found, as time went on, that the effects of LSD-25 on the corticogram were curiously dependent upon the environment in which the animal was placed. Thus, for example, in our early experiments the animal was observed in a soundproofed box placed in the open laboratory. In these experiments (in which all manner of trivial stimuli must have got into the box), low-voltage fast activity, much of the "alert" type of pattern, was seen practically every time the experiment was performed. In later experiments, the box was placed in a small soundproofed room specially built for the purpose. Here, the pattern was somewhat different, there being patches of "alert" activity, interspersed with slow rhythmic activity at from 5 to 7 cycles per second which supervened periodically, and which readily changed into the "alert" pattern in response to sensory stimuli. Although by no means clear at first, it thus became apparent that one was dealing with a rather important difference between the state induced by amphetamine and that induced by LSD-25.

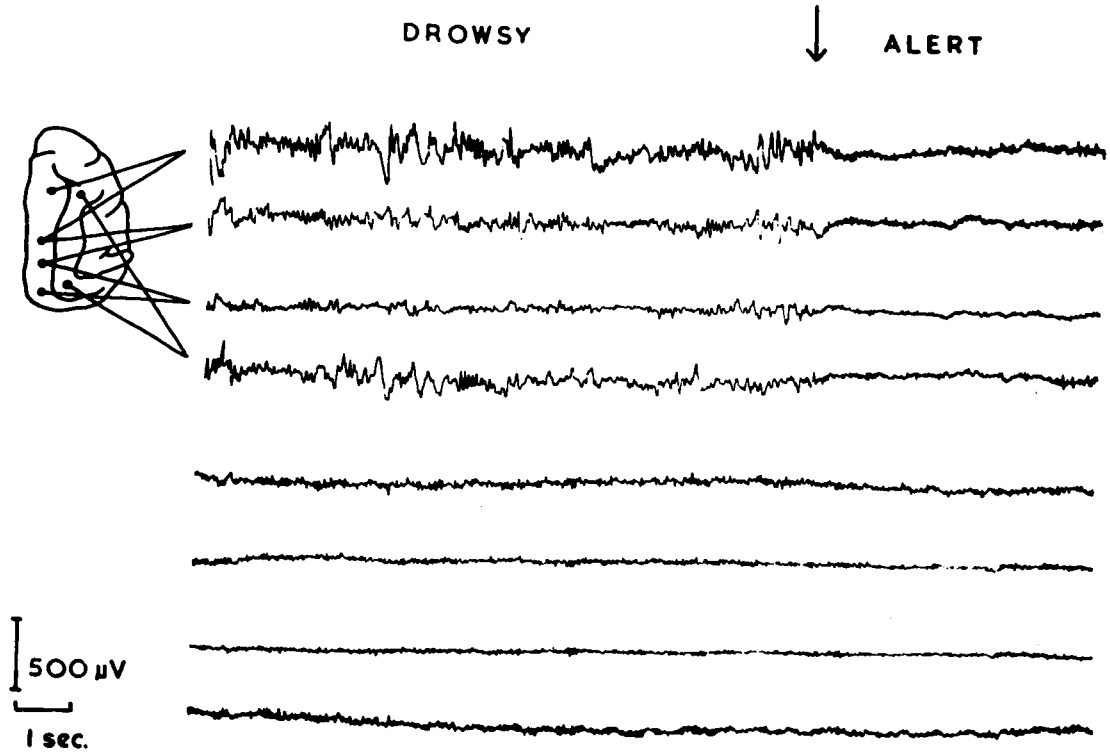

FIGURE 84. To show the effect of amphetamine on the electrocorticogram of the conscious intact cat preparation. (*A*) normal cat and (*B*) after *dl*-amphetamine, 2.3 mg./kg. I.P. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

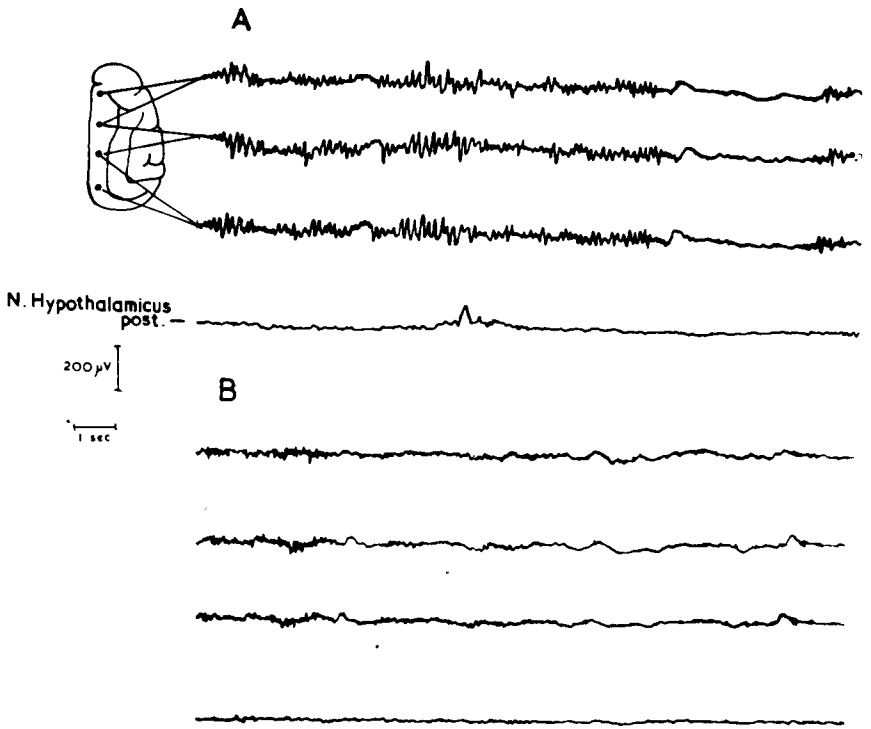

FIGURE 85. To show the effect of LSD-25 on the electrocorticogram of the conscious cat preparation: (*A*) normal, quiet and (*B*) 20 min. after 15 μg./kg. LSD-25 I.P. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

The "alert" state following amphetamine, both in terms of electrical activity and behavior, appeared to be much less dependent upon environment than that induced by LSD-25. We formed the impression, even in those early experiments, that LSD-25 sensitizes the animal to environment much more than amphetamine does.

Marrazzi: I might second that point, that there is no drug that we found that was more susceptible to the environmental influences than LSD-25.

Elkes: There are several findings in man which suggest much the same thing.

Brodie: Could you explain in more detail how much LSD-25 was given? How long after it was given did you make these measurements?

Elkes: We gave approximately from 10 to 15 μg. of LSD-25 per kg. intraperitoneally. The effects usually appear within 20 to 30 minutes.

Unna: What was the behavior of the cat at that time?

Elkes: We thought that it was unusually susceptible to environment.

Magoun: Is that a frequency analysis record of the hypothalamus? Will you tell us about it?

Elkes: We used frequency analysis in our earlier experiments. The yield, however, was scanty and we abandoned it after a time.

You will recall that in addition to the conscious animal the effects of drugs were tested in the barbitone anesthetized animal and in the *encéphale* and *cerveau isolé.* Figure 86 illustrates an example of the interaction between parasympathomimetic drug (physostigmine) and pentobarbital. A small dose of physostigmine (0.2 mg./kg.), when administered to an animal anesthetized with 30 mg./kg. pentobarbital, will change the characteristic barbiturate "spindling" to a very striking rhythmic activity which is seen in all leads. Atropine in doses of 1 mg./kg. will abolish these effects and bring back the characteristic barbiturate activity.

The activity and characteristics of the *encéphale* and *cerveau isolé* have been described by Bremer (51). As is well known, these preparations alternate behaviorally and in terms of electrical activity between the so-called sleeping and waking states. In the waking state (in which movements of the eyes and ears and vibrissae are seen, and the pupillary response to light is readily elicited), the electrical activity consists mainly of low-amplitude fast activity in all regions. This closely resembles the type of activity seen in the conscious animal in the "alert" state. In the sleeping *encéphale*, the eyes are rotated upward and the pupils are constricted and fixed. The preponderant cortical rhythms in this state are characterized by bursts of "spindle" activity, somewhat resembling the type of activity seen in the drowsy intact animal. The frequency range of the spindle activity is of the order of from 10 to 15 cycles per second. The *encéphale isolé* preparation can readily be roused by auditory or tactile stimuli. In the few acute experiments performed on monkeys, visual stimuli, too, were found to be effective.

Brazier: Did you have any difficulty in avoiding monkeys going into shock?

Elkes: Yes. Because of the expense we could use only six monkeys. The blood pressure drop is inclined to be severe. Is that what you mean, Dr. Brazier?

Brazier: Yes.

Elkes: In the *encéphale isolé* (Figure 87) of the cat, atropine and physostigmine still exerted their effects, and the changes produced by atropine (2 mg./kg.) were still reversed by physostigmine (0.4 mg./kg.). These effects were not necessarily related to the transient

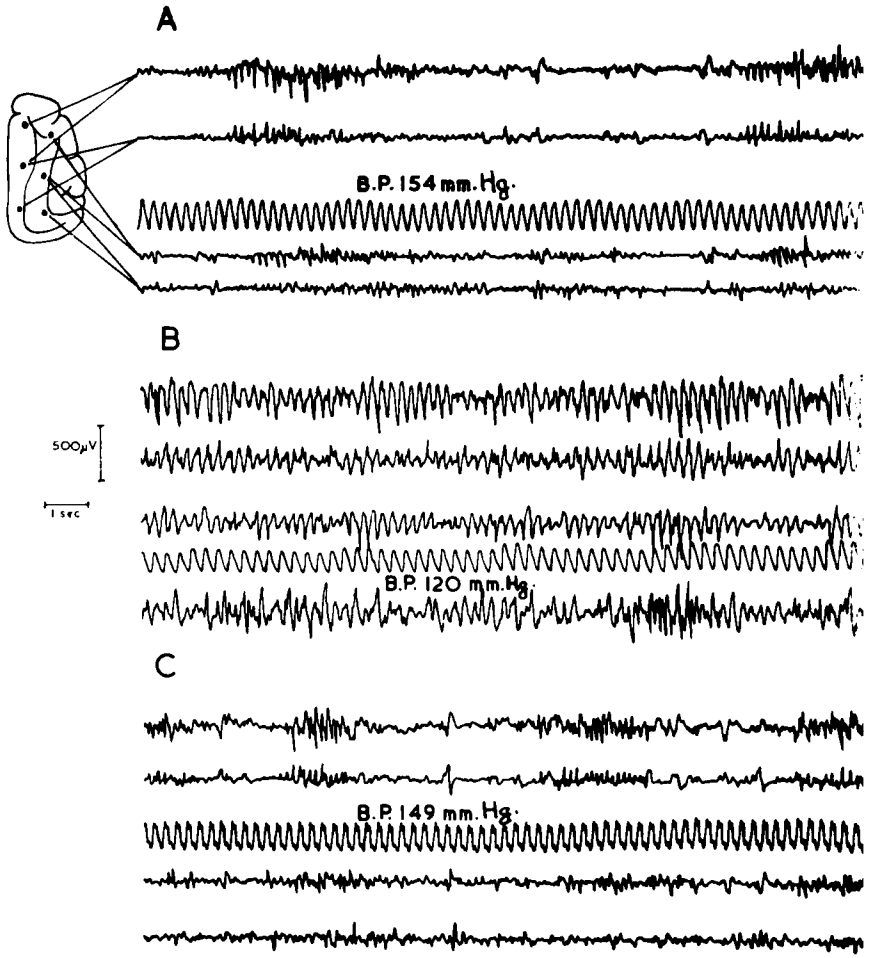

FIGURE 86. To show the effect of physostigmine on the electrical activity induced by pentobarbital. Rhythmic activity appears but is abolished by a subsequent injection of atropine: (*A*) pentobarbitone anesthesia 30 mg./kg.; (*B*) 3 min. after 0.2 mg./kg. physostigmine I.V.; and (*C*) 2 min. after 1.0 mg./kg. atropine I.V. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effect of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

effects on blood pressure produced by these drugs. For if norepinephrine (2 μg.) is infused to raise the low blood pressure observed in this particular experiment, the characteristic atropine activity is still obtained (Figure 87). It is, however, most important to distinguish sharply between a lack of correlation of the drug effects observed and changes in the systemic blood pressure on the one hand and, on the

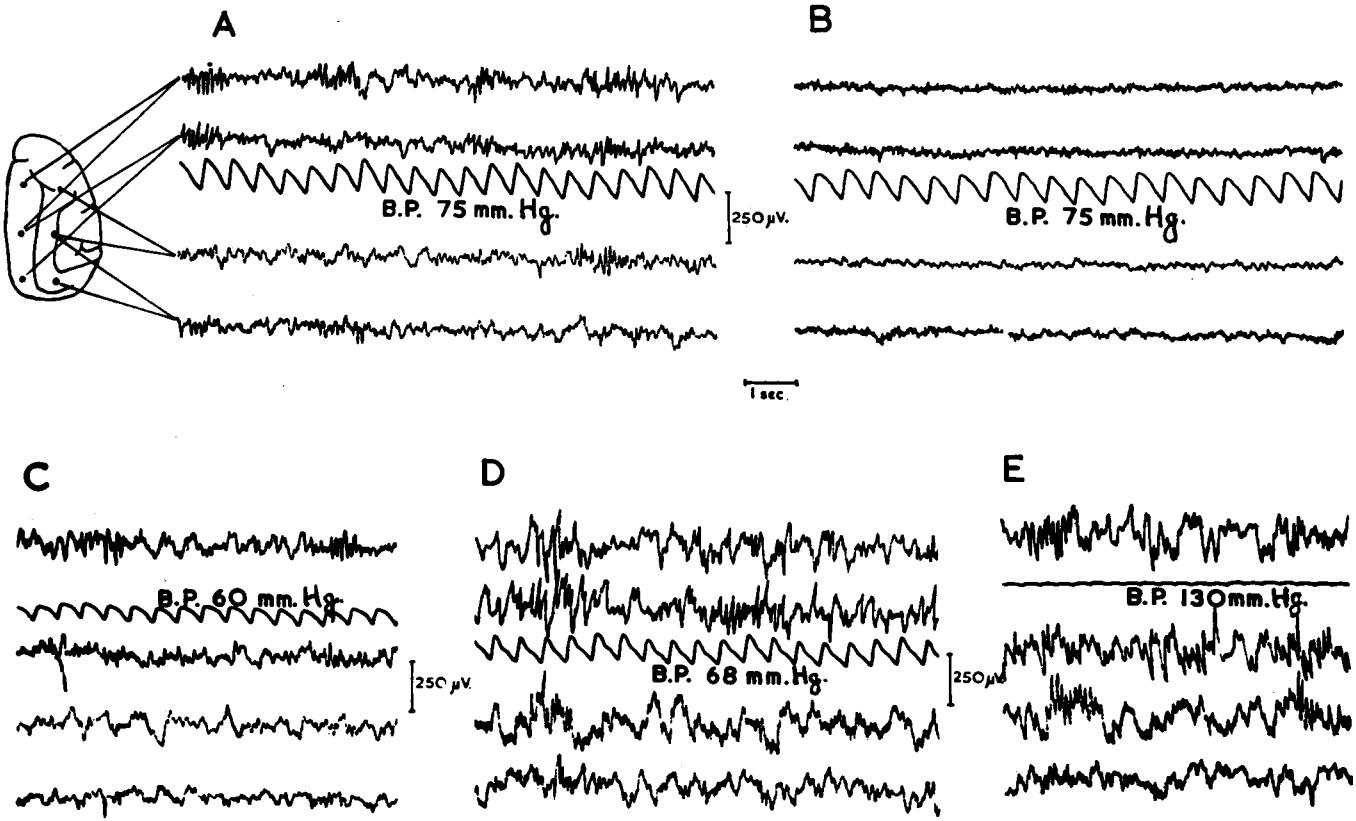

FIGURE 87. To show the effects of atropine and physostigmine on the electrocortical activity of the *encéphale isolé* preparation. Persistent atropine activity is evident despite an infusion of 2 μg. of norepinephrine (Section E): (*A*) normal-sleeping; (*B*) 40 sec. after 0.1 mg./kg. physostigmine I.V.; (*C*) 5 min. after 2.5 mg./kg. atropine I.V.; (*D*) 6 min. after 4.0 mg./kg. atropine I.V.; and (*E*) 60 sec. after 2 μg. norepinephrine I.V. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

other hand, a possible effect on *local* cerebral vascular tone and *local* cerebral circulation brought about by these agents. The latter, of course, has not been studied, and therefore can by no means be excluded.

Beecher: That is mean blood pressure, isn't it?

Elkes: Yes, these are mean excursions recorded by an electronic manometer.

Beecher: I mean that the systolic pressure might be normal and still not be doing anything.

Elkes: Yes, it is quite low.

Beecher: Not necessarily.

Unna: The blood pressure is quite low.

Bain: What is the magnitude of the excursion?

Beecher: What is the swing in the systolic and diastolic? Isn't this mean pressure you have shown? It might be almost anything.

Elkes: The effects of LSD-25 and of amphetamine were studied in the *encéphale* preparation. In thirteen experiments in this preparation (11 cats and 2 monkeys), *dl*-amphetamine was injected intravenously in doses of from 1 to 3 mg./kg. In all cases, there was a striking and consistent abolition of the slow activity, which did not reappear once the injection had been given. There were also behavioral signs of arousal, such as an opening of the eyes and an occasional moving of the ears, jaws, and vibrissae. In six cats and both monkey preparations, blood pressure was recorded at the same time as the electrical activity. The expected rise of blood pressure (approximately 20 mm./mg.) was seen following the injection of amphetamine and maintained throughout the experiment. In two experiments, therefore, a smaller dose of amphetamine (from 0.2 to 0.5 mg./kg.) was injected first. These doses produced a blood pressure rise and temporarily blocked the "spindle" activity. After about 30 seconds, however, this activity returned, despite the maintenance of a higher level of blood pressure. A second larger dose of amphetamine (from 1 to 2 mg./kg.) was therefore administered. The "spindling" was then permanently abolished, and no further rise in blood pressure was observed. Thus, once again, the "alerting" effects of amphetamine did not appear to be related to the general circulatory effects, though the reservations mentioned above when discussing physostigmine and atropine must be very clearly borne in mind.

The effects of LSD-25 on the *encéphale isolé* are shown in Figure 88. A high dose (35 μg./kg.) of LSD-25 did not exert any effect on either the electrical activity or the behavior of this preparation. Even when much higher doses were used, no effects were seen. In the monkey preparation, a somewhat different condition was obtained. Here the

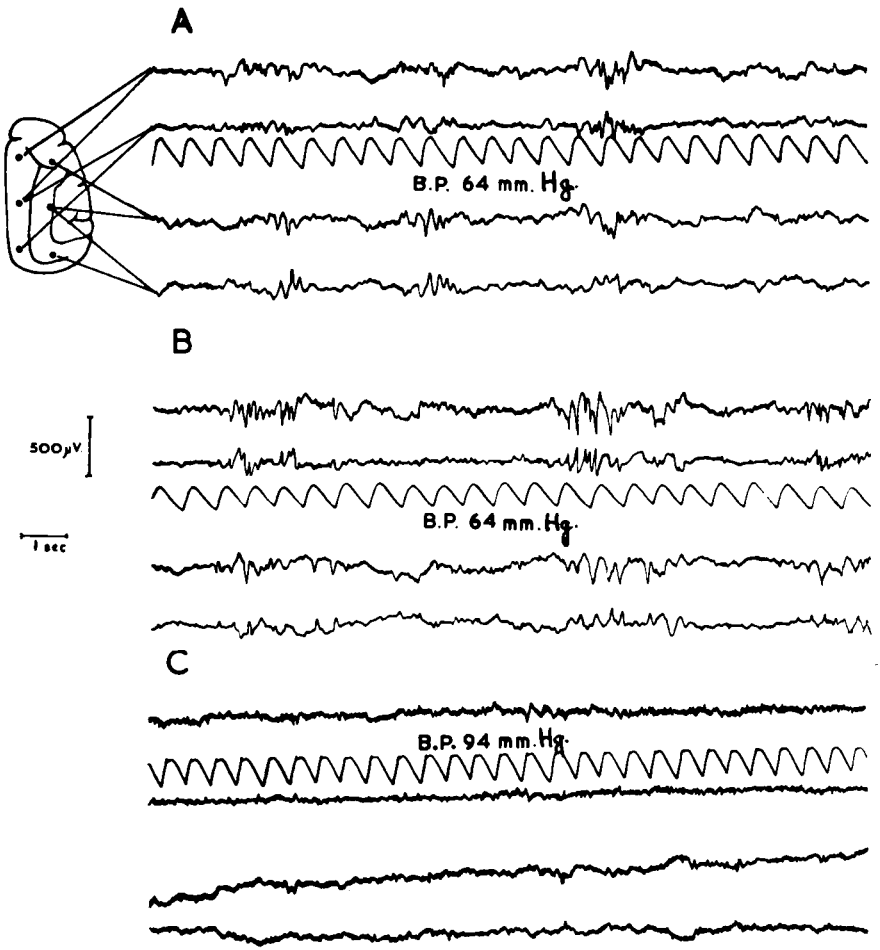

FIGURE 88. To show the lack of effect of LSD-25 in the *encéphale isolé* preparation (cat). The effect of *dl*-amphetamine is evident on this preparation: (*A*) normal sleeping; (*B*) 3 min. after 35 μg./kg. LSD-25 I.V.; and (*C*) 2 min. after 1 mg./kg. *dl*-amphetamine I.V. Reprinted, by permission, from Bradley, P. B., and Elkes, J.: The effects of some drugs on the electrical activity of the brain. *Brain* **80**, 77 *(1957)*.

characteristic intermittent rhythmic activity was replaced by bursts of activity at from 7 to 8 cycles per sec. waxing and waning in amplitude. In both the cat and monkey preparations, however, amphetamine, despite previous administration of LSD-25, still exerted its full alerting effects, both in terms of electrical activity and of behavior. Thus, once again the distinction between amphetamine and LSD-25 is encountered.

The effects of physostigmine and atropine on the *cerveau isolé* preparation are shown in Figure 89. It will be noted that the characteristic effects of these two drugs (in doses of 0.1 mg./kg. and 1 mg./kg., respectively) can be observed quite clearly. When, however, the effects of amphetamine and LSD-25 on the *cerveau* preparation are examined, a striking change in the susceptibility to amphetamine is noted, for despite heavy doses, ranging in a few instances to as high as 15 mg./kg.,

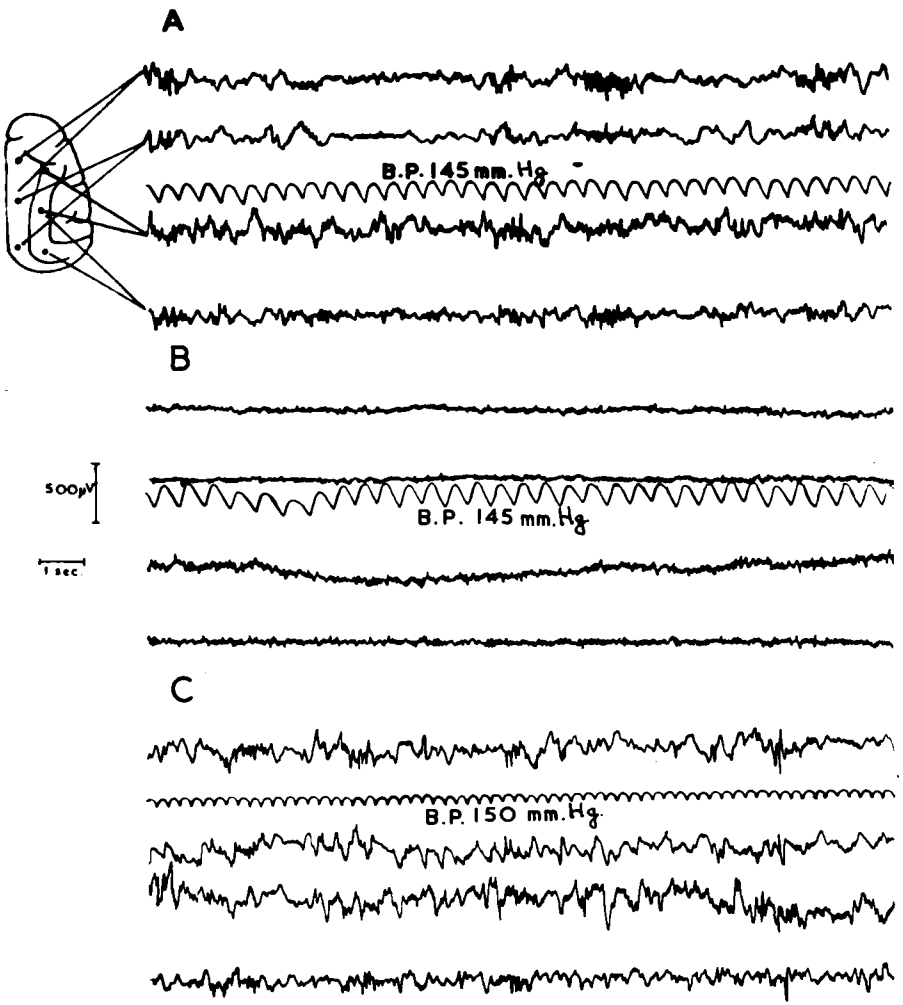

FIGURE 89. To show the effect of physostigmine and atropine on the electrocortical activity of the *cerveau isolé* preparation: (*A*) normal; (*B*) 3 min. after 0.1 mg./kg. physostigmine I.V.; and (*C*) 5 min. after 1 mg./kg. atropine I.V.

no behavioral or electrical arousal could be seen. Similarly, high graded doses of LSD-25 (up to 300 μg/kg.) produced no effects on this preparation.

Evarts: That is, a dose up around 100 γ/kg. does not affect the *cerveau isolé?*

Elkes: I presume you mean LSD-25? Yes, high doses were ineffective on our preparations.

Glees: Could this mean that LSD-25 acts on receptor sites which are cut off?

Elkes: Yes, though not quite in the sense which I believe you are implying. May I take up your comment in a later part of the discussion?

The importance of the locus of injection of LSD-25 in relation to its effects on electrical activity and on behavior was also recently shown by Bradley and Hance (61). In these experiments LSD-25 injected intraventricularly in doses of from 100 to 200 μg. produced high amplitude rhythmic activity at about 4 to 7 cycles per second. This activity was only transitorily blocked by sensory stimuli. Intraventricularly injected 5-hydroxytryptamine (from 200 to 250 μg.) on the other hand, led to an increase in slow rhythms which still responded to sensory stimuli. These effects of serotonin were not antagonized by an intraperitoneal injection of LSD-25. However, a combination of these two drugs given intraperitoneally led, irrespective of the order in which the drugs were administered, to the type of electrical activity seen following intraventricular injection of LSD-25 alone, i.e., high amplitude rhythmic activity at from 4 to 7 cycles per second. Brom-lysergic acid diethylamide, a powerful inhibitor of serotonin (but lacking its mental effects), produced mild sedation when given intraventricularly or intraperitoneally. None of the rhythmic activity seen with LSD-25 was observed with brom-LSD. The effects of LSD-25 on the electrical activity thus appear to be peculiar to this drug and may conceivably be related to its mental effects. In any event such evidence as is in our possession suggested a synergism rather than an antagonism between the central effects of LSD-25 and serotonin.

Brodie: Could your statement be modified to say that brom-LSD is a powerful antagonist of serotonin in smooth muscle preparations but lacks some of the mental effects of LSD-25 on the brain?

Elkes: I agree. *In vitro* evidence obtained in suitable serotonin-sensitive preparations can be applied only with the greatest caution to the antagonism of *locally* liberated serotonin *in vivo.* The reservations mentioned earlier in regard to acetylcholine apply with equal force here.

It would perhaps be appropriate at this point to review the data so

far. The drugs studied fall into two broad groups: First, those like physostigmine, atropine, and *l*-hyoscyamine, in which there appears to be little correspondence between the effect on electrical activity and on behavior; and second, those like the amphetamines, LSD-25 and, with some reservations, 5-hydroxytryptamine, where there is a much closer correlation between electrical activity and behavior. Furthermore, when interaction between these two groups of drugs was studied in the conscious animal, it was found that whereas the electrical activity was dominated by members of the first group, the behavior, in as far as this could be judged by gross observation, was always dominated by members of the second group irrespective of the order in which the drugs were given. This further emphasized the distinction between these two groups of agents.

One further aspect is relevant in the context, namely, the susceptibility of the drug effects observed to the plane of section in the acute preparations we have studied, i.e., the *encéphale* and *cerveau isolé*. Whereas in the cat the effects of LSD-25 on the electrocorticogram were abolished by high spinal and mesencephalic section, the effects of amphetamine, though still observed in the *encéphale isolé* could no longer be elicited in the *cerveau* preparation.

The concept of the reticular activating system was formulated by Dr. Moruzzi and Dr. Magoun (62), and in Figure 90 I have superimposed the planes of section of the *encéphale* and *cerveau isolé* preparations upon the suggested distribution of the reticular activating system and indicated also the effect of these sections upon the observed effects of the drugs mentioned.

Some 4 years ago, when we first came up against these findings (64) we wondered whether amphetamine might not act on receptors related to the upper part of the reticular activating system, and whether in fact, the action of this drug might not be brought about by interference with a naturally occurring catechol amine present in this area, and, in some way be related to the physiology of wakefulness and sleep. The old German term for amphetamine, namely *Weckamin* (i.e., waking amine), would certainly seem appropriate to a drug acting on receptors in the arousal system. The chemical relationship of amphetamine and methylamphetamine to tyramine and other catechol amines is well known. Furthermore, we have already mentioned earlier the presence of catechol amines in the central nervous system as demonstrated by Dr. Vogt (29). The distribution of norepinephrine as determined by her in the brain of the dog is not out of keeping with the possible presence of this amine in some elements of the arousal system.

At that stage we were thinking in terms of two broad groups of

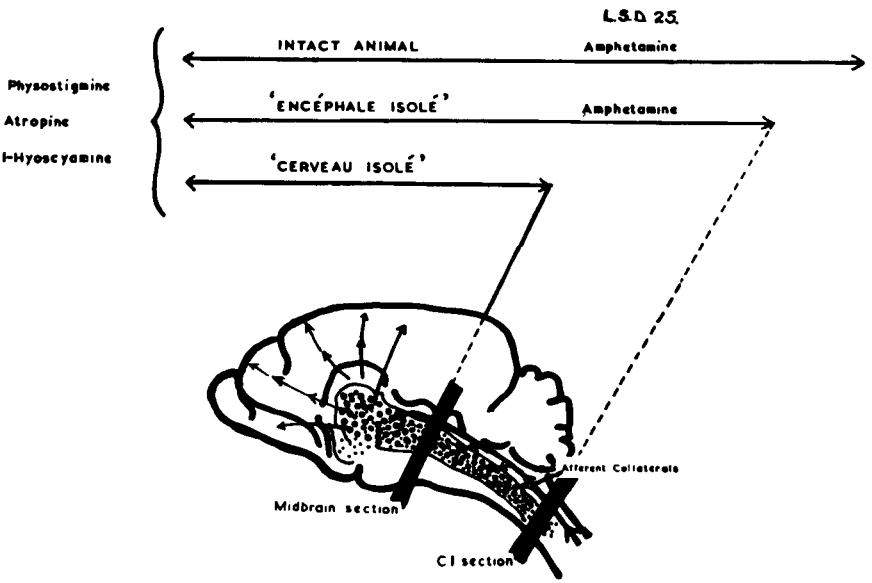

FIGURE 90. Summary diagram to indicate the effects of the level of section in the acute preparation upon the drug effects observed (63).

receptors: one for acetylcholine or a related ester and another for a catechol amine. The first appeared to have a diffuse distribution throughout the brain and, though possibly represented in the reticular activating system, did not predominantly determine its activity. The second was built into this system much more intimately and its effect on behavior was presumably brought about by a dense representation in the system. As time went on, however, and the experiments with LSD-25 accumulated, we were impressed by the possible existence of yet a third type of receptor. This receptor might be the biochemical target of LSD-25, and in turn it might reflect a central mechanism depending upon the natural turnover of an indole derivative with which LSD-25 specifically interfered. We have already noted the peculiar susceptibility both in terms of behavior and of electrical activity of the effects of LSD-25 to environment. Equally, in the cat the effects of LSD-25 were abolished by high spinal section with its massive reduction of afferent tone. Moreover, in man (65) the subjective effects of LSD-25 were found to be enhanced by one form of afferent, namely, rhythmic photic, stimulation. We wondered therefore whether LSD-25 might not act on a receptor particularly and peculiarly related to the afferent pathways, and whether, in fact, it interfered with the turnover of a naturally occurring substance (possibly an indole derivative) the

liberation of which played a dominant part in the organization of sensory experience. Such a receptor might be related to the medial (non-sense linked) collaterals of the great afferent pathways and also be built into wider thalamocortical and even cortical nets. Its essential feature, or so it appeared from the evidence on hand, was its relation to the afferent system, and although we wondered whether its action might be inhibitory (i.e., suppressing nonessential information in the organization of temporal and spatial experience), the evidence was not nearly as cogent as that of Dr. Purpura, who no doubt will be telling us of his experiments.

In a sense the distinction between the two groups of drugs is borne out by observations on chlorpromazine (66). Chlorpromazine administered intravenously in graded doses of up to 4 mg./kg. resulted in the appearance of slow waves at from 1 to 2 cycles per second in all regions and bursts of rhythmic activity at from 5 to 8 cycles per second. In this state the animals showed motor retardation and appeared indifferent to sensory stimuli; nor could an electrical arousal response be elicited. Chlorpromazine given either before or after the administration of *dl*-amphetamine or LSD-25 in full doses blocked the effects characteristic for these drugs. On the other hand, even a high dose of chlorpromazine did not prevent a small dose (0.2 mg./kg.) of physostigmine from exerting its characteristic effect on the corticogram (low-voltage fast activity). Chlorpromazine was also found to potentiate the slow activity produced by atropine. On the other hand, the behavioral effects of chlorpromazine were not susceptible to either physostigmine or atropine.

Fremont-Smith: What happens to the behavior of the animal when you give physostigmine?

Elkes: No gross change in the chlorpromazine behavior could be detected. Once again, therefore, one encounters a dissociation between electrical activity and behavior.

Thus, it would appear that those drugs (physostigmine, atropine, and *l*-hyoscyamine) in which there is a dissociation between electrical activity and behavior and the action of which appears independent of mesencephalic and spinal connections exert their effect on electrical activity (though not on behavior) in the presence of high doses of chlorpromazine; whereas those drugs (amphetamine and LSD-25) whose effect on electrical activity is related to the effects on behavior, and whose action appears to be dependent on mesencephalic and spinal connections are susceptible to chlorpromazine. This again is in keeping with the supposition that chlorpromazine acts on receptors related to the brain stem reticular activating system (67,68).

Evarts: Dr. Magoun, would you describe the work that has been done by Dr. Killam on the effects of chlorpromazine?

Magoun: The following is a brief summary of the Killams's (69) findings with chlorpromazine. They presented this information at the New York Academy of Sciences Conference on Psychotomimetic and Psychotherapeutic Drugs and have kindly allowed me to include it here:

In cats with chronically implanted electrodes, chlorpromazine in doses of 1 to 5 mg./kg. markedly elevated the threshold at which EEG desynchronization could be obtained by high-frequency stimulation of the diffuse thalamic projection system. The threshold for behavioral arousal from sleep was even more strikingly elevated indicating a separation of EEG and behavioral response under the influence of the compound. In the same experiments, the same doses of chlorpromazine only slightly elevated the thresholds for EEG and behavioral arousal following stimulation of the midbrain reticular formation, and there was no dissociation of electrical and behavioral responses. The effect on thalamocortical arousal circuits in these chronic preparations was in marked contrast to the relatively small change in the threshold for EEG activation following peripheral nerve, reticular formation, or thalamic stimulation in acute curarized cats (70). Electroencephalographic patterns following these doses of chlorpromazine in noncurarized, nonanesthetized cats allowed to set their own sleep-wakefulness cycles were not different from those in untreated cats under similar conditions, although behavioral depression and some ataxia did appear following drug administration.

Glees: Have you had any experience with reserpine in a similar study?

Elkes: Our evidence on reserpine is limited since, in our chronic preparations, the long-lasting effects of this drug made its continued use difficult. A total of fourteen experiments were performed (71) and the dose used was approximately from 100 to 250 μg./kg., although in some experiments a cumulative dose of up to 2 mg./kg. was employed. Perhaps the most noteworthy observation was the change in the corticogram observed in two experiments in which LSD-25 (25 μg./kg.) was given some 3 hours after a dose of 150 mg./kg. of intraperitoneal reserpine. Within 10 minutes of the injection of LSD-25 high-voltage rhythmic activity at from 5 to 7 cycles per sec. appeared in the record, and there was only transient abolition of this rhythmic activity by sensory stimuli. The rhythmic activity was, in fact, not unlike that seen with the injection of LSD-25 combined with 5-hydroxytryptamine (61).

Evarts: It might be interesting to mention the work of Dr. Killam (69), though, in terms of the effects of chlorpromazine and reserpine on threshold of EEG arousal by peripheral nerve stimulation or by stimulation of the reticular formation itself. In the preparation she used, she did not observe any marked effects on threshold following administration of these drugs. She did not observe effects on the threshold for production of alerting responses in the EEG, which is interesting in terms of the notion that these drugs act on the reticular formation; here is some evidence which does not seem to support that notion.

Elkes: There is some evidence from our laboratory which does not quite support that notion either. Dr. Bradley and Mr. Key (54) have studied the thresholds for arousal by electrical stimulation of the reticular formation both in terms of electrical activity and of behavior, and they have also studied the effects of intravenously injected drugs on these thresholds. In agreement with Arduini and Arduini (72), they found that pentobarbitone caused a marked rise of both thresholds. Chlorpromazine, however, even in very large doses (25 mg./kg.), produced only a slight rise in both thresholds. *dl*-Amphetamine, even in quite small doses, caused a progressive fall in both EEG and behavior thresholds, until with larger doses, the preparation remained permanently awake. LSD-25, on the other hand, caused no change in the thresholds to direct stimulation of the reticular formation in doses of up to 60 μg./kg., but even with as little as from 1 to 2 μg./kg. there was a marked fall in the threshold for arousal produced by afferent (auditory) stimulation. Once again, therefore, one comes up against a distinction between arousal produced by direct stimulation of elements in the reticular formation and arousal produced by afferent stimulation. The first is susceptible to amphetamine and barbiturates, the second to LSD-25 and chlorpromazine. I believe this is in accordance with what Dr. Evarts has in mind.

Evarts: Yes.

Glees: Has anyone studied the effects of these drugs on muscle or skin receptor discharge? I am thinking of the tremor resulting from small doses of reserpine, as Dr. Windle has shown. Could this tremor, in part at least, be caused by the action of reserpine on muscle spindle discharge?

Magoun: The possibility is likely that this effect is a result of action on the brain. The Killams find that chlorpromazine has a depressing action on evoked seizure activity in the rhinencephalic system while reserpine had a marked facilitating action on the seizure discharge. LSD-25 had some effects in this latter direction. This work is in contrast

to their failure to detect marked impairment of lower brain stem function, suggesting the possibility that these drugs, if they have any specific site of action, have it in the basal forebrain, by contrast with the lower brain stem. Perhaps, Dr. Olds can make some remarks about behavioral actions of these substances.

Olds: Yes, I will discuss that presently with illustrations. But I think I can bear out Dr. Magoun with a different study, which seems to indicate action of these drugs in the basomedial hypothalamus, just anterior to the mammillary bodies.

Marrazzi: We can also show slides, demonstrating action on the cortex.

Glees: I feel that we overemphasize central action. I am thinking of Apter's paper (73) on the effect on the retinal receptor itself. Unless we are quite certain that the actions which you, Dr. Magoun, think are located within the central housing, which have already been peripheral receptors, we might draw some wrong conclusions.

Hoagland: The retina is part of the central nervous system. As Dr. Brazier pointed out, it could be a matter of a feedback from the brain to the retina. There is nothing that would indicate that it is specific for a given receptor. Dr. Brazier, do you want to comment further on this?

Brazier: We should not forget that there are centrifugal fibers to the retina, so that if "spontaneous activity" in the retina is being recorded, it is indubitably there; but as shown by Granit (74), with microelectrodes, this may be activity initiated by a centrally located nerve cell.

Brodie: We should not overlook the evidence which might be discussed later, that LSD and amphetamine have many effects in common, but act on different receptor sites. The same may be said for chlorpromazine and reserpine.

Elkes: Dr. Brodie will remember that I carefully distinguished between two and possibly three types of receptors.

Unna: Your tracing of chlorpromazine, the effects which you had with chlorpromazine on the receptor, look like those which were shown by Rinaldi and Himwich (75) and also by Longo, von Berger, and Bovet (67). In relation to Dr. Magoun's remarks about the work of Dr. Killam (76), Dr. Killam failed, in most of her preparations, to get a marked slowing of the waves in the electroencephalogram. We have taken that up, and we have records which are very much like the ones that you presented. Also, we found that on sensory stimulation of the end of the peripheral nerve, when these impulses were monitored in the neighborhood of the red nucleus, we obtained potentials which were distinguishable, under chlorpromazine, from those seen with the barbiturates, in that the first potential always came through either

unaltered or enhanced, but the sequence of evoked responses was diminished in dependence to the frequency of the stimulations. We believe we can demonstrate clearly that there was this effect of chlorpromazine.

Elkes: I now wish to mention some studies of Dr. Ginzel's (77) in our laboratory on the central effects of LSD-25 on vasomotor responses. These were studied in cats under chloralose anesthesia. LSD-25 was injected into the lateral ventricle of the brain using a Feldberg-Sherwood cannula. The carotid pressor responses were elicited by a number of maneuvers. These included the temporary occlusion of both common carotid arteries, temporary asphyxia, close arterial injection of a nicotine-like substance, sebacinyl-bis-choline (78) into the carotid sinus region, electrical stimulation of one carotid sinus nerve, and the electrical stimulation of the central stump of the cut sciatic nerve. In a number of experiments differences in the size of the carotid occlusion reflex were also studied while the animal was allowed to breathe air and pure oxygen alternately, so as to determine the participation of chemoreceptor stimulation in this reflex (79,80). It was found that doses of from 100 to 200 μg. of LSD-25 (dissolved in 0.2 ml. of saline) when injected intraventricularly reduced or abolished the pressor responses within 1 to 3 minutes. Complete recovery was usually obtained within 30 to 60 minutes, and the effects of LSD-25 could then be reproduced in the same experiment. The reduction by LSD-25 of the pressor response produced by stimulation of the carotid chemoreceptors was more pronounced and long lasting than the reduction of the carotid occlusion reflex. The difference between the carotid occlusion responses obtained when the animal was allowed to breathe air and oxygen alternately were also reduced or abolished by LSD-25. Similarly, intraventricular LSD-25 reversed the pressor response, normally produced by stimulation of the carotid sinus nerve, into a depressor response. These findings tend to suggest that, at a high central level, LSD-25 probably discriminates between the afferent impulses arising from the baroreceptors and the chemoreceptors, respectively, and that the chemoreceptor pathway may be more susceptible to LSD-25 than that originating in the baroreceptors.

Brodie: Are there the same effects if the LSD is given parenterally?

Elkes: With somewhat higher doses. Also in such experiments the systemic effects of LSD-25 on blood pressure have to be taken into account.

Brodie: I often wonder how to interpret the over-all action of a drug following its intraventricular injection, unless the resulting distribution of the drug in the brain is determined.

Elkes: I think it is fair to assume that the areas involved in the

first instance lie around the third and fourth ventricle. In an isolated experiment Dr. Ginzel succeeded in putting a fine catheter into the region of the fourth ventricle. In this experiment a lower dose was sufficient to produce the effect.

Brodie: The reason I am bringing up this point about distribution is that generally we do not know what happens to a drug after intraventricular injection. It may easily penetrate the wall of the ventricles and be distributed extensively in the brain. On the other hand, it may pass into the brain only to a limited degree, most of it spreading throughout the subarachnoid space and disappearing via the blood stream. Without knowing the distribution of the drug in the nervous tissue, after intraventricular injection, it may be difficult to draw definitive conclusions as to its general action on the brain.

Fremont-Smith: A preparation that Putnam (81) showed may bear on the mechanism of the action of drugs given intraventricularly. He had a cat with the cisterna magna exposed. When under the microscope it was observed to have many blood vessels in the meninges, arterioles, and venules. He was giving fluorescein intravenously. What was observed immediately was that there was a collar of fluorescein around not only the veins but also the arterioles, in the subarachnoid space. From this, if a molecule as large as fluorescein can immediately diffuse out through relatively good-sized arterioles in the meninges, it should be possible for molecules of a similar size to diffuse into those arterioles from the subarachnoid space or from the ventricular fluid. This suggests that the mechanism of operation, which is so extremely rapid, of intraventricularly injected drugs is through diffusion into arterioles and direct transmission to capillary beds immediately adjacent. I can think only of some such mechanism of this kind to explain the smallness of dose which is effective so rapidly.

Bain: The data on fluorescent dyes which Mayer* has accumulated in Dr. Brodie's laboratory do not support such a supposition. Further, there is the common experience that if a tracer substance like P^{32} is administered, intravenously or intraperitoneally, it takes a considerable period of time for enough of that P^{32} to get into the central nervous system to yield an appreciable count. On the other hand, if P^{32} is put in intracisternally, the count in ATP, for example, is maximal in 7 minutes.

Fremont-Smith: Does that prove the dose doesn't get there by the blood stream?

Bain: I think so, because if it is put into the blood stream, it doesn't get there. If it is put in intracisternally, it does.

*Personal communication.

Fremont-Smith: But, if it is put in intracisternally, there is a high concentration in the immediately adjacent blood stream, a very high concentration, relatively speaking, to the concentration there would be if it was given intravenously.

Brodie: I would think that a lot depended on the physical properties of the drug. When it is assumed that the ventricular wall behaves toward the drug like a lipoid membrane, the lipoid solubility of the drug at physiologic pH would be a major factor in the ease of its penetration into the brain.

Bain: It is not possible to generalize from one drug like fluorescein to all drugs, or from one substance like inorganic phosphate to all drugs, and so on. Each one must be studied individually in order to determine where it goes.

Fremont-Smith: The suggestion was not to generalize, but merely that the diffusion of drugs into arterioles is a mechanism which, apparently, has not received too much attention. Obviously, this will vary with the drug and circumstances. It is something that should be looked at, because it certainly is open to experiment. Dyes and other experiments can show this diffusion.

Masserman: I have a footnote to Dr. Fremont-Smith's comment, which is simply this: that we forget the peri-arteriolar cerebrospinal fluid spaces. What you saw, possibly, in the collar around the arterioles was actually the drug in these Virchow-Robin structures.

Fremont-Smith: But we know that fluorescein gets into the spinal fluid, and the main point here is that it was crossing the wall of the artery.

Masserman: In one direction. Normally, it comes out of the arterioles into the Virchow-Robin space, and then enters the cellular metabolism of the brain.

Fremont-Smith: But these blood vessels are lying free in the subarachnoid space.

Masserman: But they still have a collar around them.

Fremont-Smith: That is true.

Brodie: I would like to bring up once again the possible importance of the penetration of a drug into the brain after intraventricular administration. Let us assume that a drug injected intraventricularly localizes appreciably in only a small part of the brain. If stimulatory effects are seen, can definite conclusions be drawn as to what would happen if a drug were distributed throughout the whole of the brain? The brain is normally in a complicated balance of excitatory and inhibitory processes. By being present in only one part of the brain, the drug might

block certain processes but in doing so unmask processes in those parts of the brain where the drug is absent. Thus, a false impression of the real action of the drug could be obtained.

Hoagland: We have studied the uptake by brain of K^{42} from the circulation. It is extremely slow compared to that of other organs.

We have studied the accumulation of C^{14} labelled steroids in the cerebrospinal fluid of dogs. The passage of steroids through the blood-brain barrier from the circulation is slow. It therefore seems to me that Dr. Bain's point would be relevant here; substances injected intracisternally really act upon the brain more effectively than via the circulation but perhaps not in the same manner.

Fremont-Smith: Putting a micropipette into these arterioles would be experimentally feasible. I am only suggesting these processes should be looked at, because molecules go one way and they may go the other. It opens the door to an experiment that should be done.

Purpura: What I think Dr. Brodie is commenting on is that he feels one should not have prejudices about putting drugs in special parts of the brain. But I doubt whether the fact that a drug exerts a certain effect when injected in one way and entirely opposite effects when administered another way indicates that both effects do not involve the same mechanism of action. For example, *d*-tubocurarine when introduced intraventricularly in conscious cats produces marked motor effects and convulsions (82). Application of *d*-tubocurarine to the cerebral cortex produces paroxysmal activity (83). If the drug is injected in rather high concentrations (from 2 to 3 mg./kg.) in unanesthetized artificially ventilated animals, the EEG and all dendritic activity are abolished, reversibly (84). The best way to stop the paroxysmal effect of topically applied *d*-tubocurarine is by intravenous injection of the same drug. This indicates that different effects may be obtained, depending on which synaptic systems are preferentially blocked by the *d*-tubocurarine.

Brodie: Will any *d*-tubocurarine pass into the brain when it is given parenterally?

Purpura: In high enough concentrations a central action of *d*-tubocurarine is demonstrable. As Dr. Grundfest and I have shown, and Dr. Marrazzi also, it does get in. Systemic administration, however, permits the drug to reach all synaptic sites at approximately the same time, resulting in total synaptic blockade. When the drug is selectively administered by intraventricular injection, the convulsant action so obtained would seem to indicate a blockade of inhibitory synapses in periventricular loci. We have obtained evidence that *d*-tubocurarine,

like strychnine, exerts a selective action on inhibitory synapses and this accounts for the convulsant action of *d*-tubocurarine when applied topically to the cerebral cortex. Whereas the differential for blockade of inhibitory and excitatory synapses is small in the case of *d*-tubocurarine, it is considerable for strychnine. In other words, *d*-tubocurarine is a blocking agent at all synapses. Quantitative differences are a result of differences between synapses on the same neuron or neurons in different regions of the brain, as well as central or peripheral location.

Kety: Feldberg's data cannot be ruled out, on the basis that the pharmacologic effects obtained that way are different from those obtained on intravenous administration. After all, the action of substances which act in the brain may not be dependent on their getting there by way of the circulation; a substance produced within the brain may have an effect which can only be duplicated by getting it into the brain. If the drug does not get across the blood-brain barrier, there would be no chance of demonstrating its effects by injecting intravenously.

Marrazzi: Could you tell us what the time lag in Figure 88 is?

Unna: And the dose of LSD-25?

Elkes: The dose is 180 μg. Recovery is complete within half an hour.

Marrazzi: But recovery is beginning much sooner in that figure, isn't it?

Elkes: Yes, within 10 or 15 minutes.

Marrazzi: In other words, this is one of the short actions? It comes on very promptly and tends to disappear in a short time?

Elkes: Yes, in a relatively short time.

Marrazzi: This is much more similar to the ones we have described in time course.

Kety: Can you demonstrate that by stimulation of the carotid sinus nerve, rather than by blood pressure changes? I am a little confused by the fact that this inhibition occurs at the time the blood pressure is up.

Elkes: Yes. The effects observed are not necessarily related to changes in blood pressure. In answer to your question, Dr. Ginzel has shown, as mentioned earlier, that following LSD-25, the pressor response brought about by stimulation of the carotid sinus nerve is converted into a depressor response. Would the explanation we gave be acceptable?

Kety: The carotid sinus nerve is a mixed nerve. By preferentially blocking it, a depressor effect may be obtained.

Elkes: Yes. Dr. Ginzel believes that there is a preferential blockade of the chemoreceptor component of the reflex.

Kety: That could easily explain it.

Elkes: We now come to the third and most critical area of investigation, namely, the effect of drugs on the human subject. For, whatever its merits, the animal experiment is but a necessary preliminary; and whatever data it may yield must be complementary to data obtained in man, where subjective sensations communicated by means of speech, gesture, or creative expression, or those sensations that are incommunicable by their very nature, provide an indispensable segment in the circle of evidence. In what follows I can only hope to indicate briefly the various lines which such investigations can follow inasmuch as they illustrate method. Thus, for example, there can be studies of the acute effects of intoxication in normal subjects; there can be observations of the acute affects of drugs in the mentally abnormal; there can be the sustained and controlled therapeutical trial; and there can be a systematic study of drug reactivity patterns in terms, not only of their psychologic consequences, but of their metabolic effects and corollaries. I would like to ask that we consider familiar facts in the light of the considerations which preceded them.

For example, let us examine the clinical effects of lysergic acid diethylamide: The first sample of the drug came into our hands in Britain toward the end of 1951. In our early experiments we were less interested in the florid manifestations of the full intoxication than the early signs, the precise patterning and sequence of the various symptoms, and the effect of one form of afferent stimulation (namely rhythmic, photic stimulation) upon the course of the intoxication. The electroencephalogram was also recorded (65).

Fifteen volunteers, twelve male and three female, drawn from the medical research staff of the Medical School served as subjects. The experiments (which were in charge of Dr. Charmian Elkes) were conducted in a small semidarkened room, and all relevant verbal material was sound-recorded and the transcript subsequently analyzed for relevant elements. At the beginning of the experiment the subject rested comfortably on a couch and was exposed (first in a run with eyes open, and then in a repeat run with eyes shut) to regular flashes of white light coming from a stroboscope lamp at a rate of from 4 to 24 flashes per second. Sufficient time was given at each frequency for the subject to describe, or more precisely attempt to describe, the subjective sensations of form, color, and movement experienced at each frequency. The stroboscope run was then repeated with the subject silent, while an EEG record was being taken. The electrodes were then removed, the subject allowed up, and the drug (in doses of from 0.5 to 1.0 μg./kg.) administered, usually at 11:30 a.m.

The symptoms generally began to appear within 20 minutes and,

with three exceptions, were quite apparent within the hour. These are common knowledge, but illustrate material here and there. It must be stressed that although the symptoms of intoxication became increasingly familiar to us as the experiments proceeded, the patterns into which they formed themselves in individual subjects varied as greatly as the subjects themselves. At no time could the precise mosaic into which they fitted themselves be predicted.

Disturbances of *affect* occurred relatively early, and of these a fluctuating affect, unmotivated laughter, and depersonalization were the commonest manifestations. Fluctuation of affect was almost universal. Thus, a subject would be suddenly seized with restlessness, pace the floor and become perhaps somewhat aggressive, argumentative, and demanding. Then he would sink into his chair in a phase of silence and withdrawal. Or halfway through the experiment a subject would feel good. He would be convinced that his mind was clear and that he was in control of himself and free from intoxication, only to have the symptoms return after a short walk around the block. Unmotivated laughter, sometimes of gargantuan proportions, was another symptom. But perhaps the most vividly recalled and disturbing of all sensations was the depersonalization which at some time or other would steal over all subjects. This was described in various terms. "As I was sitting and watching you," said one subject some 25 minutes after taking the drug, "I felt suddenly as if I had been put out of gear. I saw and heard the hum of your activities, but I could not engage, I could not feel. If I had not chosen to tell you, you would not have known of my state. But to me you looked like actors going through your cues in a play; and I too, was a player watching myself from outside." These, then, are the main affective symptoms. Anxiety, aggression, incongruity, indifference, and withdrawal, all playing against each other according to endopsychic and environmental factors. I would add that two of our fifteen subjects experienced a mild paranoid state.

Beecher: Do the subjects think those visions they had were real? Were they divorced from reality or in touch with reality? There is a crucial point there.

Elkes: This is a very crucial point. To my mind it depends on dosage, personality structure, and, to no little extent, on the way the subject's environment is manipulated.

Beecher: Have you seen anyone who, with this dose, was divorced from reality?

Elkes: I have seen one.

Beecher: We have seen none, in all the subjects we had. They all knew the visions and sounds were not real.

Elkes: The subject I have in mind received only a small dose (25 μg.). The response was delayed and exceptionally severe. I wonder whether this is your experience?

Beecher: I wonder how normal he was.

Elkes: He was one of our subjects, selected with some care. You asked whether we had seen loss of insight in a subject under experiment. We have.

Beecher: It is not the customary thing, is it?

Elkes: No, most certainly not.

Fremont-Smith: What was the response he showed? Let us hear the response.

Elkes: This subject showed a striking time lag in the development of his symptoms. These were only few and relatively slight during the first 4 hours. During the late afternoon, however, the symptoms increased steeply in severity. Affective disturbances came first; these merged rapidly with paranoid coloring. Visual hallucinations became quite evident and the delusional symptoms were subsequently projected into the hallucinations.

Beecher: What is his subsequent history?

Elkes: The aftermath of the experiment lasted for at least 3 days. The subject was good enough to let us have a very detailed account of these aftereffects. The subsequent history was uneventful, though the subject still thinks of the LSD experience "as the experience of a lifetime."

Hoagland: If a person took the drug daily over a long period of time, or if he had a constant titer maintained in his blood, and he did not know he was getting a drug, it is likely that the parallel between psychotic hallucinations and the drug hallucinations would be close or even identical. In the experimental psychosis, a normal subject comes in, gets a dose of medicine, is told that he is there for a particular experiment, and therefore the experiences are part of this total pattern. But if these hallucinogens were being continuously administered without his knowledge over a period of time, it seems to me likely that such a man would come to objectify and believe in the reality of his hallucinations, as he does not do in the acute laboratory experiment.

Brodie: However, Dr. Hoagland, don't you think that Dr. Beecher brought up a cogent point in reminding us that the psychoses induced by LSD are not like those suffered by patients with mental disease, an important difference being that the experimental subject is not completely divorced from reality?

Hoagland: Yes. I think that is the reason for the difference but it

is not fundamental to the nature of the drug action *per se* for this very reason.

Fremont-Smith: Dr. Hoagland has made a good point, that there is a form of testing reality in these situations in which the patient can see he is in an experiment and therefore he can constantly remind himself that his sensations and hallucinations are a result of the experimental procedure he is in, whereas, if he did not know he was being given the drug and experienced the same degree of internalization of feelings, of hallucinations, etc., he would have no way of understanding how it happened, and he would wonder what was happening. I remember one time suddenly feeling an acute sense of nausea and instability and wondering what was happening to me. It was a few seconds before I realized that I was in a small earthquake, and, immediately, I felt entirely different about the sensation. I was now able to project it to the outside environment. Actually, in psychotic patients, it is possible to observe the gradation from the time when they are able to test their sensations against reality to the time when they become more intense and when they cannot test them. Dr. Beecher pointed out again that the dosage depends upon their testing reality, or recognizing reality depends upon the dosage.

Beecher: But it seems to me that there is a crucial point here which leads to a great deal of confusion. If a drug is used which, under given circumstances in an experiment, will separate a man from reality, and then, under the same circumstances, other individuals who are not separated from reality are tested, different situations are in question. The reports about a drug producing experimental schizophrenia are misleading, because if these hallucinations are not real, in the sense that the individual is not divorced from reality, then a lot of confusion has arisen in the area where it need not arise.

Brodie: Hashish is taken by some individuals practically every day of their lives and they presumably experience hallucinations every day of their lives, but as far as I know they never really become divorced from reality.

Masserman: There is some contributory evidence, however, from another sphere, that is, from the isolation techniques. A person deprived of stimuli in a dark room, floating around in a warm bath, at first recognizes his beginning hallucinations as such, but soon he begins to live in them. The transition is not at all precise.

Hoagland: That is right. If he were allowed to go under the influence of hashish continuously without having to do anything about taking it, a different situation might result.

Brodie: The fact remains, though, that the only evidence that we

have now is to the contrary, that is, that the hallucinations induced by LSD-25 do not completely divorce the subject from reality. They are a reflection of the pharmacologic action of the drug and as such are presumably repeatable from day to day. I would just as soon accept this evidence until it is proven to be wrong.

Fabing: There is one exception. In the experiments that Hoffer and Osmond (85) made on adrenochrome and adrenolutin, they report that subjects lost the connecting thread with the reality situation with these two drugs, and that they lost the comforting realization that they were in an experimental situation. This is different from experiences usually reported with LSD and mescaline, etc.

Fremont-Smith: And it has not been possible to repeat these tests yet?

Fabing: No.

Glees: What would happen if you found the beginning of schizophrenia in a patient and you told him that this was the result of an experimental condition (LSD)? Would you be helping him somehow to exteriorize his whole being?

Fremont-Smith: Therapy can help a person transiently, so I would assume that this form of suggestion could also help a person transiently in the early stages.

Brodie: Does this mean that a person might have schizophrenia but if he is told that he is merely under the influence of LSD, he might actually get better?

Fremont-Smith: This is a little overstated!

We argue whether or not the effects of LSD-25 are an experimental form of schizophrenia, but no one has defined real schizophrenia as yet so it is naturally difficult to compare the two.

Mirsky: Until definitions are made, as you claim, the opposite should not be done, as Dr. Brodie has indicated. From the pharmacologic effect of a drug producing hallucinatory phenomena to schizophrenia is a stupendous jump.

Brodie: Therefore, we should not use the term, "psychotomimetic"?

Hoagland: Why not?

Brodie: Because we don't know what a psychosis is!

Abramson: I think that this can be accepted: that LSD in certain individuals produces a psychotic state, often more like schizophrenia than is found in the naturally occurring schizophrenic state in some clinically diagnosed patients.

Kety: Dr. Beecher and Dr. Hoagland brought up an interesting point and I do not think the entire answer is at hand. However, while

we are contributing evidence on this controversy, we should add the report by Charles Savage (86) that one of his subjects who isolated himself after taking LSD-25, and in that way destroyed a great deal of the experimental situation by having no observers around, became extremely paranoid and delusional.

Elkes: Dr. Kety's point is very important. We know, as yet, very little about the individual variations in the reaction to LSD-25. In Britain some authors (87,88) are getting wide experience of the therapeutic uses of LSD-25. In this context one is repeatedly and most forcibly struck by the way in which the structure of the environment (of which the therapist or investigator is a part) can play a vital role in determining the course of the LSD-25 intoxication. The picture of the intoxication can be influenced practically by the way one enters the room or by the way one talks, encourages, or discourages the subject. In the wider sense, the precise experimental setting is of equal moment. For example, the experiments I am reporting were carried out in an almost dark and silent room, since stroboscopic stimulation was an important element in the situation. We found, if we left subjects alone in this dark cubicle under the influence of LSD-25, that there was often an extraordinary and bizarre accentuation, particularly of the affective symptoms. This showed itself in a fear of not being able to "get back" to the reality of the adjoining room. In some subjects this amounted to panic even while the symptoms were wearing off. The dependence on sensory input is interesting. It looks (though I am anticipating the trend of the discussion) as if LSD-25 in some way may alter the coding of sensory information: information coming from one's own body—the so-called body image which probably represents one of the earliest frames of reference laid down to distinguish the "me" from the "not me," and thus be related to ego formation and ego structure; sensory information coming from the distance receptors which are presumably also built into the perception of time; and the affective toning of these experiences in terms of pleasurable, displeasurable, or neutral setting. Something further which comes to mind in connection with Dr. Kety's remarks is the possible relationship of LSD-25 intoxication to the states described by Dr. Hebb (89) and Dr. Lilly, on the effects of massive sensory isolation. At this stage, of course, one can but speculate, but I wonder whether, in line with the points I made earlier, we are not physiologically kept coded and oriented, as it were focused on the "here" and "now," by virtue of an active inhibitory state which is reciprocally related to afferent sensory input, and is in fact, inseparable from transforming input into information. This state may be chemically mediated and related to the physiologic liberation of an LSD-sensitive

indole derivative as a result of afferent bombardment. Reduction of afferent input may conceivably lead to an imbalance in the production of this agent, and a disinhibition of the inhibitory tone essential for the organization of experience.

The effects of rhythmic photic stimulation on the picture of LSD-25 intoxication were not out of keeping with the supposition that the mechanism interfered with by LSD-25 was related to the afferent system. In twelve out of 15 subjects, photic stimulation, carried out in the way described above, enhanced the symptoms of intoxication, and in three out of these twelve, symptoms were brought out where no symptoms of any kind had been experienced before. Visual imagery was particularly affected by this stimulation. This was shown by a heightened and, in some instances, altered color perception. Colors were described as having a peculiar brilliance and luminosity. There was an increased vividness, and organization of the geometric mosaic-like patterns in eleven out of fifteen subjects. They were described as having a polished marquisette-like quality about them. Repeatedly, subjects felt that they could not possibly communicate the state which they were experiencing, and at times the illusions (and ultimately, hallucinations) became invested with a strange and bizarre, frightening, and by no means pleasant emotional tone.

Beecher: What was the dose?

Elkes: It was 1 μg./kg. To continue, six out of fifteen subjects experienced a sensation of body movement ("lightness, floating, levitation"), and six subjects showed also a gross disturbance of body image. As one of our female subjects put it, "I feel like the original muse of Mr. Henry Moore."

Six subjects, as a result of photic stimulation, went into an incommunicable trance-like state from which they refused to be roused. This state, and this is quite important, persisted after photic stimulation had ceased.

Fremont-Smith: How long did these persist after the photic stimulation?

Elkes: For up to 30 minutes after photic stimulation. Although only showing slight symptoms before, they emerged from the room after the stroboscope run heavily intoxicated.

Abramson: These are nonpsychotics, so-called normal subjects?

Elkes: Yes, these were normal volunteers. A further feature was that these subjects, as a result of photic stimulation, showed a slight, but clinically quite definite alteration in muscle tone not unlike that seen in catatonia, which, as you recall, has a characteristic "feel" of its own. This state too persisted after photic stimulation had ceased.

Pfeiffer: What was the frequency of the photic stimulation in these subjects?

Elkes: We started from four and went up to twenty-four, giving at each frequency time enough for the subject to describe his subjective sensations. The motor effects were seen particularly around the ten to fourteen range.

Pfeiffer: Did you notice any effect on the facial muscles? The reason I ask is because George Mueller,* working with mescaline, had photic-stimulation-induced seizures in normal subjects.

Elkes: No, we have not seen any effects on facial musculature. On the other hand, during photic stimulation in the one instance we saw a rising of the lower limbs while the photic stimulation was in progress. This ceased when the stimulation was discontinued.

Jarvik: Do subjects get these symptoms without LSD-25?

Elkes: No. Photic stimulation, of course, induces subjective states and increases visual imagery considerably (90,91). However, by the common consent of our subjects, the LSD-25 experience under photic stimulation was different from either the LSD experience or the experience of photic stimulation alone.

Jarvik: You raise an interesting point. You say that some of the subjects, even with 1 γ/kg., experienced no symptoms?

Elkes: Yes.

Jarvik: How frequently did you find this and how do you explain it? We have several subjects that were similarly resistant, and we gave some of them up to 225 γ without any symptoms.

Beecher: Normal subjects?

Jarvik: Yes.

Elkes: Did they take amphetamine? I ask because one of our subjects was taking amphetamine at the time of the experiment as part of an attempt to lose weight.

Jarvik: No. They tended to become drowsy with LSD-25.

Abramson: We consider the threshold dose to be 25 γ, roughly, independent of the body weight. With our method of study, for the group I am running now (five normal subjects at my home, for the last 2 years), three out of five always detect between 20 and 25 γ, and two out of five usually detect 35 γ. We consider the latter as especially resistant. A so-called 35-γ resistant woman of 40 showed a marked reaction under 75 γ.

Jarvik: I would like to say that what would be most desirable would be some statement as to the $E.D._{50}$ of particular symptoms, so that we might know how effective these so-called hallucinogens are. I would say

*Personal communication.

that for a sign such as pupillary dilation or other autonomic signs, the E.D.$_{50}$ was probably rather low, and for such things as hallucinations, it was probably high.

Hoagland: That has been our experience, too.

Unna: Dr. Abramson, didn't you report that there is a rapid development of tolerance?

Abramson: Yes.

Unna: How can you reconcile that with the statement that you give extremely small doses?

Abramson: That is because tolerance disappears in 5 days (92). These subjects are studied at least 1 week apart. Dr. Jarvik, our co-workers, and I (93) published a rank order of the appearance of symptoms (Table XIII). If your method of observation is inclusive enough, I think you can easily detect, in most normal subjects, from 25 to 30 γ, independent of the body weight.

Fabing: Do you mean minimal symptom response?

Abramson: No, with suitable placebos and with at least 50 per cent of the subjects, using the questionnaire technique as the operational method of observation, 25 γ by mouth is a detectable amount.

Beecher: For the first symptom?

Elkes: For dilation of pupils?

Abramson: No, not for dilation of pupils. The rank order depends on the personality of the subject. It does not depend on his pupils. It depends on the reaction of the individual to drugs, as measured by our technique of observation, the questionnaire.

I should like to describe an experiment that we made. We had administered, in an experiment, tap water plus X-LSD, to a young Chinese girl. She telephoned the next morning to say that her legs were paralyzed. She had gotten just tap water. We also had one man on a tap-water placebo who became so agitated that I had to spend 8 hours with him to calm him. Therefore, if there is going to be an LSD reaction by mouth, it will occur within 2 hours and will not last, in general, on low doses (about 50 γ), more than 8 hours. Any other phenomena, I believe, are superimposed, as psychologic phenomena, and they are a result of the personality of the individual and his reaction to the total situation rather than of the pharmacologic action of the drug itself.

Fremont-Smith: You said "in general," and I think there is room for exceptions. I am inclined to feel that one should take cases with low tolerance seriously and, although it might well be that their reaction was not a result of the drug, the possibility that the reaction was a result of the drug is a sort of therapeutic assumption that we should

keep in mind, and the warning is of extreme importance to have on the record. Until we have gone much further, I do not think we can justifiably assume that anything that takes place 5 or 6 hours after administration of the drug or within the next 24 hours must inevitably be caused by something else. There can be suggestion, to be sure, but that does not prove that each case is a result of suggestion, and it is at least possible that there are delayed reactions, delayed absorption, and especially environmental circumstances that may induce, at a later date or a few hours later, a special sensitivity to the dosage that the organ is then carrying.

Abramson: As a matter of fact, I have studied the effect of delayed absorption. I grant, if the stomach is filled with certain adsorbents, absorption may be prevented. But, under normal fasting conditions, I doubt if the absorption will be delayed much.

Fremont-Smith: Is there any evidence that people are more easily hypnotized under LSD-25?

Elkes: I am not aware of any clear evidence in this respect. It is, however, our strong impression that subjects receiving LSD-25 are more susceptible to cues in the environment and by inference may be more suggestible.

Beecher: That is true of a number of other drugs. Hirschlaff (94), in 1921, wrote a book about this, and he said that ether, morphine, and alcohol all increase hypnotizability.

Fremont-Smith: But LSD-25 has not been tried yet, as far as we know.

Elkes: Not systematically, as far as I know.

Fabing: In a bibliography to one of his papers on mescaline (90), Klüver gives a reference that states that the patient who has been given mescaline is more easily hypnotized (95).

Elkes: There is another aspect which Klüver touched on in his masterly and prophetic paper (90), namely, the broad resemblance between the colored geometric patterns seen under stroboscopic stimulation in the absence of any drug and those seen in deep mescal intoxication in the absence of stroboscopic stimulation. Again, one wonders whether there is an interference with the coding (in time) of sensory information either at retinal or more central levels. Both phenomena may represent a shift in the time base in the activity of some elements along the visual pathway, the shift being induced in one instance by rhythmic stimulation of the visual fields, and in the other by selective interference with a cycle of chemical events essential for the function of neurons occupying a key part in the sensory system. It must remain an open problem whether or not such changes are related to the evidence of a triple pathway along the visual system (96,97).

TABLE XIII

Rank of Each Question for Each of Three LSD-25 Dosage Groups*

Question Number	Question	N = 13 0 γ	Rank of question N = 21 25 to 75 γ	N = 14 100 to 225 γ
1	Do you feel ill in any way?	10.5	32	15
2	Are you nauseated?	28	24.5	13
3	Have you a feeling of choking?	37.5	32	34
4	Is salivation increased?	8	24.5	44
5	Or decreased?	16	28.5	19
6	Is your appetite increased?	19.5	32	27.5
7	Or decreased?	5.5	26.5	23.5
8	Do you have a "dry" taste in your mouth?	19.5	21	29
9	Do you have a funny taste in your mouth?	10.5	18.5	23.5
10	Is it a bitter taste?	24	43	35
11	Are your lips numb?	37.5	23	20.5
12	Or drawn back as if you were smiling?	43.5	18.5	31.5
13	Does your head ache?	3	11	22
14	Are things moving around you?	43.5	37	27.5
15	Do you feel dizzy?	13	10	5.5
16	Or unsteady?	28	2	1
17	Is there difficulty in breathing?	28	37	40
18	Do you pass more urine than usual?	19.5	43	44
19	Are you aware of your heart beat?	32.5	21	40
20	Is it faster than usual?	19.5	35	36.5
21	Are you sweating?	37.5	21	25.5
22	Are you hot?	13	12	11.5
23	Or cold?	43.5	37	36.5
24	Are your palms moist?	1	1	3.5

*Rank 1 indicates highest per cent of positive responses. Succeeding numbers represent decreasing per cents.

TABLE XIII (Contd.)

Question Number	Question	N = 13 0 γ	Rank of question N = 21 25 to 75 γ	N = 14 100 to 225 γ
25	Are your palms dry?	43.5	46	40
26	Or cold?	8	43	40
27	Is your skin sensitive?	24	32	25.5
28	Do you have funny feelings on your skin?	32.5	9	7
29	Do your hands and feet feel peculiar?	8	3	5.5
30	Do they feel heavy?	13	13	9
31	Or light?	32.5	14	17.5
32	Is there pressure in your ears?	24	17	15
33	Is your hearing abnormal?	24	28.5	46
34	Is it more acute than usual?	24	43	30
35	Is your eyesight blurred?	32.5	15.5	15
36	Do you have difficulty in focusing your vision?	43.5	26.5	11.5
37	Do you see double?	43.5	47	47
38	Are shapes and colors altered in any way?	37.5	32	31.5
39	Does light bother you?	32.5	39.5	44
40	Do things seem too close?	43.5	43	40
41	Or too far away?	43.5	39.5	33
42	Do you tremble inside?	16	4	2
43	Do you feel weak?	32.5	5.5	3.5
44	Or fatigued?	5.5	5.5	17.5
45	Do you feel drowsy?	2	8	10
46	Do you feel as if in a dream?	16	7	8
47	Are you anxious?	4	15.5	11.5

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There is one related area of investigation which cannot be left unmentioned. The strange disturbances of ego function, the apparent reactivation of infantile modes of thinking, and the occasional emergence of repressed childhood memories in heavy intoxication suggest a most fruitful avenue of work for the serious, research-minded psychoanalyst. I have never really understood what is meant by the alleged contradiction between Freudian theory and the so-called "organic" approach. To my mind this implies a parallax, an optical illusion. The growth, maturation, organization, and economy of the mental apparatus as envisaged in Freud's studies may yet turn out to be one of the most powerful and penetrating predictions made in the absence of adequate evidence at the time. Yet, with few exceptions, little attempt has been made to validate and predict events in psychoanalytic sessions, and in the long-term course of psychoanalytic therapy. The tools of psychoanalysis have so far been those of natural history rather than of science. Yet the use of chemically activated states of altered consciousness provide a therapeutic setting which, if so wished, could be made the setting of an experiment, that is, a situation designed to test a hypothesis. I hope I will not be misunderstood. I do not mean to imply that we are even remotely within sight of a neural or neurochemical substrate of mental phenomena. All I mean is that an understanding of the grouping, the economy, and the autonomic functioning of purely psychologic forces may well be facilitated by the use of a chemical tool of this kind; and no area could benefit more in this regard than an understanding of ego structure and organization.

There is a further area of work which I wish to indicate, namely, the effect of some drugs on catatonic schizophrenic stupor. The reasonably well defined, and in suitable cases, stable character of the syndrome lends itself well to a comparative study of the effects of drugs, more particularly in view of the transient reduction in stupor affected by amylobarbital. This phenomenon formed a convenient base line for the study of drugs other than barbiturates. The study was carried out by Dr. Charmian Elkes at All Saints Hospital, Birmingham.

Catatonia is a comparatively rare condition in a modern mental hospital, and an initial survey produced twenty-four patients (twenty-one female and three male) who presented a clinical picture sufficiently representative of catatonia to warrant further study. Quite early it became apparent that rigorous criteria had to be applied in the further selection and analysis of suitable cases. The following criteria were agreed upon: (*a*) Maintained inaccessibility and indifference to environment, in terms of speech, gesture, and drawing. This was based on a ward report, observation during interview, and the response on the

part of the patient to a request to draw. Patients subject to attacks of catatonic excitement were excluded from this study; (*b*) Akinesis, i.e., the lack of spontaneous movement; (*c*) Increased muscle "tone," as judged by the "feel" of passive movement and the maintenance of imposed and abnormal limb posture for long periods; (*d*) Cyanosis of the extremities (the so-called acrocyanosis); (*e*) Reasonable pre-psychotic level of intelligence. This was assessed on the basis of the admission record, and ensured that mental defectives were excluded from the series; (*f*) Lack of obvious secondary dementia. This seemed important as it would have been difficult to judge an increase in accessibility in the presence of dementia; (*g*) Adequate sphincter control. This excluded patients who were obviously degraded and incontinent; (*h*) Adequate nutrition and general health. This criterion was agreed upon to minimize any risk attached to the use of drugs in these patients; and (*i*) Increased accessibility in terms of speech, gesture, and drawing following the intravenous injection of small (from 210 to 300 mg.) doses of amylobarbital sodium. It was this response to amylobarbital which formed the base line of a comparative study of the other drugs.

The above criteria were discussed with staff members of All Saints Hospital. All twenty-four patients (and some additional ones) were examined independently by four physicians and the findings of each observer were noted on a scoring card. The results were then discussed at a further meeting and formed the basis of selection. Four patients were rejected because of physical ill health. Amylobarbital sodium (from 180 to 300 mg.) was then administered intravenously to the remaining patients. Six were rejected because they failed to react by an increase in accessibility following this injection. The remaining fourteen cases were then observed for a further period of 3 weeks and ultimately reduced to a stable group of nine patients, one male and eight females (ranging in age from 32 to 63) who complied with all the above criteria in some degree. The male patient had later to be rejected on the grounds of physical ill health.

The experiment was carried out in a small room that was used solely for the purpose. This contained a bed, on which the patient rested throughout the whole of the experiment, and only such apparatus as was needed at the time. Each experiment lasted about 3 hours. The patient was brought to the room from the ward, allowed to rest on the bed, and a period of equilibration of at least one-half hour was allowed for the blood pressure and pulse rate to settle. During this time, the patient was not talked to, but was observed quietly and readings of foot temperature were taken. The mental state was then further assessed by interview and by the response of the patient to a request

to draw. In the interview, special attention was paid to facial expression, gesture, presence or absence of spontaneous movement, tone of voice, form and flow of speech; the content of the response was gaged in terms of orientation, any alteration of cognitive functions, of affect, and the presence of hallucinatory or delusional material. In testing willingness and ability to draw, a drawing board, together with a collection of colored pencils, was put in front of the patient for 10 minutes, and the patient was allowed to draw or write as he pleased, without prompting. The mental response to a drug was graded into six broad categories:

Zero (0) Response, which applied to experiments where there was no detectable mental change; −1 Response, which denoted increased stupor, as shown by increased retardation, resistiveness, inaccessibility and mutism, and a poor drawing response; −2 Response, which showed gross retardation, a set, rigid, mask-like expression, complete akinesis and absence of associated movements, occasionally coupled with an increase in muscle tone, and development of a fine tremor; +1 Response, which denoted the uttering of a few more or less connected words or monosyllables, or an appropriate response (in terms of gesture and expression) in reply to questions where none had been shown before (e.g., nodding, smiling, laughing, or shaking of head); and +2 Response, which indicated a marked response where there was a definite increase in accessibility and awareness after the drug had been given (the change of expression and gesture was appropriate, there was an increase in spontaneous and purposeful movements, contact was maintained, and questions were answered in connected sentences); and +3 Response, which marked an exceptional response of only one patient. Here the response, in terms of rapport, speech, and gesture, was so startling as to warrant a special category.

As far as possible, the following somatic changes were also recorded: Blood pressure and pulse rate were noted every 10 minutes. Skin color was noted continuously; and skin temperature was measured by means of thermocouple junctions attached to the left ankle and right big toe. Muscle "tone" was recorded in the flexors of the left forearm by means of a simple weighting device. This consisted of a plaster of Paris cradle molded at an angle of 100 degrees to accommodate the elbow. The left arm rested comfortably in this cradle with the upper arm lightly strapped into position to ensure fixation. A leather band was strapped round the wrist, and two lengths of thin strong thread (fishing line) were attached to this wristlet on its flexor and extensor sides, respectively. The former thread was carried back over a ball bearing pulley which was fixed to the bed above and behind the patient's left shoulder.

A small weight was tied to the end of this thread. The other line was taken over a similar pulley fixed at the foot of the bed and carried a tin which exactly counterbalanced the weight at the other end. At the beginning of the experiment, the patient's arm was fully flexed. Lead disks, each weighing 15 gm. were then dropped into the tin until the forearm had come to rest in the position of semiextension of approximately 100 degrees, or where (because of high flexor tone) this was not possible, the tin had dropped a distance of 15 cm. down a measuring scale fixed at the foot of the bed. This became necessary because, in some cases, resistance was so great that the weight required exceeded the maximum used in these experiments, i.e., 4 kilograms. Higher weights were never used because of the potential damage to tissues around the wrist, and because weight readings were being taken at 10-minute intervals, and the actual time of observation was thus limited. All the observations were made by one observer (C.E.), the pulse, blood pressure, and skin temperature readings alternating with the "weighting" procedure.

The pain threshold was tested in initial experiments by a manometric pressure method operating on the nail bed, but in view of the marked indifference to pain in these patients, this method was later abandoned as being potentially traumatic.

We performed 146 experiments, and the following drugs were administered: (*a*) Amylobarbital sodium was given intravenously. This was injected slowly, at the rate of approximately 60 mg. per minute. In the first few experiments as much as 360 mg. were given, but the optimum dose later was found to lie between 180 and 240 mg. (*b*) Sodium amytal was given, preceded by atropine and neostigmine. Atropine, in doses of 0.8 mg., was given by subcutaneous injection in initial experiments, to exert a peripheral protective action in view of hypotension shown by some of the patients at the beginning of this study. It was given after about a 30-minute equilibration period, and followed 20 minutes later by 0.5 mg. of neostigmine methylsulfate (prostigmine). The dose of neostigmine was increased to 0.75 mg. in a further series of experiments. In three cases, in whom the initial blood pressure was at a normal level, neostigmine was followed by sodium amytal without the initial dose of atropine. (*c*) Sodium amytal was also given, preceded by physostigmine (eserine). In this series, no atropine was given. Physostigmine was administered subcutaneously in doses of 1 mg. This was followed some 20 minutes later by the usual dose of amytal. (*d*) Amphetamine was given by slow intravenous injection (spread over from 5 to 8 minutes) in doses of from 7 to 15 mg. (*e*) Mephenesin at first was given orally in doses of from

1 to 2 mg. Later it was given intravenously (as a 5 per cent solution in saline) by slow injection. The total dose averaged 1 gm. except in one instance where 750 mg. were given. (*f*) Lysergic acid diethylamide was given orally in doses of 100 μg., with the exception of one patient who received 50 μg. (*g*) Control injections of 3.5 ml. of sterile saline were given intravenously in two separate series of experiments.

The results are summarized in Table XIV. Figures 91 and 92 show

FIGURE 91. Unprompted drawing response (over a period of 10 min.) by patient in catatonic schizophrenic stupor before the administration of amylobarbital sodium.

a typical drawing response as a result of the administration of amylobarbital. Figures 93, 94, and 95 illustrate the effects of amylobarbital, amphetamine, and mephenesin on blood pressure, foot temperature, and muscle tone in single experiments. Cumulative effects of amylobarbital and mephenesin are illustrated in Figures 96 and 97.

Inasmuch as a response to amylobarbital was a criterion of selection, an increase in accessibility was seen in every case examined. The precise extent of the response varied from patient to patient. In every case, however, there was an increase in rapport, a marked change in expression and gesture, and a striking increase in verbal productivity. The content of the response remained the same for each patient. It was also noted that with repeated administration there was a blunting of the response to amylobarbital. Once such blunting was observed, there was little change in threshold. Higher doses did not lead to an increase in accessibility, but would merely result in sleep.

TABLE XIV*

Summary of Mental Effects of Amylobarbital, Amphetamine, Mephenesin, and LSD-25 in Nine Cases of Catatonic Stupor

Case No.	Amylobarbital	Saline Control	Amylobarbital + Neostigmine, and Atropine	Saline Control	Amylobarbital + Physostigmine	Amphet-amine	Mephen-esin	Amylobarbital	LSD-25	Saline Control	Amphet-amine	LSD-25	Amylobarbital	Mephen-esin
1	+2	0	+3	0	+2	0	0	+1	+1	0	−1	†	0	0
2	+2	0	+2	0	+2	−1	0	+2	†	0	−1	†	+1	0
3	+2	0	+2	0	+1	−2	0	+2	†	0	−2	0	0	0
4	+3	+1	+2	0	+2	−2	0	+1	†	+1	−2	0	+1	0
5	+2	0	+2	+1	+1	−1	0	+1	†	0	−2	†	0	0
6	+2	0	+2	0	+2	−2	0	+1	†	0	−1	†	+1	0
7	+2	0	+2	0	+1	−2	0	0	+1	0	−1	†	+1	0
8	+2	0	+2	0	+1	−2	0	0	†	0	−2	0	0	0
9	+1	0	+2	—	—	−2	0	—	—	—	—	—	—	—

†Signifies a reaction peculiar to LSD-25 and unlike that seen either with amylobarbital or amphetamine.

*From the data obtained by C. Elkes. The criteria for the scoring figures are referred to in the text.

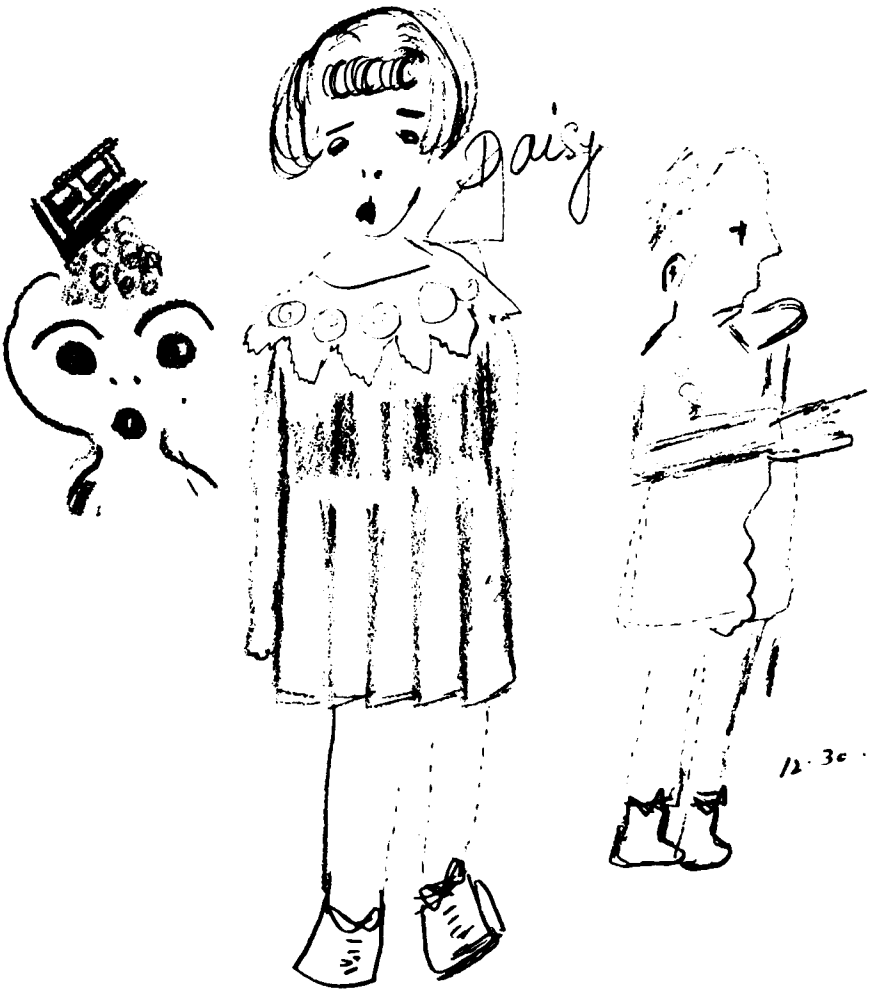

FIGURE 92. Unprompted drawing response (over a period of 10 min.) by patient 12 min. after the intravenous administration of 260 mg. of amylobarbital.

Combining amylobarbital with neostigmine or physostigmine was tried in view of the modification of the characteristic barbiturate activity brought about by anticholinesterases in the experimental animal (56). The initial findings tended to suggest that neostigmine reinforces the transient clinical effects of amylobarbital. This, however, was not borne out by later findings with physostigmine. Neither the threshold nor the extent of the observed effects was consistently influenced by these anticholinesterases.

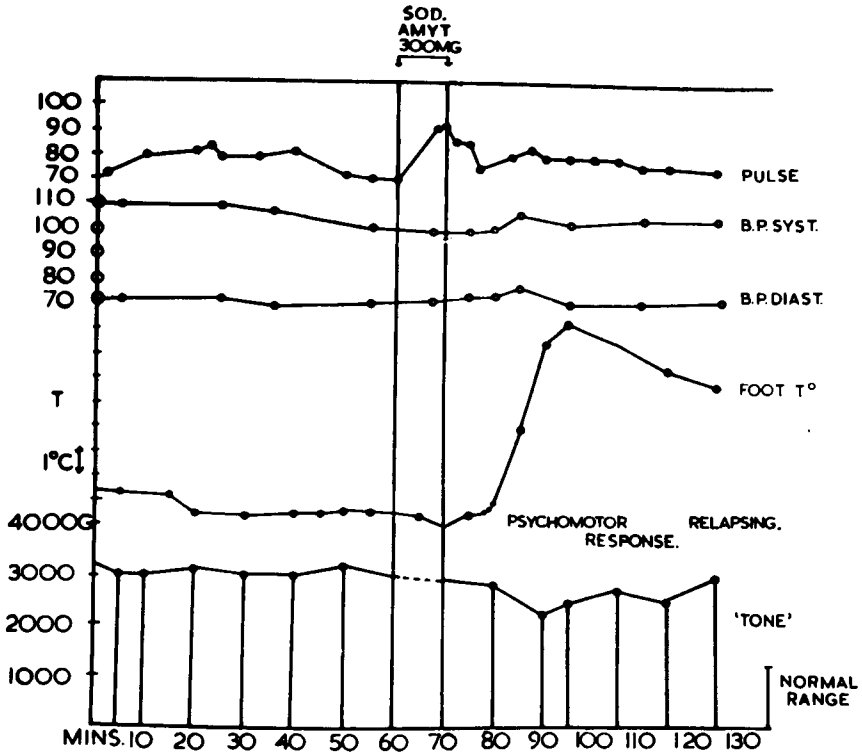

FIGURE 93. To show the effect of the intravenous injection of 300 mg. of amylobarbital sodium on pulse rate, blood pressure, foot temperature, and forearm flexor "tone," as measured by weighting device described in text. The psychomotor response coincides broadly with the rise in foot temperature. The fall in the weighting figures is only slight.

Amphetamine invariably led to an increase in stupor as judged by increased retardation, resistiveness, inaccessibility and mutism, and a poor drawing response. In some cases (those scored −2) there were also increased somatic signs of catatonia. Mephenesin exerted little obvious effect on the mental state of the patients. LSD-25, on the other hand, led to a reaction which was quite unlike that seen either with amphetamine or amylobarbital. This was characterized by an apparent increase in autistic withdrawal, unmotivated laughter and crying, dissociated, bizarre behavior, and apparently an activation of delusional and hallucinatory material. In some cases it also seemed as though new symptoms had been added to the established psychotic picture. In three cases when amylobarbital was superimposed upon LSD-25 the symptoms ceased abruptly, but they returned after from 20 to 30 minutes. The inert saline injections produced no noticeable mental effects.

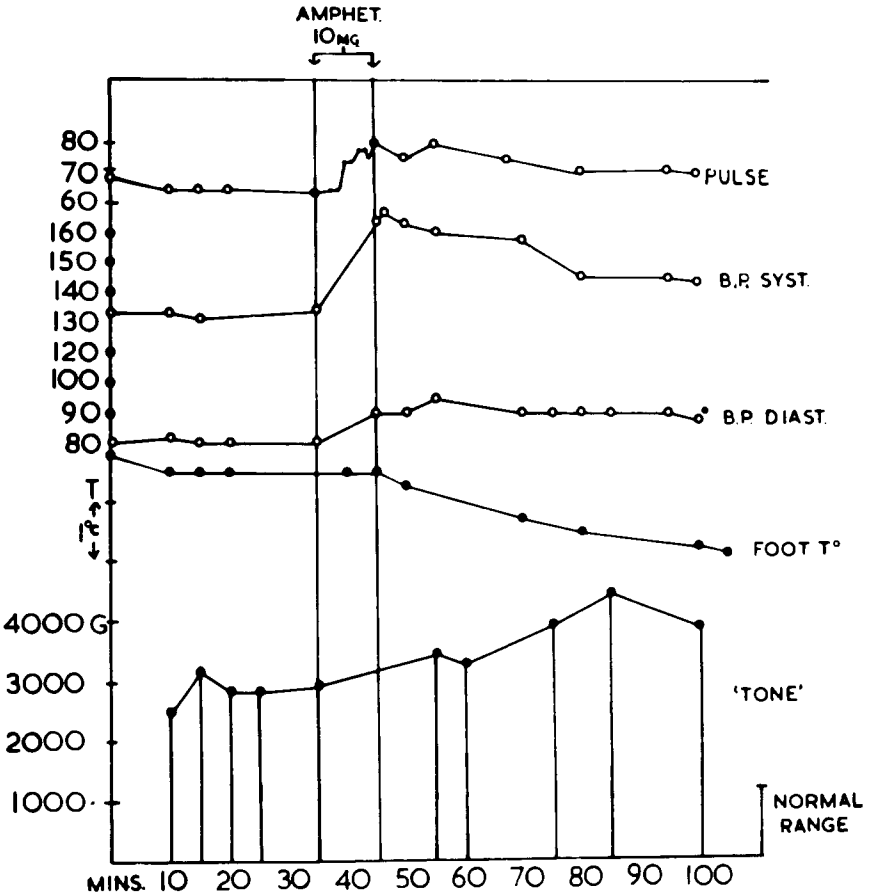

FIGURE 94. To show the effects of the intravenous injection of 10 mg. of amphetamine on pulse rate, blood pressure, foot temperature, and forearm flexor "tone," as measured by weighting device. There was a decrease in accessibility in this experiment (score of —2). The increase in forearm flexor "tone" is evident.

The somatic corollaries, particularly in regard to the effects of drugs on foot temperature and muscle "tone" (as measured by the weighting device) may be of interest. The administration of amylobarbital was often followed by a striking change of color in the skin of the extremities, the usual slate-grey turning to pronounced pink, accompanied by a rise in foot temperature (Figure 93). Both in terms of time course in the individual experiment (Figure 93) and in terms of incidence in repeated experiments in the several subjects (Figure 96), there was good correspondence between the mental response and the rise in foot temperature. On the other hand, there was (Figure 93) relatively little effect

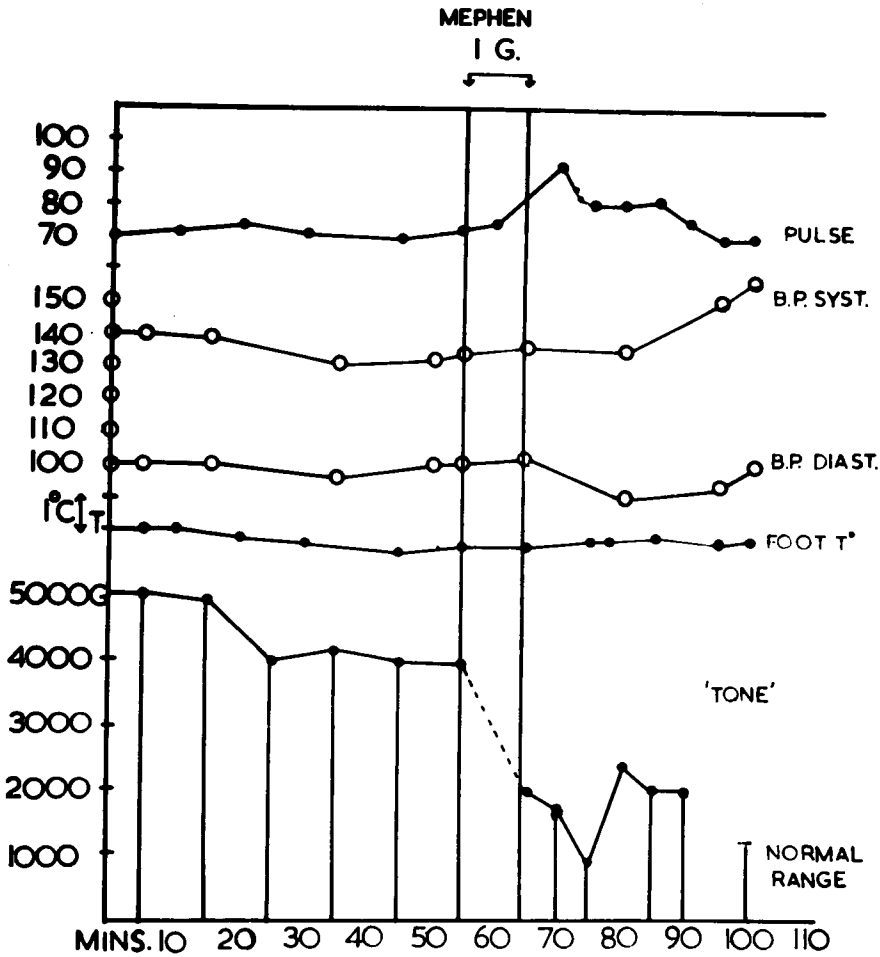

FIGURE 95. To show the effects of the intravenous injection of 1000 mg. of mephenesin on pulse rate, blood pressure, foot temperature, and forearm flexor "tone," as measured by weighting device described in text. No appreciable mental response was noted in this experiment. There was, however, a reduction in muscle "tone."

of amylobarbital in the doses used upon muscle "tone," as measured by the weighting figures. Only with doses of amylobarbital sufficient to induce sleep did noticeable relaxation supervene. Amphetamine (Figure 94) increased the weighting figures, and, in those cases scored −2 a fine tremor could be observed. Mephenesin led to a regular and marked reduction in the weighting figures (Figures 95 and 97), but as mentioned previously there was little noticeable effect in terms of mental function.

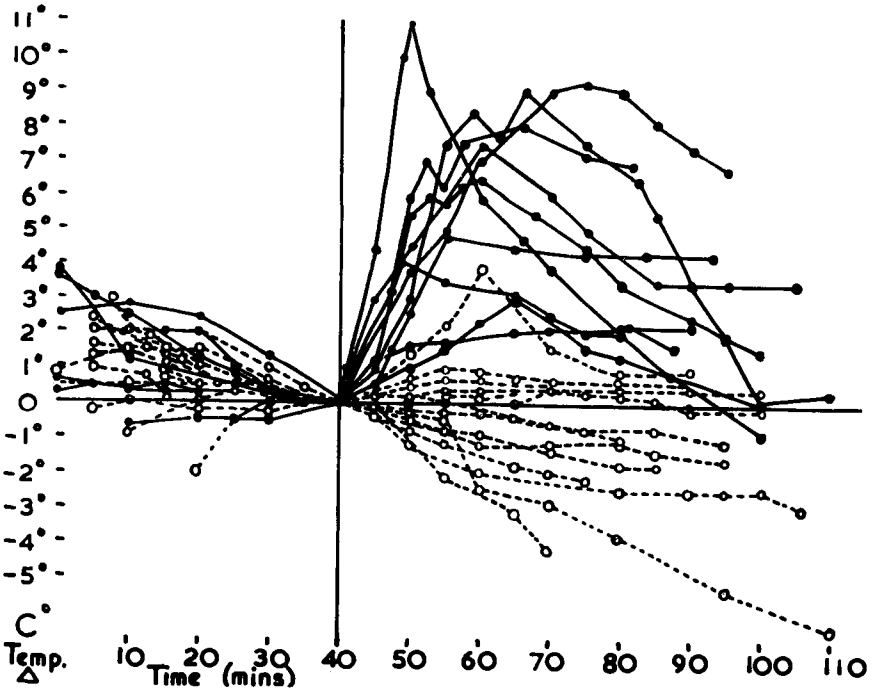

FIGURE 96. To show the effect of amylobarbital on foot temperature reading in catatonic schizophrenic stupor. Only difference in temperature (△ T°) from resting temperature before the administration of barbiturate is shown. The time of administration of amylobarbital, which averaged 40 min. after the beginning of the experiment, is taken as zero point for purposes of plotting:

Continuous line, solid circles: experiments in which the mental response was scored as +2 or +3.

Broken line, solid circles: experiments in which the mental response was scored as 0 or +1.

The effects of these drugs in catatonic stupor are paradoxical and unlike the effects in normal subjects. Amylobarbital, in doses which normally produce somnolence or sleep, leads to an increase in accessibility and, in terms of motor performance, a state which may be likened to awakening, though it certainly is not true awakening any more than stupor is like normal sleep. Amphetamine, which normally leads to euphoria and overactivity, leads to a deepening of stupor in these patients. The increase in accessibility produced by amylobarbital appears to be related to a rise in foot temperature without being related to a reduction of the grossly excessive muscle tone as measured in the flexors of the forearm. This lack of correlation between muscle tone and mental effects is brought out further by the effects of mephenesin.

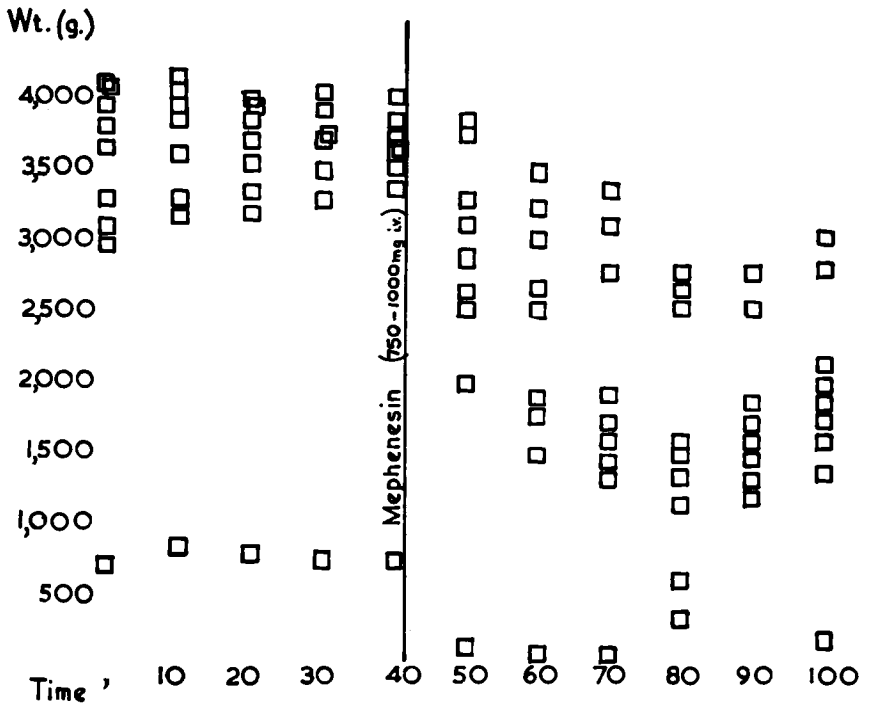

FIGURE 97. To show the effects of intravenous mephenesin on forearm flexor "tone" weighting figures, in eight cases of catatonic schizophrenic stupor. Fall in weighting figures is shown. There was little mental response in these experiments.

Here a consistent reduction of muscle tone is not accompanied by any psychomotor effects, nor was any rise in foot temperature observed in these experiments. The effects of LSD-25 are peculiar and unlike those seen with any other drug. That the effects observed are attributable to the drugs used is shown by the lack of effects in the control series.

It would seem that catatonic stupor represents a purposeful and in some ways unusually stable homeostatic reaction which in the present experiments could only be partly and temporarily modified by chemical means. The blunting of the mental and somatic responses with repeated administration of the drug bears out the stability of the original reaction. Its physiology must remain as obscure as it is interesting and remarkable, and it is much too early to indulge in anything but gross speculation at this stage. There seems, however, little doubt that one is dealing with an active inhibitory state.

Ingram, Barris, and Ranson (98) produced what they called catalepsy in cats by lesions in the region of the mammillary bodies. In a recently

extended finding by Sherwood (99) it was shown that diisopropyl fluorophosphate and acetylcholine produce catatonia following intraventricular injection (40). Akinetic mutism and obstinate progression were observed by Bailey and Davis after lesion in the central grey and interpeduncular fossa (100); and Ward (101) has shown how area 24, through its connection with the reticular formation of the brain stem, could inhibit all motor activity. Such findings and many others serve to emphasize the strategic position of hippocampal, fornix, mammillary, thalamic, and cingulate loops. The role of the ventromedial hypothalamic nuclei, as has been shown by Adey and Meyer (102), is to receive fibers from the amygdaloid complex and the tip of the temporal lobe. The connection (by way of the periventricular fibers) of the hypothalamic nuclei and the dorsomedial nuclei of the thalamus connects in turn, probably both upward and downward, with the reticular substance of the brain stem, which profoundly affects sleep, wakefulness, and posture. Is it possible, one wonders, that once again we are dealing with a continuously maintained imbalance between some elements in these circuits, and that receptors sensitive to amphetamine play a part in this imbalance and may in fact be locally represented in the brain stem? The fact that a drug such as amylobarbital reduces the catatonic state, and that this response is more related to the autonomic response than to alteration in muscle tone, suggests that the effect may be exerted principally at this level. Mephenesin, too, is an internuncial blocking agent: but as shown by Unna (103), and as borne out by the experiments described earlier, it acts principally lower down on the activating and inhibiting formation of the brain stem. Perhaps significantly, it did not particularly affect autonomic or mental function in the doses used.

Only the briefest remarks will have to suffice concerning the fourth area of work. The clinical trial is the most universally used instrument of investigation, yet only design and repeated calibration can make it a sensitive research tool. Dr. Charmian Elkes and I encountered this difficulty when faced with an early trial of chlorpromazine (104). Certain simple *desiderata* seemed advisable at the outset, although these could only be regarded as minimal ones.

We felt, for example, that a trial should be long enough for the observer-error and the spontaneous fluctuation in the clinical state to be adequately allowed for.

Marrazzi: How long?

Elkes: At least a year. Our initial chlorpromazine trial lasted 47 weeks, and the reserpine trial that we have just completed took con-

siderably longer. A further *desideratum* is that a trial should be blind, and a third, that it should be self-controlled.

Beecher: Is that double-blind?

Elkes: Of course.

Beecher: To the observer as well as the subject?

Elkes: Yes, the dispenser knows only the code. The term self-controlled is used to denote that the patient acts as his own control, placebo and drug being administered alternately. This, we feel, is preferable to using matched groups, although by changing over matched groups a self-controlled pattern can be built into a matched group design. A fourth *desideratum* is that the results should be recorded on agreed documents, and that they should be multiply documented: by several physicians (to minimize observer error); by the day and night nursing staff, who have the advantage of seeing patients in their natural environment for much longer periods than the physicians; by the psychiatric social worker; by the family of the patient; and by the patient himself, if he is accessible enough to complete the document. Lastly, the documents should be analyzed blindly in retrospect, and only when this analysis is completed, matched against the code of the trial. It is not necessary to labor the importance of statistics in either design or in retrospective analysis.

In the therapeutic field the trial is, without question, the most powerful research instrument we have. Its efficacy depends upon design and communication between a group of observers; and thus the calibration of terms, the construction of glossaries, and of documents fashioned on the basis of terms used by professional personnel to describe change, becomes an important part of any accurate pharmacology of human behavior. The placebo reaction observed not only in the patient, but also in the observers during our chlorpromazine trial (104) bears witness to the importance that must be attached to such factors.

A summary may perhaps now be in place. To my mind, the above remarks point to the existence of elective chemical affinities within the nervous system, which, though influenced by factors of access such as vascularity and permeability, reflect a differential and uneven distribution in the receptors themselves, uneven not merely in terms of anatomical location, but extending to finest level of histologic organization. Evidence coming from electrical stimulation studies, both in animals and man, the effects of ablation in animals, and lesions in man, though still a silhouette, shares points and even areas of correspondence with the drug effects we have described. One cannot, of course, be sure, but it is possible that the basic states of consciousness, and the storage and release, the dormant or nascent character of patterns of affective

behavior in response to afferent stimulation, may be governed by the neurochemical milieu of nodal cell groups in midline key areas. There are two points which come to mind in this context: First, the mosaic of cells and cell groups we know as the brain stem reticular activating system, which is really a system of systems, and their rostral and septal continuation bear a peculiar and reciprocal relation to the afferent pathways which feed into them by way of collaterals. They are activated by these, but, equally, through their activity determine the ultimate perception of the cortical signal. Magoun's (105) work on anesthesia has shown this clearly. Second, though resting on exquisitely complex juxtaposition of excitatory and inhibitory states, these processes involve large, and widely separated cell groups, in what might be called field effects in emanating from these midline key areas.

This latter point is perhaps worth taking further. The patterning and apposition of excitatory and inhibitory states in adjacent cell assemblies are of the very essence of nervous function. One need not proceed beyond the Sherringtonian reciprocal inhibition in a monosynaptic spinal reflex (106) to witness the operation of this remarkable rule. The prolonged inhibitory states following prolonged afferent inhibitory volleys justify the assumption of the existence of an as yet unidentified inhibitory substances in the spinal cord (107). The manufacture, storage, and release of this substance are presumably vested in an element ubiquitously present throughout the spinal cord.

Evolution may have made use of this element. Inhibition, delay of immediate response, the storage of patterns, and of the matching of patterns, are aspects of evolution which, in an ever expanding cell population can only have been pursued by weaving inhibition more closely into its patterns of activity and grouping and modulating it in a way which would not only allow a patterning of excitatory and inhibitory states within a small neural field, but also, by making provision for massive modulation of large neuron pools at a distance. The facilitatory and inhibitory brain stem reticular formation defined by Magoun (108) most obviously illustrates this. But what lies buried in their obviousness is the massive influence of a relatively small cell assembly upon a large neuron pool. A chemical field operating around these key areas may thus exercise field effects spreading deeply into nervous tissue, and profoundly affecting the "set" of vast cell assemblies with which it is connected. Muscle tone, stance, posture, and locomotion may be its most obvious expression in the motor field. But if it is looked at rostrally, and if its peculiar and intimate connection with the great afferent pathways is taken into account, other more complex modalities may, in fact, be governed by the same principle. We either

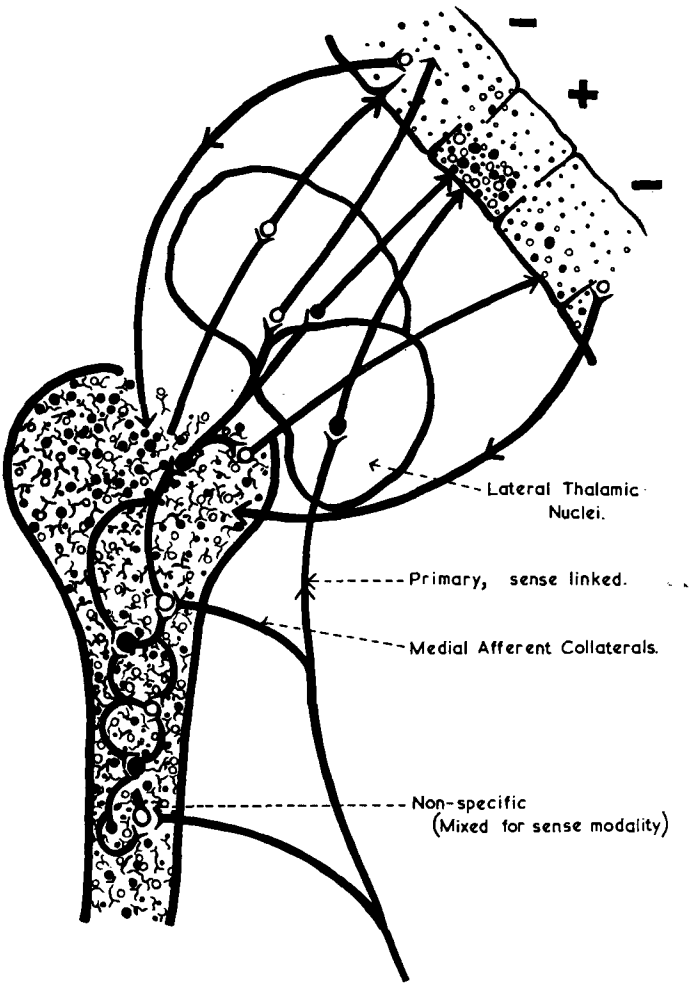

FIGURE 98. Hypothetical diagram to indicate the presence of the uneven distribution of three types of receptors (marked respectively by small solid circles, large solid circles, and open circles) in the reticular formation, and the thalamic, thalamocortical, and cortical nets. Self-excitation (indicated by closed neuron loops) may account for the autogenic generator activity within portions of the reticular activating system. Afferent signals feed into the reticular mixing pool by way of afferent collaterals. The excitation (indicated by +) of elements projecting diffusely into the cortex coincides with excitation of the primary sense-linked pathways (by way of the lateral thalamic nuclei) and with reciprocal patterned inhibition (indicated by —) emanating from midline structures. Inhibitory elements also populate the cortex itself, and the inhibitory tone exercised by cortical or subcortical elements is also shown.

wake, or sleep; we either attend or drift; we are either engaged (taken up, engagé), at grips with our outer or inner world, or we are "available"; we either "hold on" or "let go." These are crude and familiar examples and are merely cited to illustrate the obvious: namely, that seen in their extremes, they are mutually occlusive. Attention to something involves inattention and suppression of other things. Deep sleep excludes perceptual wakefulness. And it is only when we sleep lightly, when we daydream, when we are either possessed, or exhausted, or delirious, or drug intoxicated, or subject to a psychomotor attack, that this unique selecting, filtering, and coding device which we carry at the base of our skull falters. It is then that old stored patterns gain access to neurons, the contact with which renders them conscious or dimly conscious.

Standing between the afferent pathways and the vast cell assembly of the neocortical mantle, the various elements making up the upper brain stem reticular formations and the diffuse projection systems (109), and quite possibly some elements of the rhinencephalon, appear to have been favored by evolution just precisely for this filtering, and coding and patterning of afferent signals, patterns which probably involve a selective excitation and inhibition of fields of thalamocortical neurons at cortical level, simultaneously with the arrival of impulses over the specific projection systems, also at the cortical level.

I submit that the operation of these functions is dependent upon the presence of a group, and possibly quite a small group, of neurohumoral agents selected by evolution, and either identical with or more likely evolved from neuroeffector agents familiar to us at the periphery. Equally, I submit that in certain and as yet undetermined nodal areas in the brain stem, a neurosecretory process may govern the local titer of one or several of these agents and thus profoundly modulate the over-all activity of the area and the neuron pools with which it is connected. The reticular formations act as transmitter, as integrator, and as autogenic generator (110). Their neurons are dense, the axons are short, and somatodendritic potentials predominate. This would make for self-excitatory phenomena within a limited field and the powerful operation of vectorial spatial influences within this field. In such fields it is impossible to conceive of neurons functioning in isolation.

In a very crude, qualitative, and approximate way, I have tried to indicate what I mean in Figure 98, where neurons of three kinds, related but not identical, may be operating within the narrow field of the mesencephalic and diencephalic funnel, and, equally, may be represented at high subcortical and cortical levels. The great afferent pathways are sense-linked; the great reticular mixing pool is mixed for

sense modality (111). Self-excitation, possibly by branching neurons, may account for the rhythmic autogenic generator activity in this area. The excitability of cell groups within this narrow field may be regulated and modulated by delicate shifts in thresholds of concentration of neurohumoral agents locally manufactured and held in this area, and having a rather long decay time. An organized colloidal element, of special properties, part of the ground substance in this area, may perhaps be responsible for this anchoring and protection of the locally liberated neurohormone. The arrival of an afferent signal along the sense-linked path at the cortex would, according to this view, coincide not only with the excitation by way of an afferent collateral of elements in the upper reticular formation (and a consequent diffuse alteration in cortical tone) but also the simultaneous and highly patterned inhibition of other cell assemblies in the thalamocortical circuits. It is this apposition of excitation and inhibition which may, by suppressing the "ground" and bringing out the "figure," provide the basis of the analysis of perception. In this context, one recalls Pavlov's (112) ideas on the cortical analyzer, though a patterned inhibition emanating from the subcortex seems more likely. By contrast, the non-sense-linked component in the midline subcortical structures may also make for patterned, i.e., meaningful linking of sensory information coming from different sensory sources, and thus may well provide the physical substrate for the simple conditioned response. The connection of these elements with mesencephalic nuclei, the hypothalamus, and the archipallium may provide direct pathways for motor expression in the somatic, autonomic, and affective fields which govern behavior. The discrepancies between electrical activity and behavior noted previously may perhaps be accounted for by the representation of a cholinergic neuron at high brain stem level or even in the deeper layers of the cortex itself.

I have said little of the phenomenon of which we, as psychiatrists, are most familiar. Every time I administer amytal or ether to a patient and witness the emergence of a memory, and its appropriate effect, arising like Atlantis out of an ocean of oblivion, I am astonished anew by the physiologic phenomenon I am witnessing. And I experience, I confess, the same curious sentiments when I compare the phenomena seen just before going to sleep with those of hypnosis, drug intoxication, or psychomotor epilepsy. Our ignorance in this field far outweighs our body of knowledge, and the only merit of the drugs I have been discussing over the ones I have not discussed is that perhaps we are a little less ignorant of them than of the others. For some of their central actions may depend upon the possession of an overriding

property, which, by slow trial and error, may lead to a better understanding of these central effects, and, if used singly or in combination with other drugs, to an enhancement of similar effects in possible analogues. The possession of a few pharmacologic precision tools, scalpels of chemical dissection, may be helpful in interpreting the less definite, composite statistical states so common in the central nervous system.

But this is not new. Let me read you a passage written in 1867 (113). "It is deserving to remark that different nervous centers of the body manifest elective affinities for particular poisons. That medicinal substances do display these elective affinities is a proof, at any rate, that there are important, though delicate, differences in the constitution, or composition, of the different nervous centres, notwithstanding that we are unable to detect the nature of these . . . It may be that there is shadowed out in these different effects of poisons on the nervous system a means which may, ultimately, be of use in the investigation of the constitution of the latter." The author of this remarkable passage was Henry Maudsley, for whom we have named one of our great hospitals. There is little one can add to such mature reflections, except perhaps to say that hypotheses carry the seed of their own growth or decay in the experiments which quite inevitably follow them.

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