Chemical Coding of Behavior in the Brain

Stimulating the same place in the brain with different chemicals can elicit different types of behavior.

Neal E. Miller

Two classical methods for studying how the brain controls behavior have been to study the effects of destroying specified areas and to study the effects of stimulating specific sites. These methods, which have led to a great increase in our knowledge, can be illustrated by work on the hypothalamus. This is a primitive structure deep in the base of the brain, which first appears in vertebrates, such as primitive fish, and has quite similar structure in mammals from the rat to man.

If a certain small area is destroyed on both sides of the hypothalamus, the animal will stop eating and will starve to death (*1*). Conversely, electrical stimulation in this same lateral area will cause an animal which has just eaten to satiation to eat voraciously (*2*). Various studies have shown that such stimulation does not elicit mere reflex gnawing, but that it also can elicit learned food-seeking habits, and that it has many, and perhaps all, of the properties of the strong hunger that normally develops during a fast (*3*). The results of destruction and of stimulation agree in showing that the lateral hypothalamus is significantly involved in food-seeking behavior.

Additional studies, however, have shown that the effects of lesions and of stimulation in the lateral hypothalamus are not as simple as was originally supposed. Lesions in this area cause animals to stop drinking water as well as to stop eating food. Tests in which water is tubed directly into the stomach show that the interference with eating is not secondary to dehydration produced by failure to drink (*4*). Furthermore, electrical stimulation of certain sites in the lateral hypothalamus will cause animals which have just drunk water to satiation to resume drinking. Also, stimulation of this area can evoke sexual responses, such as penile erection and ejaculation (*5*). It can serve, also, as a reward: animals learn to press a bar to get a few moments of electrical stimulation in this area (*6*); yet, paradoxically, if exactly the same stimulation is provided for too long, the same animals learn to press a bar to turn it off (*7*). Lesions in the lateral hypothalamus will abolish the rewarding effect of electrical stimulation in another region of the brain, and will abolish the appetitive response to a deficiency of salt (*8*). Thus, it becomes painfully obvious that a number of diverse motivational and emotional functions all involve this tiny area of the brain, and that the techniques of making lesions or of providing electrical stimulation are relatively crude and indiscriminate in that they are likely to affect all of these diverse functions.

When diverse effects are produced from the same site, one may obscure the other. For example, stimulating the lateral hypothalamus with electric current at higher voltages produces frantic, escape-like activity which precludes the possibility of observing anything else. Perhaps we are observing only a few of the most dominant functions.

We first attempted to deal with these difficulties by using electrodes with tinier bare tips for stimulating smaller regions of the lateral hypothalamus of rats. Instead of getting more specific, better effects, we got almost none at all, and we tentatively concluded that one must stimulate a fairly large population of cells or fibers in order to produce observable behavioral effects.

Other investigators have thought it might be easier to isolate functions in the larger brain of the monkey. However, it appears that the probability of getting a given specific effect from electrical stimulation via an electrode in the hypothalamus is lower in the monkey than it is in the rat. Furthermore, stimulation of a site in the hypothalamus may elicit several effects in relatively unpredictable combinations (*9*). The picture is one of networks that are spread out and interlaced, rather than of functions that are completely separated into distinctive areas. While this diffusion of function is adaptive in preventing a small lesion produced by a tiny blood clot or infection from completely interrupting any vital function, it necessarily makes investigation of the organization of the brain more difficult.

Yet another difficulty arises from the fact that lesions and electrical stimulation both affect fibers that are merely passing through an area, as well as the synapses of neurons, at which information from various fibers is brought together and processed. To use a loose analogy, they affect the telephone cables as well as the switchboard.

The foregoing difficulties have caused workers at our laboratory, among others, recently to explore the possibility that chemical stimulation may produce effects which are more specific to given functional systems than effects produced by electrical stimulation, and may act on synapses or cells without affecting fibers of passage.

Early Evidence for Chemical Specificity

Some early behavioral evidence for "chemical" specificity came from the work of Andersson, who anesthetized goats and cemented needles into place through their skulls, by means of which minute injections could later be made into the brain of the recovered, normal, unanesthetized animal (*10*). He found that injection into the medial anterior region of the hypothalamus of goats of a solution of sodium chloride which had slightly higher osmotic pressure than body fluids have would cause an animal that had just drunk to satiation to resume drinking. We obtained similar results with cats; we showed that such an injection would elicit not only drinking but also the performance of a learned response of working for water, and that an injection of pure

The author is James Rowland Angell Professor of Psychology, Yale University, New Haven, Connecticut.

water would have the opposite effect, that of stopping either drinking or working for water (*5*). These specific behavioral effects, which are not elicited from other regions of the brain, presumably are due to the osmotic, rather than the chemical, action of the solution and are mediated by specialized sense organs or osmoreceptors in the brain.

Alan Fisher used a similar technique to test the effects of a soluble form of a male hormone, sodium testosterone sulfate, on different parts of the rat's brain (*11*). He thought that this hormone might elicit male sexual behavior. What he first observed, however, in both male and female rats, was the maternal behavior of nest-building and retrieving infant rats, activities that male rats ordinarily do not engage in. In an extensive series of subsequent investigations he has found that when this hormone is applied to the medial preoptic region of the hypothalamus it can elicit the maternal-like behavior of nest-building and of carrying infants to the nest in either male or female rats (*12*). When the same hormone is applied to a slightly different area, the lateral preoptic region, it can elicit male sexual behavior in either male or female rats. Such injections can cause male rats to mount nonreceptive females and even other males or infant rats, going through as much as is physically possible of the complete sex pattern, including ejaculation. It can cause similar behavior in female rats, with the exception, of course, of intromission and ejaculation.

When the needle delivers the hormone to a site between these two areas, mixed behavior may result; a male, for example, may alternately mate with a female and carry strips of paper to build a nest, his responses apparently being determined by the type of object that happens to fall in his sensory field.

These effects occur within from 20 seconds to 5 minutes after injection of the hormone. They are not produced by injections in other parts of the brain. Interestingly enough, the female hormone, estrogen, which has a related chemical structure, produces similar effects but is apparently less potent. These effects are produced by from 1 to 4 micrograms of either hormone, irrespective of whether it is administered in the form of crystals or as a liquid in 1-microliter amounts.

Many other chemical substances, ranging widely in osmotic pressure and

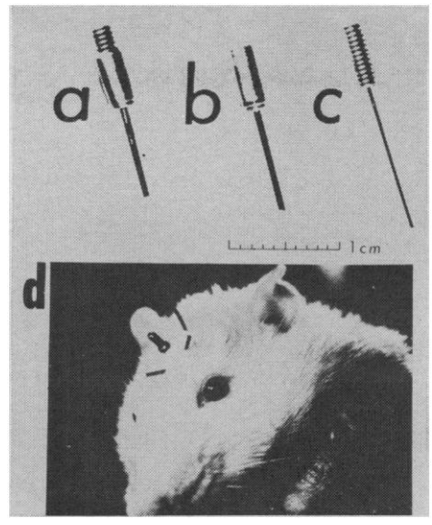

Fig. 1. Double cannula system for chemostimulation: (*a*) the complete implant; (*b*) the outer cannula; (*c*) the inner cannula; (*d*) rat with double cannula implant and three additional pins for electrical stimulation and recording. [From Grossman (*38*)]

*p*H, have been tried, with negative results. So far, the only substance that has been found to produce similar behavior is the chelating agent sodium versenate. The behavioral effects of this agent are highly similar to those of sex hormones and occur after approximately the same delay. In line with previous notions about the normal action of endogenous sex hormones, Fisher interprets their effect as being that of lowering thresholds, so that external cues will be more likely to elicit sexual responses. He does not believe that the hormone is itself a direct excitant of the brain. He advances the tentative hypothesis that a chelating agent may produce its effect of lowering threshold, or reducing inhibition, by removing calcium ions from the nerve cells.

One perplexing aspect of these experiments is the fact that in only about 10 percent of the implants does Fisher get any effect. Once he finds an animal which shows an effect, however, he often is able to reproduce this effect many times, sometimes over a period of weeks. Thus far he has been unable to determine the cause of this low percentage of effective placements—to learn whether it is due to individual differences in the degree to which cells responsible for such behavior are concentrated in one location or to other factors. The very rareness of the effect has prevented detailed analysis.

Fisher's experiments have shown that the neural circuits necessary for complex patterns of distinctively male or female behavior are present in the brains of both male and female rats. He has also shown that these patterns can be activated by the presence of sex hormones. But the fact that both of these distinctively different types of behavior can be activated by the same hormone is puzzling. What, under normal circumstances, causes activation of characteristically male responses in the brain of the male and of characteristically maternal responses in the brain of the female? It may be that the threshold is lower for the pattern appropriate to the sex of the animal in question, or that two different hormones are normally involved. In either case, since hormones, especially the steroids, frequently are not completely specific, it is entirely possible that a local application of an abnormally high dose activates a response not normally governed by the particular hormone applied. Thus, testosterone might be the normal hormone for eliciting male sexual behavior and some other, as yet untested, hormone might change the normal one for eliciting maternal behavior.

Somewhat similar experiments have been performed by Harris and Michael on cats previously subjected to ovariectomy and hence not sexually receptive (*13*). These workers made up a tiny pellet of paraffin containing a synthetic female hormone, stilbestrol; when this was implanted in the hypothalamus the stilbestrol would slowly diffuse into the body. They found that when large pellets were implanted outside the brain or in inactive sites in the brain, changes in vaginal cells always occurred at doses smaller than those required to restore sexual receptivity.

When pellets were implanted in the hypothalamus, however, doses too small to produce changes in vaginal cells restored complete receptivity. In fact, the cats could be described as hypersexual; they would repeatedly accept successive males at any time of the day or night, without showing any signs of the refractoriness which normally follows mating. These effects first appeared only several days after implantation of the pellets containing the slowly diffusing form of the hormone. However, the proportion of cases in which the effects occurred was much higher than the proportion of cases in which the male sexual behavior or the maternal behavior was elicited in rats by the soluble hormones which Fisher used.

Differential Effects of Presumptive Transmitter Agents

The results just described show that specific forms of behavior can be produced by applying directly to the proper part of the brain certain chemical substances that are normally found in the body. The experiments described below differ in that they involve use of a special class of substances believed to act as transmitters in the synapses of the more readily accessible and more thoroughly studied peripheral nerves. Transmission in the synapses of the parasympathetic nervous system is via acetylcholine. Chemicals having this effect are called cholinergic. Similarly, norepinephrine seems to be the transmitter for at least some of the synapses in the sympathetic nervous system. Chemicals having this effect are called adrenergic. Does similar chemical coding of transmission occur in the brain, and if it does, is it related to specific forms of behavior? Biochemists have shown that the hypothalamus is especially rich in both acetylcholine and norepinephrine. What are they doing there?

Effects of same agent in different sites. Investigators such as Delgado, Feldberg, and MacLean have shown that injection of acetylcholine into the brain of a cat can evoke a variety of responses (*14*). When injected at one site it produced motor responses such as circling. When injected at another it produced catatonic-like postures; at another, rage; and at yet another, convulsions, followed by purring and other apparent manifestations of pleasure. In addition to the naturally occurring substance acetylcholine, these investigators used a synthetic substance, carbachol, which is similar in structure and in cholinergic effects. Because of a slight difference in its molecule, carbachol is not disposed of by the deactivating enzymes as rapidly as acetylcholine is, hence its effects last longer.

Effects of different agents in the same site. Encouraged by these results, Grossman, in our laboratory, tried implanting, in the "feeding-drinking" area of the lateral hypothalamus, minute crystals (weighing from 1 to 4 μg) of various substances thought likely to act as transmitter agents (*15*). An anesthetized rat was placed in a stereotaxic instrument with its head at the proper angle for insertion of a tiny cannula through a hole drilled in the skull. When the tip of this cannula was in the desired location the cannula was cemented into place and the skin was sewed together; the rat recovered and appeared perfectly normal and healthy. Figure 1 (top) shows photographs of the cannula. It consisted of a tiny hypodermic needle fitting snugly inside a slightly larger one; the two tips were cut off flush and the hubs were machined down so that the inner needle could screw into the outer one. After the rat had completely recovered, the inner cannula was withdrawn, tiny crystals were tapped into the tip, and the cannula was reinserted.

By this means Grossman found that either acetylcholine (mixed with eserine to delay its destruction by enzymes in the brain) or carbachol would cause rats that had just eaten or drunk to satiation to start drinking again within 5 to 10 minutes after implantation of the cholinergic agent and to consume an average of 12 milliliters of water during the next hour. These cholinergic agents would also cause satiated rats to work at the learned response of pressing a bar to get water. Subsequent implantation of tiny crystals of the adrenergic substances epinephrine or norepinephrine into the brains of the same satiated rats, at exactly the site where the cholinergic agent had been implanted, via the same cannulas, elicited a different response—eating, or pressing a different bar, one that delivered food.

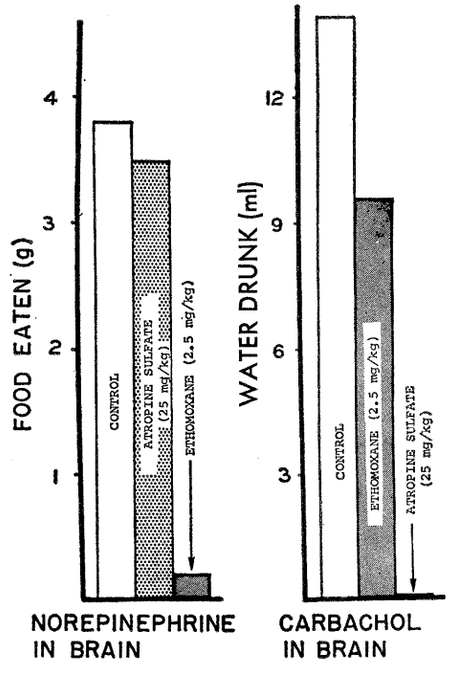

Fig. 2. Differential effects of blocking agents on eating and drinking elicited by application of norepinephrine and carbachol, respectively, in the lateral hypothalamus of the rat. First, blocking agent is injected systemically, then crystals of norepinephrine or carbachol are implanted in the brain. [Data from Grossman (*16*)]

When carbachol was implanted in the brains of thirsty rats, the amount of water drunk was increased and the amount of food eaten was decreased, relative to the results for nonthirsty rats. Implantation of norepinephrine in the brains of hungry rats had the opposite effect, that of decreasing water consumption and increasing food consumption.

Whereas in previous studies different types of behavior were induced by implanting the same substance at different sites, in Grossman's study different types of behavior were induced by implanting different substances at the same site. These results clearly showed that systems located in the same region of the brain but controlling different types of behavior can be selectively affected by cholinergic or adrenergic substances.

Effects of blocking agents. It is conceivable that the different behaviors induced by the administration of different transmitter agents to the brain could be due to some differential side effects, rather than to the transmitter, or even due to a disinhibitory or modulating effect in the brain. Therefore, a number of control studies were made; these ruled out osmotic pressure, *p*H, and vasoconstriction or vasodilation as factors in the behavioral effects observed.

The most convincing control studies, however, were of a different nature. Studies on more easily isolated and manipulated peripheral nerves have shown that atropine blocks only the transmitting effect of carbachol or acetylcholine in such nerves and fails to block that of norepinephrine; conversely, ethomoxane blocks the effect of epinephrine or norepinephrine without affecting that of acetylcholine or carbachol. Therefore, Grossman investigated the effects of these blocking agents on the behavioral effects of chemostimulation in the brain (*16*). He injected the blocking agent into the peritoneal cavity so that it was circulating throughout the body before the crystals were applied to the brain.

He found that either blocking agent,

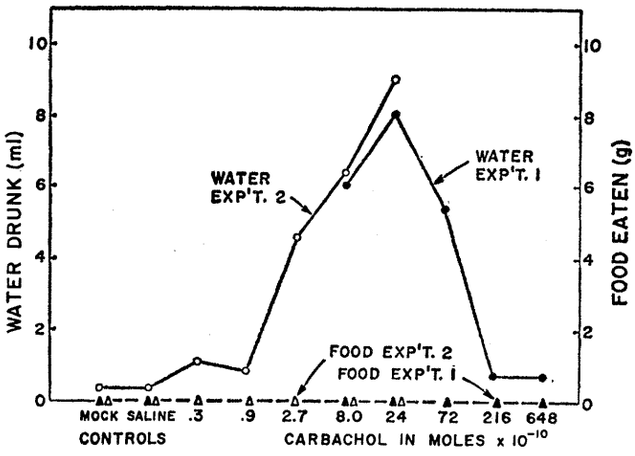

Fig. 3 (left). Dose-response curve for drinking elicited by injections of carbachol into the lateral hypothalamus of rats that had eaten and drunk to satiation. No eating is elicited. [From Miller *et al.* (*18*)]

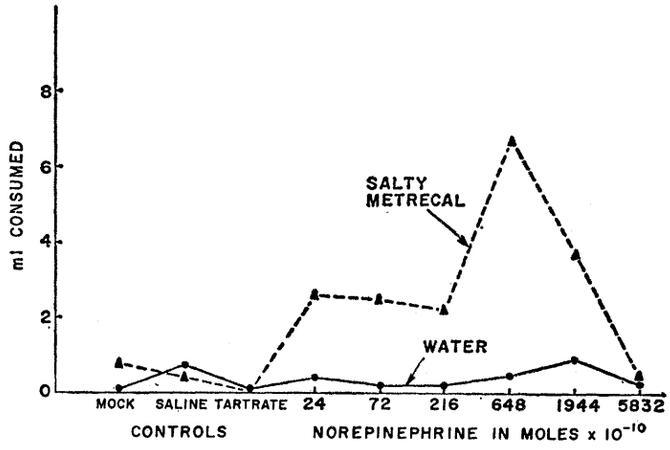

Fig. 4 (right). Dose-response curve for consumption of a liquid food (salty Metrecal) elicited by injection of norepinephrine into the lateral hypothalamus of rats that had eaten and drunk to satiation. Negligible amounts of water are consumed. [From Miller *et al.* (*18*)]

if administered at sufficiently high dosage, would prostrate the rat and eliminate all behavior. But at an intermediate dose, results were differential. Results for such a dose are summarized in Fig. 2. It may be seen that the cholinergic blocking agent atropine produced little reduction in the eating elicited by norepinephrine, while completely eliminating the drinking elicited by carbachol. Conversely, the adrenergic blocking agent ethomoxane produced only a moderate reduction in the drinking elicited by carbachol but a much larger reduction in the eating elicited by norepinephrine. These results strongly indicate that the differential behavioral effects are indeed due to the cholinergic and adrenergic properties, respectively, of the implanted substances.

While these results make it clear that the systems responsible for the eating and drinking are differentially susceptible to acetylcholine and norepinephrine, it is possible that these drugs produce their effects in some abnormal way and are not the normal transmitter substances for these systems. If they are indeed the normal transmitters, we would expect the blocking agents to have differential effects on normally elicited hunger and thirst analogous to their effects on hunger elicited by norepinephrine and their effects on thirst elicited by carbachol. And, indeed, Grossman found that such differential effects on normally elicited hunger and thirst could be induced either by systemic injection of the blocking agent or by direct introduction of the blocking agent into the lateral, or feeding-drinking, area of the hypothalamus. While these differential effects were not as complete as those illustrated in Fig. 2, the differences were statistically highly reliable.

Furthermore, Coons and I have found that an injection, into the body, of atropine methyl nitrate, which does not readily cross the blood-brain barrier, produces much less reduction in drinking by water-deprived rats than does an injection of atropine sulfate, the form of atropine used by Grossman, which readily gets into the brain (*17*). This shows that the interference with thirst is primarily due to the action of atropine in the brain, rather than to its action on peripheral structures.

Effect of enzyme inhibitor. It is known that eserine inhibits the action of the cholinesterases, the enzymes that normally deactivate acetylcholine soon after it is released at the synapse. Therefore, if acetylcholine is indeed the transmitter normally involved in the thirst system, one would expect an injection of eserine into a synaptic region of this system to cause the normal acetylcholine to last longer, so that it would stimulate more transmission and thus cause more drinking. And indeed Chun-Wuei Chien and I have recently found that an injection of 3×10^{-8} mole of eserine into the preoptic area of the brain of a rat very slightly deprived of water will increase the amount it drinks during the next 30 minutes from an average of 0.3 milliliter to 10.5 milliliters. In addition to being an independent test of our conclusions, this is a more powerful test than that of injecting a cholinergic substance for demonstrating the normal participation of such a substance in thirst.

Dose-response curves. A dose-response curve is useful in determining whether an apparent differential effect is an artifact of a particular dose-threshold interaction and whether the amount of substance needed to produce an effect is of the order of magnitude one might reasonably expect for the normal process.

While Grossman's use of crystals prevents spread up the needle shaft, with crystals the concentration is highly abnormal and it is impossible accurately to control the dose. Therefore, in the dose-response study performed in our laboratory, aqueous solutions were used (*18*). The results are presented in Figs. 3 and 4. It may be seen that there is a marked differential effect of all active doses of the carbachol and norepinephrine. Increasing the dose of carbachol produces marked increases in drinking but not in eating, while increasing the dose of norepinephrine produces marked increases in eating but not in drinking. With either substance, the highest doses produce side effects, such as convulsions, which interfere with eating and drinking.

The carbachol elicits drinking at doses as low as 2.7×10^{-10} mole (0.047 μg); the smallest dose of norepinephrine that elicits eating is 24×10^{-10} mole (0.8 μg). The effective dose for carbachol seems to be some-

where near the content of the normal substance, acetylcholine, in the volume of tissue probably reached by our injections. The norepinephrine content of this part of the brain probably is no higher (in micrograms) than the acetylcholine content, but our effective dose of norepinephrine is approximately 20 times as high. This seems to be a high dose, but in our present state of considerable ignorance we cannot be sure that more of the norepinephrine reaches the receptor sites after an injection than is involved in normal transmission.

It should be noted that both dose-response curves cover the whole range, from the dose producing no effect to that producing prostrating side effects. In neither case is there any suggestion that low doses activate one behavioral system while higher doses cause the other system to be dominant. Thus, these results agree with those of the previous experiments in indicating that the difference between the systems is indeed a qualitative one, rather than the product of a mere quantitative interaction of the thresholds and the dominance of the systems with a nonspecific difference in the potency of the cholinergic and adrenergic compounds used.

Central versus peripheral effects. Dextroamphetamine is a compound closely resembling norepinephrine in its general structure and many of its physiological effects. But extensive clinical experience with people and careful experimental work with animals consistently show that dextroamphetamine lessens appetite. Why should these effects be opposite to those just described for norepinephrine?

There are two plausible possibilities. One is that the dextroamphetamine molecule is similar enough to the norepinephrine molecule to attach itself to the same receptor sites in the brain, but not similar enough to excite them. According to this hypothesis, dextroamphetamine acts like a bad key which jams in a lock without opening it. The fact that dextroamphetamine is administered peripherally, either by mouth or by injection, suggests the other major possibility: the peripheral action of substances of this class may be opposite to, and may occur at lower thresholds or be stronger than, their action in the feeding-drinking area of the brain; thus, when the substance is administered to the whole body, the peripheral effects may override the effects of the substance diffusing from the blood to the brain.

In order to test these hypotheses, Coons, Quartermain, and I used the dose of norepinephrine (22 μg) that had been shown by the dose-response study to be optimum for eliciting eating when injected into the lateral hypothalamus. We injected exactly this dose into the jugular vein, via a permanently implanted catheter, and washed it down with isotonic saline. This injection caused hungry rats to stop eating, or to stop pressing a bar for food. These results demonstrate that the central and the peripheral effects of the same dose of norepinephrine are indeed antagonistic. They fit in with previous unpublished results, obtained by Coons in our laboratory, which show that peripheral administration of another adrenergic compound, epinephrine, raises the threshold for eating elicited by electrical stimulation of the lateral hypothalamus, and with results of Russek and Tina which show that peripheral administration of epinephrine will stop eating (*19*). Since we found that the same peripheral dose of norepinephrine stopped drinking as well as eating, it seems likely that it produces some general disturbance, perhaps as a result of a sudden increase in blood pressure, rather than a specific inhibition of a drive.

The norepinephrine injected into the blood stream becomes vastly more diluted than that affecting the cells adjacent to the cannula in the brain. Thus, the fact that the same dose is quite effective by either route is surprising. Perhaps the peripheral receptors are much more sensitive than the central ones, or the central receptors may be so well protected that only a minute fraction of the chemicals in the surrounding tissue reaches them. We must also consider the possibility that the consistent pattern of central-nervous-system results we have described is only an effect of a dose that is abnormally high, and thus does not necessarily mean that norepinephrine is involved in normal transmission in this part of the brain.

If norepinephrine does indeed act as a normal neural transmitter in the brain, its natural release should be limited to the active sites and not be diffused widely. Some of the norepinephrine which we inject into the lateral hypothalamus must diffuse into the bloodstream, where, presumably, it produces appetite-inhibiting peripheral effects. These effects may well mask some of the central hunger-arousing effects of the norepinephrine injected into the brain. If we could eliminate the peripheral effects by administering to the body a blocking agent for norepinephrine that would have difficulty in getting into the brain across the blood-brain barrier, we could eliminate or greatly reduce the antagonistic peripheral effects and might get a clearer picture of how norepinephrine works in the brain. To date, we have been unable to find such an agent, but it seems likely that side chains could be attached to the molecule of a suitable adrenergic blocking agent to produce a drug that would have difficulty in diffusing through the blood-brain barrier.

Activating Various Elements of a General Homeostatic System

Although carbachol in the brain elicits drinking and norepinephrine elicits eating, it is conceivable that these drugs do not directly stimulate thirst and hunger systems in the brain but, instead, produce their effects indirectly. For example, carbachol could stimulate nuclei in the brain which, in turn, stimulate a sudden water loss via the kidney, the subsequent thirst and drinking being a by-product of the normal reaction of the body to the rapid dehydration. Similarly, norepinephrine could stimulate nuclei to send neural impulses that would cause the pancreas to secrete insulin; this would produce a drop in blood sugar and elicit eating. It is known that a peripheral injection of insulin can cause rats just fed to satiation to eat. Thus, it is possible that the thirst and hunger mechanisms are not themselves chemically coded, but that the differential effect of these chemicals is on some completely different systems which activate the thirst and hunger mechanisms in some indirect way. In the examples cited, which are the most plausible ones, we would expect the carbachol in the brain to produce a sudden excessive secretion of urine (in other words, diuresis), and the norepinephrine to produce a drop in the blood sugar, or glucose.

There is another possibility, however, from which one would predict the opposite results. Perhaps we are activating general homeostatic sys-

tems, which regulate food and water balance by activating a variety of mechanisms, some physiological and some behavioral, to deal with any deficit. If we are activating the entire system that normally responds to water deficit, we would expect one corrective measure to be stimulation of the secretion of the antidiuretic hormone which causes the kidney to reabsorb water from the urine, so that a smaller volume of more concentrated urine is lost. While thus conserving water, the animal would also go out looking for water to drink to replenish its supply. Similarly, the activation of an entire system for responding to a nutritional deficit would mobilize stores, such as those in the liver and fat, pouring them out as glucose into the blood, and at the same time would elicit hunger which would motivate the animal to find and eat food as a new source of nutrition. While the hypothesis given in the preceding paragraph predicts increased secretion of urine and a drop in blood sugar in response to stimulation of the brain by carbachol and norepinephrine, respectively, this second hypothesis predicts the opposite results.

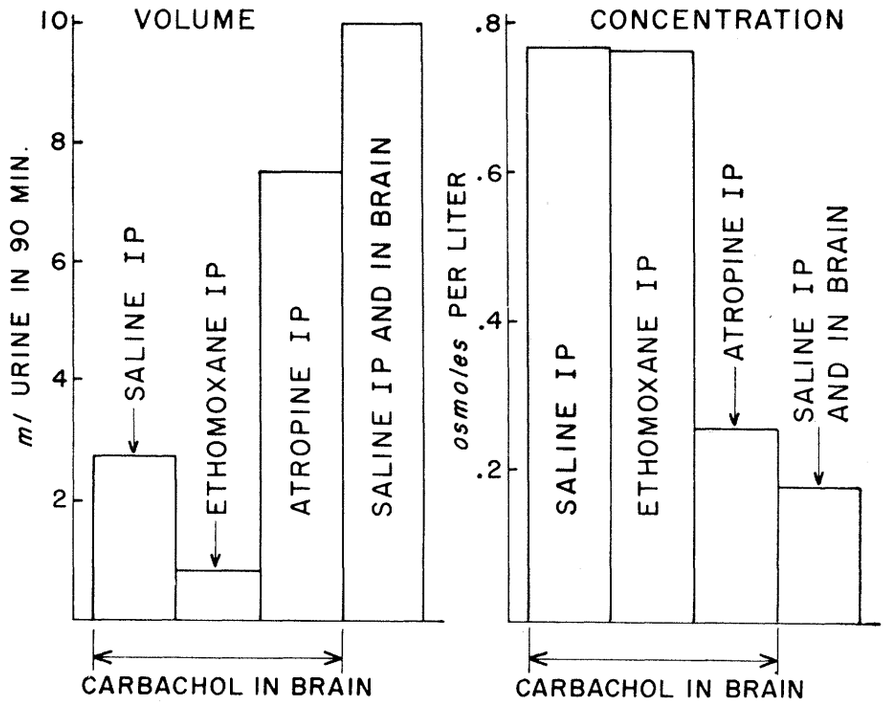

Fig. 5. Effects of injecting carbachol into the lateral hypothalamus of unanesthetized rats. Such injection reduces the volume and increases the concentration of urine. Prior intraperitoneal (*IP*) injection of the cholinergic blocker atropine reduces these effects, but injection of the adrenergic blocker ethomoxane does not. Injections of the isotonic saline used as the vehicle serve as controls. [Unpublished recent data of Miller and Chien]

Effects on secretion of urine. Chun-Wuei Chien and I have recently tested the effects on urine secretion of injecting into the lateral hypothalamus the optimum dose of carbachol (0.43 μg) for eliciting drinking. First, we briefly anesthetized water-satiated rats with ether and gently squeezed them to remove the residual urine from their bladders; we then administered, via stomach tube, 15 milliliters of water and injected into the hypothalamus either 1 microliter of carbachol solution or, as a control, the isotonic saline used as the vehicle. After this the rats were put in metabolism cages without food or water, and the urine was collected via stainless steel funnels underneath the cages.

We found that the carbachol greatly reduced the volume of urine. It also increased the concentration, as measured by the freezing-point-depression test of osmolarity; this increase in turn showed that the decrease in volume was caused by reabsorption stimulated by the secretion of the antidiuretic hormone normally released in response to water deprivation (*20*). If the carbachol had merely caused some interference with kidney function—for example, by producing a drop in blood pressure that interfered with the initial secretion by the kidneys—the decrease in volume would not have been accompanied by an increase in concentration.

Similar results were obtained from another part of the brain, the preoptic area; carbachol in this area, too, elicits drinking. Injection into the jugular vein (via an implanted cannula) of exactly the same dose as that given in the earlier studies (the optimum dose for eliciting drinking when injected into the brain) neither appreciably elicited drinking in water-satiated rats nor decreased the volume and increased the concentration of urine of water-satiated rats that had been given additional water by stomach tube. Administration of this same dose to thirsty rats produced no obvious lessening of drinking. Thus, with carbachol, at least at this dose level, we do not find the marked differences in the behavioral effects of central and peripheral administration that we observed with norepinephrine.

To return to the antidiuretic effect of carbachol injected into the brain, if this is indeed a cholinergic effect, it should be blocked by atropine but not by ethomoxane. Chun-Wuei Chien and I tested these expectations. First we injected intraperitoneally either the blocking agent or isotonic saline as a control. We used the doses that had produced the greatest differential effects on drinking and eating. Then, 20 minutes later, after the agent had had time to diffuse through the body, we gave the water-satiated rats additional water by stomach tube and injected into the lateral hypothalamus either the optimum dose of carbachol or a control dose of the isotonic saline used as the vehicle.

The results are shown in Fig. 5. It may be seen that, as compared with the injection of saline, both intraperitoneally and into the brain, injection of carbachol into the brain (preceded by a control injection of saline) greatly reduced the volume and increased the concentration of the urine. Previous injection of the adrenergic blocking agent ethomoxane did not interfere with this antidiuretic effect, but previous injection of the cholinergic blocking agent atropine markedly reduced it. In another experiment we injected these two agents into water-satiated rats given additional water by stomach tube but not injected with carbachol. Neither agent produced a marked effect. Thus, the effects ob-

served in the preceding experiment presumably were due to differential blocking of the effects of carbachol rather than to any other direct effects of these agents.

Our results confirm those of previous investigators who have concluded, from work on anesthetized dogs, that secretion of the antidiuretic hormone is mediated by a cholinergic link in the preoptic area (*21*). Our results show that such links exist in at least two areas—the lateral hypothalamus and the preoptic area—in which carbachol can elicit drinking and that they are strongly stimulated by the dose that elicits the most drinking. The results show that the carbachol does not produce behavioral manifestations of thirst in some indirect way, but that it activates a general water-conserving mechanism, one aspect of which is conservation of water by reabsorption in the kidneys and another aspect of which is motivation of animals to perform learned water-seeking habits.

As an extension of the foregoing study we injected norepinephrine into the lateral hypothalamus of a number of rats at the dosage (22 μg) that we had found optimum for producing eating. While the major response of the rats was eating, to our surprise some of them drank a few milliliters of water before starting to eat. When we investigated the effects of norepinephrine on the secretion of urine, we found in general little, if any, effect in the animals whose first response had been eating but some reduction in the volume, and some increase in the concentration, of urine in the rats which had done a little preliminary drinking. These antidiuretic effects observed to date have been considerably less than those elicited in our experiments with carbachol, but we cannot draw any definitive conclusions until we have completed dose-response studies. We must also determine the action of blocking agents on these effects. When stained slides of these rats' brains are completed, it will be interesting to see whether or not the location of the implants differs slightly for the animals which showed only an eating effect and for those which showed also a slight drinking effect.

Effects on blood sugar. Coons, Booth, Pitt, and I have shown recently that the same electrical stimulation in the hypothalamus that causes satiated rats to eat produces a marked elevation in blood sugar during tests in which no food is present. This result suggests that stimulation in that area of the brain activates a general homeostatic mechanism which deals with a nutritional deficit both by increasing blood sugar and by motivating the animal to get food to replenish the deficit.

We are using chemostimulation to further investigate this problem. Some recent results are shown in Fig. 6. In these tests, no food is present. Samples of blood are taken from the tip of the animal's tail at specified intervals and analyzed by the glucose oxidase method. The portions of the curve for time prior to injection of substances into the lateral hypothalamus show a gradual rise; this rise continues with negative acceleration following the injection of isotonic saline into the brain, suggesting that the stress of handling and the taking of successive samples of blood produces some increase in the level of blood glucose. This increase, however, is definitely less than that which follows injection of the norepinephrine into the brain, a finding which shows that the mechanism by which the norepinephrine injection elicits eating is not that of stimulating insulin secretion and thus producing a drop in blood sugar. The results obtained thus far are symmetrical and esthetically satisfying and favor the hypothesis that norepinephrine activates a general mechanism for correcting nutritional deficits.

From such an hypothesis, one would not expect carbachol to produce an increase in blood sugar. But, as Fig. 6 clearly shows, the carbachol produces a considerably greater increase than the norepinephrine does. This unexpected result shows that our understanding of what is going on is still far from complete; it presents us with a puzzle that we would not have discovered had we confined our studies to electrical stimulation.

When the same dose of carbachol or of norepinephrine that was injected into the brain is injected into the jugular vein by means of an implanted cannula, the carbachol has no measurable effect, but the norepinephrine has approximately the same effect that it has when injected into the brain; the latter result suggests that at least some of the increase in blood sugar following the injection of norepinephrine into the brain could be caused by peripheral effects after it has been absorbed into the blood.

Effects on shivering and temperature. Feldberg and Myers recently have shown that both shivering and an increase in body temperature are elicited by the injection into the anterior hypothalamus of a cat of a few micrograms of serotonin, an amine normally found in this area (*22*). Conversely, injections of either epinephrine or norepinephrine inhibit such shivering and reduce the fever. They also cause a drop in the temperature of normal cats. Injections outside this general area of the brain do not produce such effects.

Evidence that serotonin is indeed involved in normal temperature regulation comes from a study in which Canal and Ornesi showed that cyproheptadine, an agent that blocks serotonin, inhibits a fever induced by injecting typhoid vaccine into the ventricles of the brain (*23*). These studies suggest that the central mechanisms controlling shivering and temperature regulation may be chemically coded, and the results add a new substance, serotonin, to the list of substances known to produce differential effects.

But there is an intimate relationship between temperature, on the one hand, and food and water regulation on the other. For example, Andersson and Larson found in the goat that local cooling in the preoptic area, which is adjacent to the anterior hypothalamus, will elicit eating and shivering, while heating will stop eating and elicit panting and drinking (*24*).

These facts suggested to us the desirability of studying, in the rat, the effects on body temperature of injecting carbachol and norepinephrine into the lateral hypothalamus in the doses that are optimum for eliciting drinking and eating, respectively. Coons, Levak, Wechsler, and I found that the norepinephrine produces a drop in temperature of approximately 0.5°C, while the carbachol produces approximately twice this drop in temperature. Except for the fact that the effect is a drop instead of a rise, the curves look strikingly like those of Fig. 6. Furthermore, as was found for blood sugar, the effects of this dose of norepinephrine are much the same regardless of whether it is injected in the vein or in the brain, but injection of this dose of carbachol by vein has no measurable effect.

To summarize, the effects on body temperature of injecting norepinephrine in the vein, norepinephrine in the brain, and carbachol in the brain are similar to each other and opposite to those of injecting serotonin in the brain. The effects on blood sugar of injecting carbachol in the brain and norepinephrine in the brain and in the vein are also similar to each other. But the effects on food intake of injecting norepinephrine in the vein and in the brain are opposite. Injection of norepinephrine in the lateral hypothalamus typically increases food intake and decreases water intake, while such injection of carbachol typically has the opposite effects.

It seems unlikely that the drinking elicited by injection of carbachol in the lateral hypothalamus and the eating elicited by injection of norepinephrine are secondary to temperature changes, since these two substances change temperature in the same direction, while a given change in temperature has opposite effects on eating and drinking. The fact that norepinephrine in the central system and norepinephrine in the peripheral system produce similar effects on body temperature but opposite ones on eating also argues against the hypothesis that the effect of this substance on temperature produces its effect on eating.

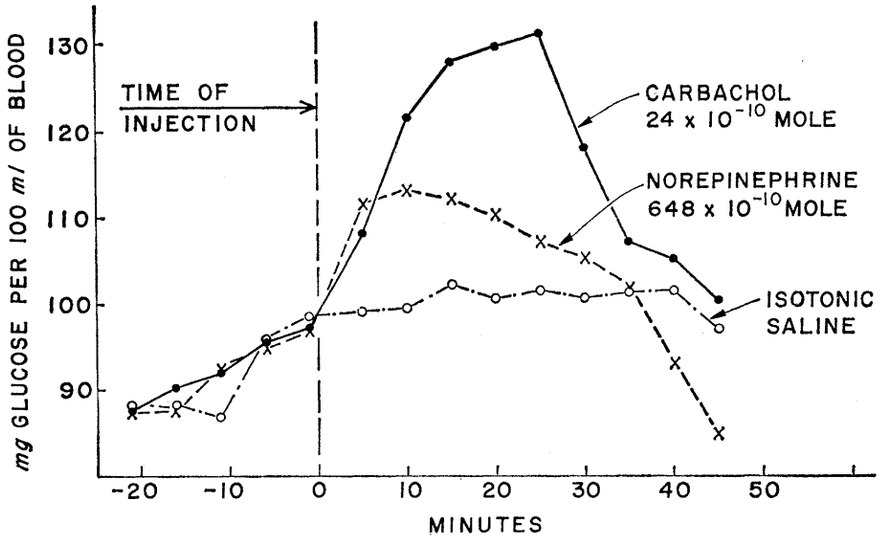

Fig. 6. Effects on blood glucose of injecting norepinephrine and carbachol into the lateral hypothalamus of rats. As compared with an injection of isotonic saline, an injection of the dose of norepinephrine that is optimum for eliciting eating produces an increase in blood glucose, but an injection of the dose of carbachol that is optimum for eliciting drinking produces an even greater increase. No food and water are given during these tests. [Unpublished recent data of Coons, Booth, Pitt, and Miller]

Use of Chemostimulation for Tracing Neural Circuits

Since Grossman's initial demonstration, a number of investigators have been using the selectivity of chemostimulation to trace the circuits involved in various behavioral effects.

The thirst system. Fisher and Coury have found that injection of carbachol into any of the ten different structures they have investigated throughout the limbic system will elicit drinking (*25*). This is a system that extends beyond the hypothalamus and is characterized by a number of closed loops and other intricate interconnections which seem to provide the anatomical basis for sustaining activity by central positive feedback, as well as providing alternate pathways and intimate intercorrelations between various functions. They have also found that carbachol in any of ten nearby areas outside this circuit does not elicit appreciable drinking.

While Fisher and Coury found that the relationship between cholinergic stimulation and drinking throughout the limbic system was remarkably specific, they did find a few cases in which carbachol increased both eating and drinking. And we have noted instances in which norepinephrine elicited some slight drinking. Myers has noted even more drinking elicited by norepinephrine (*26*). Also, as discussed earlier, the effects of carbachol and norepinephrine on blood sugar are similar. These exceptions are puzzling. The effects of blocking agents on these exceptional effects have not yet been investigated. These exceptions may indicate that the same system changes its chemical coding in different parts of the brain; they could be produced by interactions between different systems that are differently coded, or they might have still other implications. We do not yet know the answer.

The reward system. It has long been known that the limbic system is involved in emotional behavior. As Fisher and Coury point out, there is a remarkable parallelism between the brain structures from which they can elicit drinking by injection of carbachol and those from which MacLean and Ploog, by electrical stimulation, have elicited penile erection (*27, 28*). Furthermore, these are the same structures in which electrical stimulation has been shown, by Olds, to act as a reward to reinforce learning and maintain the performance of learned habits (*6*).

In addition to investigating the rewarding effects of electrical stimulation of the brain, Olds has recently tested the rewarding effects of various chemicals applied in minute amounts to different sites in the brain (*29*). In these studies the rat is in a small cage containing a bar. Every time the rat presses the bar he activates a device which injects approximately 3 millimicroliters of solution via a thin polyethylene tube leading to a special needle permanently implanted in the skull. If the injection is rewarding, the rat learns to press the bar and works to give itself injections. Olds gets rewarding effects from injecting acetylcholine or carbachol in certain brain areas, while norepinephrine, epinephrine, and serotonin have the opposite effect of reducing the rewarding effect of another solution. He also gets good rewarding effects with chelating agents, and the best of all with testosterone sulfate. It is interesting to note that these two latter agents are the only ones with which Fisher could elicit either male sexual or maternal nest-building and infant-retrieving behavior in the rat.

Specificity of responsiveness of overlapping systems. As further evidence for the specificity of response to chemostimulation of overlapping systems, Fisher and Coury have found

one animal that consistently responded to injections of carbachol by drinking, to injections of norepinephrine by eating, and to injection of a soluble sex hormone by building nests (*25*). All three chemicals were applied to the same site at the junction of the area of the diagonal band of Broca and the medial preoptic region, and all three effects were specific.

Variety of tests required. Certain studies which Grossman has made since he left our laboratory have indicated further lines of fruitful work on chemical stimulation and, at the same time, the difficulty of the survey that is needed to give us a more adequate picture of the brain (*30*). He found that carbachol in the medial septal area elicited drinking in water-satiated rats and increased the water consumption of thirsty ones. It also depressed the food intake of hungry rats and impaired the learning and the performance of responses which allowed the rat to avoid electric shocks.

Implantation of the cholinergic blocking agent atropine into this area of the brain reduced the water consumption of thirsty rats and improved the performance of a previously acquired avoidance response. Implantation of noradrenaline had no effect on intake of food or water but improved the avoidance response; hence, in this respect, noradrenaline acted oppositely to carbachol. Thus the inclusion of an avoidance-learning situation, presumably measuring fear-motivated behavior, proved to be a useful addition to the tests.

Grossman observed no marked or consistent effects of chemostimulation of the ventral amygdala of satiated rats (*31*). From this one might conclude that there were little, if any, effects of chemostimulation in this area on hunger or thirst. But when he tested animals that had been deprived of food and water, he found that norepinephrine increased the food intake and decreased the water intake, while administration of an adrenergic blocking agent, dibenzyline, in the same area of the brain had the opposite effect of decreasing food intake and increasing water intake. Cholinergic agents produced symmetrically opposite results; carbachol decreased food intake and increased water intake, while the blocking agent atropine tended to increase food intake but decrease water intake.

A number of other agents and control substances had no observable effects. Gamma-amino butyric acid, however, produced results similar to those produced by carbachol. This substance, which is normally found in the brain, is believed not to have any cholinergic effect. Thus, it is possible that it acted by blocking an inhibitory system. Recent results by Booth at Yale fit in with such an interpretation of the effects of gamma-amino butyric acid. Booth injected this substance into the ventromedial nucleus of the hypothalamus, an area known to exert an inhibitory effect on hunger. He found that such injections markedly increased the rate at which rats pressed a bar to get food without producing comparable increases in rates of pressing other bars to get water or to avoid an electric shock.

To return to Grossman's experiments on the amygdala, the fact that the effective chemicals did not elicit any appreciable results with satiated animals, but only with animals deprived of food and water, suggests that the function of the sites he stimulated is to modulate ongoing activity rather than to initiate activity.

Even when one is investigating a limited aspect of behavior, such as motivation, there are various different types of tests which are needed in initial screening investigations. Such tests should be made not only when the appropriate motive is initially absent but also when it is present. After an effect is discovered, additional tests are often needed to check alternative interpretations of the results. We have illustrated in detail elsewhere (*32*) the desirability of using a variety of behavioral tests to cross-check all conclusions.

In each of these behavioral tests one must try the effects of a number of different presumptive transmitters, and also blocking agents. Each of these must be tested at a number of dosages, since our inverted U-shaped dose-response curves in Figs. 3 and 4 show that one can fail to get results by using either too high or too low a dose. Furthermore, this process must be carried out for a great number of different points in the brain, sometimes separated by as little as 1 millimeter. It is obvious that a thorough survey will involve an enormous amount of work, and also that there are many opportunities for significant discoveries.

Combination of Classical and Chemostimulation Techniques

The classical technique of destroying a certain part of a pathway can be combined with the technique of chemostimulation to determine the direction of the pathway, or to indicate what are the most critical parts of the system. Thus, Wolf and I have shown (*33*) that a lesion in the feeding-drinking area of the lateral hypothalamus eliminates drinking elicited by chemostimulation at sites anterior or posterior to this location, while lesions in the anterior or posterior sites do not eliminate drinking elicited by chemostimulation in the lateral hypothalamus. Such results show the special importance of the lateral hypothalamic area.

Similarly, Paolino and I are combining the technique of electrical stimulation of brain areas in reward and punishment experiments with the use of reversible biochemical lesions. When a general blocking agent, such as the local anesthetic Xylocaine, is used to make such a lesion in the lateral hypothalamus, it greatly reduces the rewarding effects of electrically stimulating the anterior part of the medial forebrain bundle but seems to have less effect on the punishing effects. Having used a general blockade by a local anesthetic to confirm the location of a crucial point in the system, we are now exploring the effects of specific blocking agents to produce biochemical lesions that are not only reversible but also selective, and to throw light on the chemical coding of the system.

Additional combinations of classical and chemostimulation techniques are used in studies of the sleep and arousal systems.

Sleep versus arousal in the cat. Hernandez-Peon and his colleagues have demonstrated the usefulness of chemostimulation for dissecting out, in more detail than had been possible in work with electrical stimulation, the brain circuits involved in sleep and in arousal (*34, 35*). At the same time his work demonstrates a puzzling species difference between effects on the rat, the animal used for most of the studies so far described, and the cat, which he used. Thus, these results provide still further evidence for both the fruitfulness of work with chemostimulation and the magnitude of the task.

Hernandez-Peon and his co-workers found that the sleep system involves a number of pathways descending from the cortex through the limbic midbrain circuit, and also an ascending component coming up from the spinal cord through the medulla and pons to the midbrain. Along the pathways of this circuit, stimulation with acetylcholine or carbachol elicits sleep. As might be expected, if the normal transmitter is cholinergic, injection into this system of eserine, which is known to inhibit the enzymes that destroy natural acetylcholine, will elicit sleep. By preventing the destruction of the transmitter, presumably the eserine potentiates impulses that are a part of the normal background activity of this system, and thus tips in the direction of sleep the balance between such impulses and those of arousal.

Additional evidence that the chemostimulation is indeed cholinergic comes from the finding that it can be blocked by atropine. If one point in a circuit is stimulated by an injection of acetylcholine and a crystal of atropine is placed downstream, the atropine blocks the induction of sleep. But if the location of the injections of the two substances is reversed, no blockade occurs. Tests involving the use of atropine, production of temporary chemical lesions by a local anesthetic, or permanent destruction of the path by an electrolytic lesion have yielded similar results, confirming earlier findings concerning the direction of the pathways.

An arousal system that runs roughly parallel to the sleep system at some levels and overlaps it at others responds to injections of norepinephrine. At some levels of the brain, acetylcholine and norepinephrine elicit their effects from different sites. At these sites, presumably, the two systems do not overlap. At other brain levels both substances produce an effect from the same site; the nature of the effect is determined by the type of substance. Here, presumably, the two systems overlap. One such point of overlap is the preoptic area, where the application of noradrenaline elicits alertness, while application of acetylcholine via the same cannula into the same site elicits sleep. Another site where injection of one and then the other substance via the same cannula produces opposite results is the central gray matter of the spinal cord at the level of the 8th cervical vertebra.

A number of investigators have elicited sleep through electrical stimulation of some of these same parts of the brain *(2, 36)*. In some sites, long slow pulses have elicited sleep while short rapid ones have produced arousal. In other sites, Hernandez-Peon and his collaborators have found it easy to elicit sleep by chemostimulation but difficult or perhaps impossible to do so by electrical stimulation. Furthermore, while studies with electrical stimulation have seemed to indicate that there is an anterior center for light sleep and a posterior one for deep sleep *(37)*, Hernandez-Peon and his colleagues have found it possible to elicit light sleep from either location by weak doses of acetylcholine or carbachol, and deep sleep from either location by stronger doses. Thus, it appears that the depth of sleep is a function of the strength of stimulation of the sleep system, and that the earlier apparent finding of two different centers for these two types of sleep probably was an artifact of a difference in the degree of separation of the sleep system and the arousal system, which allowed electrical stimulation to produce a stronger differential effect in one case than in the other.

Although cholinergic stimulation of certain brain areas in the cat produces sleep, stimulation of other areas, some of them quite close to the sleep circuits, elicits a spectacular attack response: the cat growls, hisses, flattens back its ears, arches its back, raises its hair, strikes out with its claws, and bites. Similar manifestations of rage had previously been elicited by electrical stimulation of these areas. These results further illustrate that the response elicited depends on the area stimulated as well as on the "transmitter" used.

Species differences. Some of the sites at which injection of carbachol or acetylcholine elicits either sleep or rage in the cat correspond to sites at which injection of these substances elicits drinking in the rat. Nevertheless, in investigating these and many other sites in the cat's brain, Hernandez-Peon and other workers have not observed a single instance of elicited drinking *(27, 35)*. The fact that drinking can be elicited easily by application of cholinergic substances at any of a wide variety of brain sites in the rat but has not yet been elicited by these substances in the cat is puzzling. It could be (i) that carbachol and acetylcholine, although somewhat more differential in their effects than electrical stimulation, nevertheless stimulate a number of overlapping systems, the effect on thirst being dominant in the highly domesticated albino rat and effects on rage, sleep, and other systems being dominant in the cat. Perhaps stimulation of the remnants of the rage system in the domesticated rat was the basis for the unexpected rise in blood sugar that accompanied the dominant drinking elicited by carbachol. If species differences are merely a matter of the relative dominance of systems in different animals, one might expect different results from the wild cousins of laboratory rats, which show much more fear and aggression, or from certain desert rats that apparently can get along without any water. On the other hand (ii), it is conceivable that there are genuine differences in the chemical coding of the brains of different species of mammals, or that (iii) we are not yet using quite the right substances in the right way to exploit the subtleties of the chemical code.

Summary

Distinctive patterns of behavior can be elicited by directly stimulating the brain with substances that are normally found in it, or with synthetic compounds resembling these substances. Recent research shows that the response elicited depends on both the site stimulated and the type of chemical used. Compounds of different classes, applied via the same cannula to exactly the same site in the brain, can elicit different kinds of behavior, or opposite effects on the same kind of behavior. This differential sensitivity is useful in tracing the circuits in the brain that control different types of behavior, especially since some of these circuits are intimately interlaced in certain places. A better understanding of the chemical coding of behavioral systems in the brain may also help ultimately to provide a more rational foundation for the discovery of new drugs to treat certain forms of mental disorder.

Evidence is accumulating that a general homeostatic system, with both overt behavioral and internal physiological components, may use neural circuits that are chemically coded in

the same way in at least certain different regions of the brain. Stimulation of any of a number of areas of the rat brain by the cholinergic substances acetylcholine or carbachol causes water-satiated rats to drink and to perform thirst-motivated learned behavior, at the same time conserving water by stimulating the reabsorption of water by the kidneys. Cholinergic stimulation also causes water- and food-deprived rats to drink more and to eat less. On the other hand, stimulation of some of these sites by the adrenergic substances epinephrine and norepinephrine has the opposite effect of causing rats that have eaten and drunk to satiation to eat, or rats that have been deprived of food and water to eat more and to drink less.

In the cat a similar antagonism has been found between the effects of cholinergic and adrenergic substances, only in this case the cholinergic effect is sleep and the adrenergic one arousal. While cholinergic stimulation of certain areas of the cat brain elicits sleep, cholinergic stimulation of other areas elicits a spectacular rage response: the cat hisses, arches its back, raises its hair, flattens its ears, and makes an accurately directed attack.

The finding that some of the brain structures in which cholinergic stimulation elicits drinking in the rat are analogous to structures in which it elicits sleep or attack in the cat is an unsolved puzzle.

Agents that are known to block the effects of certain transmitter substances, or to have the opposite effect of inhibiting the enzymes that destroy these substances, provide a means of rigorously cross-checking conclusions. Such cross checks have provided data that are consistent with, and strongly support, the conclusions drawn from the studies described.

Although the new method of chemostimulation has revealed some esthetically satisfying symmetrical patterns of lawful relationships, it has also turned up some unexpected, apparently discordant, and tantalizing results. It is becoming increasingly clear that we need to use a variety of behavioral tests to determine the effects of different doses of a considerable number of possible transmitting, inhibitory, modulating, or blocking agents at a vast number of different sites in the brain. The chemical methods should be combined, also, with other approaches, such as electrical stimulation, the recording of evoked potentials, and the destroying of discrete pathways or nuclei by lesions. The task before us is enormous, but, by the same token, so is the opportunity for making new discoveries about one of the most miraculous products of nature—the brain.

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