Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms

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The selective sensitivity of neural tissue to a variety of chemical agents has been known for some time. Chemical mediation of neural transmission in the peripheral nervous system has been firmly established, and the manifold effects which peripherally active neurohumors exert in the central nervous system have been attributed to a similar “transmitter” action. The evidence for this interpretation is at present largely inferential, and the possibility of neurochemical “coding” of functional systems, i.e., the differential sensitivity of neural elements to specific chemical substances, requires further study.

In the present series of experiments an attempt was made to approach this problem by investigating the behavioral effects of the peripherally active neurohumors, acetylcholine and norepinephrine, applied directly to an area of the hypothalamus that appears to contain neural elements active in the control of both food and water intake.

The choice of these agents was determined by a number of factors. Both have transmitter properties at peripheral synapses and are known to be distributed nonrandomly within the central nervous system, along with enzymes for their synthesis and destruction. The temporal relationships between the release and destruction of these humors meet the criteria for chemical mediators, and both have been shown to exert a great variety of central effects. Facility as well as inhibitory actions have been reported for both drugs at all levels of the neuraxis, the direction of the effects varying among different systems (1-3).

The area chosen for this investigation is in the lateral hypothalamus, at the level of the ventromedial nuclei. Bilateral ablation of this region produces complete, although perhaps not permanent, aphagia in a variety of species (4-6), whereas electrical stimulation (7, 8) or perfusion with hypertonic solutions (9, 10) elicits drinking in satiated animals. Miller (11, 12) has shown that the behavior evoked by electrical stimulation of this region appears to have many of the motivational properties of normal hunger.

Bilateral lesions in, or very close to, this area have also produced complete and permanent adipsia (13, 14) and electrical stimulation (15, 16) as well as perfusion with hypertonic solutions (15-17) has been reported to elicit drinking in satiated animals. Ablation (6) as well as electrical stimulation of this region (7, 8, 12) frequently modifies both eating and drinking behavior.

These results suggest that this region of the hypothalamus contains neural elements active in the control of both food and water intake, but do not prove conclusively that the observed effects are results of the destruction or stimulation of localized cell concentrations rather than of fiber tracts which merely pass through this region.

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Chemical stimulation of central structures, by means of microinjections of chemicals in solution, has been employed by a number of investigators. The interpretation of the experimental results that have been obtained with this technique is complicated, however, by the problem of excessive and uncontrolled diffusion. MacLeai (18, 19) has shown that the use of chemicals in crystalline form minimizes spread and thus allows one to localize the affected neural elements more precisely, but this technique has not allowed repeated stimulation of a selected locus in a chronic animal, because the guide provided for the stimulation implant did not penetrate into the brain itself. In the present study a double-walled cannula was developed that avoids this objection.

METHODS

Subjects

Thirty-six experimentally naive, male albino rats of the Sprague-Dawley strain (Holtzman Co., Madison, Wis.), approximately 90–120 days old at the time of operation, were used. The animals were maintained on ad libitum food (Purina laboratory checkers) and water throughout the experiment. The same food and water were removed from the home cages and used for the test periods. Daily food and water consumption records were maintained.

Procedure

The general procedure of operation, testing, and histological verification remained constant in all experiments and will be summarized at this point. Procedural variations, specific to a given experiment, will be discussed in the RESULTS section of the particular study.

Construction of the implant. The double-walled cannula, which was implanted in all animals, consisted of two modified syringe needles. The outer cannula was constructed of a standard no. 21 stainless steel syringe needle, the hub of which had been reduced on a lathe to an outside diameter of approximately 2.5 mm and a total length of approximately 5 mm and was threaded on the inside. The inner cannula was made of a standard no. 27 stainless steel needle, the hub of which was turned down to a 1.5 mm outside diameter and was threaded on the outside so as to screw into the outer cannula.

The needles were then cut so that the length of the cannulas themselves, i.e., exclusive of the hub, corresponded to the desired vertical stereotaxic coordinates. When tightly screwed together the tips of the two cannulas were flush with each other, and the hub of the inner needle protruded about 3–4 mm above that of the outer cannula.

For the purpose of electroencephalographic recording, a “disposable” needle with a plastic hub was substituted for the outer cannula, and the needle shafts of both cannulas were insulated with Teflon insulating material. This procedure allowed the use of this implant as a bipolar recording and stimulating electrode. Since the resistance in this system was high, however, better recording results were obtained by using this implant as a monopolar electrode and recording between it and a conventional stainless steel electrode placed at a 2.4-mm distance in the corresponding contralateral structures.

Operative procedure. The double-walled cannula was implanted under Nembutal anesthesia (40 mg/kg) with a Johnson stereotaxic instrument into the hypothalamus of 36 albino rats, each of which weighed approximately 250–300 g at operation.

The design of this implant allowed the entire shaft of the cannulas to disappear below the skull, leaving only the reduced hub of the needle to protrude above the bone. Around this hub approximately 5–8 mm of dental cement were built up to secure the implant to the skull. Stainless steel pins, which were in contact with the recording electrode and the outer cannula, were also partially embedded in this mound of cement. Small clutch mechanisms were used to provide the connection of the leads from the recording apparatus to these pins.

Histology. All animals were sacrificed under heavy Nembutal anesthesia (60 mg/kg) and perfused with a 10% formalin solution. Since the tracts of the implants nearly reached the bottom of the brain, it was more convenient to cut the brain at an angle to the implant. Fifty-micron frozen sections were taken, and one of every group of three successive sections was selected at random for staining with Luxol blue and cresyl violet, according to a staining technique developed by Klüver and Barrera (20).

Testing procedure. In each separate experiment within a series of studies, successive treatments were administered in a counterbalanced sequence by means of a Latin-square design. This variation of the complete factorial design systematically varies the order of drug administrations between subjects. Since the order of successive treatments is different for each animal, artifacts due to a possible interaction of drugs can be statistically assessed, and their effect on the combined results from a group of subjects is minimized.

There was a 24-hr interval between successive injections. This intertreatment interval was increased whenever there was reason to suspect a more prolonged effect of the experimental manipulation. In all but the first experiment, the animals were maintained and tested in a constant, temperature- and humidity-controlled environment.

To establish a control level separately for each experiment, the animals were sham stimulated (i.e., the inner cannula was removed, cleaned and replaced as during chemical stimulation), and were then placed in the testing apparatus for 1 hr immediately before each experiment. All tests were conducted in the morning or early afternoon. None of the rats consumed measurable quantities (5 g or 1 cc) of either food or water during any one of the prestimulation control periods, and additional control data on the intake of unstimulated animals indicate that both food and water consumption remained essentially zero throughout the morning and early afternoon. The control level for all effects is therefore virtually zero and is omitted from all figures.
For the purpose of stimulation, the inner cannula was unscrewed and cleaned in order to remove the 1–2 mm crust of dried tissue fluid which formed at the tip of the inner cannula in the course of the intertreatment interval. Minute amounts (1–5 μg) of crystalline chemicals were then tapped into its tip before returning it to its usual position. The drug was then allowed to diffuse out of the cannula in order to avoid unnecessary tissue damage which would result from pushing the chemical out of the cannula by means of a stylus. Control data, obtained by microscopic analysis of the inner cannula, indicate that the minute amounts used in these studies diffused out of the cannula completely within 4–5 min, which corresponds fairly well to the latencies of the observed behavioral effects.

The quantities thus “injected” were estimated on the basis of both weight and volume (the height of the column of chemical inside the cannula). Variations in the molecular structure of the different chemical agents were not controlled. Although only relatively gross dose-response relationships could be obtained with this technique, it appeared suitable for the present purpose, since the effects observed in these experiments were reliably reproducible at relatively constant levels of magnitude in spite of slight variations in amount of stimulation. Since the concentration of the chemical at the tip of the inner cannula is always maximal, regardless of dosage, adding to the quantity of chemical inside this needle can only increase the extent of effective spread or prolong the duration of stimulation at a given site.

For all drugs used in the present series of experiments, an attempt was made to investigate gross dose-response relationships to ascertain: a) the minimal dose required for the elicitation of some overt behavioral response (i.e., eating, drinking, changes in general activity, etc.); b) the maximal dose tolerated (without evoking gross behavioral changes such as motor seizures or coma); and c) the optimal dose for the elicitation of feeding or drinking behavior. Special care was exercised to vary the dosages of all control substances over a sufficient range to assure, within the limitations of the available techniques, that the observed failure of these agents to induce reliable food or water intake was not merely a function of dosage differences.

For the control and test periods the same food and water that were always available in the animals’ home cages were transferred to the test boxes. Food intake was recorded by weighing the total amount of food in the test cages before and after a period of observation. Food lost through spillage was deducted from the total. Water intake was recorded by direct readings from graduated cylinders. The standard observation period in the present series of studies was 1 hr. The animals were tested when food and water satiated, but no special effort was made to obtain “super satiation” by special feeding schedules.

The statistical evaluation of the results is based on t tests for paired observations (correlated means) and on the product-moment coefficient of correlation.

RESULTS

Experiment 1

Double-walled cannulas were implanted in six albino rats with stereotaxic coordinates, from which Miller (11, 12) had obtained eating or drinking in response to electrical stimulation.

Three of these rats, with closely adjacent implant placements, showed a clear though relatively brief effect of the injection of crystalline acetylcholine chloride, vigorous drinking beginning 4–8 min after stimulation. This effect was greatly prolonged by the addition of small amounts of physostigmine sulfate, a cholinesterase inhibitor, to the injected acetylcholine. Control injections of equally minute amounts of physostigmine alone did not have a clear effect on water intake, suggesting that the dose was insufficient to inhibit enough cholinesterase to allow the accumulation of acetylcholine in concentrations adequate for stimulation of this system.

Since the drinking effect appeared to be limited primarily by the rapid rate of destruction of acetylcholine, carbamylcholine chloride (carbachol), a powerful parasympathomimetic agent which is not hydrolyzed by cholinesterase, was used with the expected result of greatly lengthening the drinking effect.

The injection of crystalline l-epinephrine bitartrate into the same loci in the same animals which had shown the cholinergic drinking effect produced a clear and prolonged eating effect which began after similar latencies. Levarterenol bitartrate (l-norepinephrine) produced an even more sustained and intense eating effect after comparable latencies.

Control for nonspecific activation. In order to control for the possibility of a general, nonspecific excitation of the neural elements in contact with the stimulating substances, strychnine sulfate was deposited in the hypothalamus of all animals.

A wide range of dosages, exceeding those used for the adrenergic and cholinergic substances, failed to elicit any overt behavioral changes, and no effect on subsequent food or water intake was recorded for either the standard 1-hr poststimulation period or the following 23-hr period.

Histological verification of intended placements. The three clearly positive placements were distributed in the perifornical region at the level of the ventromedial nucleus, corresponding to de Groot (91, 92) coordinates (A = 5.4; L = 1.0; H = −2.0), and these coordinates were used throughout the subsequent series of studies. One animal showed only weak effects of both adrenergic and cholinergic stimulation, and two were entirely negative. These three animals showed implant placements that were distributed more anteriorly as well as more dorsomedially.

Experiment 2

Double-walled cannulas were implanted in 12 albino rats at constant coordinates derived from the results of
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FIG. 1. Effects of chemical stimulation of the hypothalamus on food and water intake in satiated animals during a 1-hr post-stimulation period.

FIG. 2. Effects of adrenergic and cholinergic stimulation of the hypothalamus on food and water intake during a 24-hr post-stimulation period. Control levels were determined during a 24-hr period preceding each stimulation.

the preceding study. The results of this experiment are summarized in Fig. 1.

Effects of adrenergic stimulation. All the animals operated in the present study showed a reliable \( (P < .001) \) eating effect after the injection of crystalline epinephrine, food consumption beginning 5–10 min after central stimulation and persisting with variable intensity for 20–40 min. The injection of norepinephrine into the same loci in the same 12 animals produced similar, although more persistent and pronounced effects, beginning after comparable latencies. Total food consumption averaged 3.0 g after injection of epinephrine and 4.3 g after norepinephrine, with a range of 1.0–6.0 g and 2.0–11.0 g, respectively. This difference between the effects of the two adrenergic agents did not reach statistical significance in the present study, but was reliable \( (P < .002) \) when data from seven additional animals \( (exp. 3) \) were added to the analysis.

Neither epinephrine nor norepinephrine induced drinking reliably, although 4 of the 12 animals tested consumed some water after the injection of epinephrine and 3 drank after norepinephrine. Since this water consumption occurred in all but one animal only near the end of the test period, after a considerable amount of dry food had been eaten, drinking appeared to be secondary to the consumption of dry food rather than a direct effect of the adrenergic stimulation.

In order to test this conclusion and to establish the specificity of the adrenergic effect, norepinephrine was deposited in six animals which had previously shown a pronounced eating effect. These animals were then tested in cages containing only water. For 30 min after norepinephrine stimulation none of the animals consumed any water, although four of them repeatedly approached the drinking tube to gnaw at it. When food was introduced all animals began to eat almost immediately, although total intake was, of course, lower than that normally observed after adrenergic stimulation, since the pellets were introduced into the test cages only toward the end of the period of effective stimulation.

Effects of cholinergic stimulation. The injection of acetylcholine (capped by physostigmine) into the identical loci in the same 12 animals which had shown the adrenergic eating effect, reliably \( (P < .001) \) resulted in drinking in all animals, the latency, duration, and magnitude of the effect being roughly comparable with those observed for eating. Carbachol again showed similar but more persistent and pronounced effects. Total water intake...
averaged 7.4 cc after injection of acetylcholine and 12.8 cc after injection of carbachol, with a range of 2.0–10.0 cc and 8.0–19.0 cc, respectively. This difference was statistically reliable ($P < .001$). Neither substance reliably evoked eating in these animals, only one rat eating 1.0 g after injection of acetylcholine.

Dose-response relationships. Because of the relative crudeness of the measuring techniques used, only gross dose-response relationships could be investigated. Both adrenergic and cholinergic agents produced reliable and repeatable effects at relatively constant levels of magnitude over a fairly wide range of dosages (1–5 µg). Doses smaller than 1 µg either were not effective at all or produced only small and unreliable effects.

Increasing the amount of adrenergic stimulation beyond the optimal dose of 3–5 µg, either by increasing the quantity of a single injection or by repeating the stimulation before the effects of a previous injection had worn off, reduced or abolished the eating effect, and induced hypoactivity, somnolence, and general refractoriness to any form of sensory stimulation.

Increasing the amount of cholinergic stimulation, on the other hand, produced immediate and pronounced hyperactivity and increased reactivity to sensory stimulation, as well as excessive grooming, licking, and sniffing, followed by slowly developing motor seizures which persisted for 30–50 min. Even when an overdose did not elicit the seizure activity, drinking was strongly reduced or abolished.

That the failure of higher dosages to produce the eating and drinking effects may not be due primarily to these side effects on general activity, is suggested by the fact that even relatively small doses (5.8 µg), which evoked only minor changes in overt motor activity, failed to elicit the consummatory behavior.

The significant differences between the effectiveness of carbachol and acetylcholine and of norepinephrine and epinephrine do not appear to be attributable to dosage variations, because an "optimal" dose was established for each drug which was used throughout these experiments.

Long-term effects. The daily consumption records suggest that the amount of food or water consumed during the 1-hr observation period immediately following chemical stimulation was consumed in addition to the animal's normal daily intake. This conclusion is supported, at least for the adrenergic eating effect, by an increased body weight on the day after stimulation. These effects were not statistically reliable in this experiment due to temperature and humidity fluctuations which caused excessive variability in the data.

Control for osmotic stimulation. The results of the present series of studies demonstrate clearly a differential effect of adrenergic and cholinergic stimulation, but do not prove that these effects are due to the sympathomimetic and parasympathomimetic properties of norepinephrine and acetylcholine, respectively, rather than to one or
more of the many "side effects" which these drugs are known to produce.

The first question to be raised concerns the effects of osmotic stimulation which occur as a by-product of the injection of hypertonic solutions. To control for this factor, crystalline NaCl, in quantities comparable with those used with the adrenergic and cholinergic substances, was deposited in the same points that had shown the eating and drinking effects. All the animals tested responded to this treatment with varying degrees of hyperactivity, but only 3 of the 12 animals consumed any food or water. Since this effect was so much smaller than those evoked by adrenergic and cholinergic stimulation ($P < .001$) and failed to be specific to either eating or drinking, the factor of osmotic stimulation does not appear to play an important role in the determination of the specific adrenergic and cholinergic effects.

**Experiment 3**

Replication of adrenergic and cholinergic effects. In the third series of experiments the selective effects of adrenergic and cholinergic stimulation on the eating and drinking mechanisms, respectively, were fully replicated in 11 additional animals. The average magnitude of the effects corresponded very closely to those of the first experiment, the mean values being 4.3 g and 12.8 cc for the first study, and 4.4 g and 12.0 cc for the second.

**Effects of chemical stimulation on electrical activity of affected region.** EEG records taken from the tip of the double-walled cannula before, during, and after both adrenergic and cholinergic stimulation failed to show any consistent modifications of the recorded electrical activity. Carbachol spikes were recorded only when an "overdose" of carbachol (i.e., more than 8–10 μg) was injected. Under these conditions drinking did not occur and hyperactivity, followed by slowly developing motor seizures, accompanied the high-voltage activity in the EEG record.

**Long-term effects.** In this second series of studies it was possible to provide constant levels of temperature and humidity by air conditioning. The data summarized in Fig. 2 indicate that the total water intake for the 24-hr period immediately following cholinergic stimulation was significantly greater ($P < .001$) than that of a normal control period immediately preceding stimulation. The additional intake was significantly greater ($P < .001$) than the amount consumed during the 1-hr observation period that immediately followed stimulation, and was reliable ($P < .001$) even when the water consumed during that 1st 1-hr period was deducted from the daily total. Food intake did not appear to be affected, either primarily by the cholinergic stimulation or secondarily by the excessive water consumption.

The injection of norepinephrine, on the other hand, showed precisely the opposite effect. Total water con-
suggestion during the 24-hr period following adrenergic stimulation was entirely normal in spite of the fact that food intake for this same period showed a highly significant (P < .001) increase over the control level. This additional food intake was significantly greater (P < .001) than the amount consumed during the 1-hr observation period immediately following stimulation, and was reliable (P < .001) even when the amount eaten during that 1-hr test period was deducted from the total.

Although the conspicuous effects of both adrenergic and cholinergic stimulation appeared to wear off within the 1-hr observation period, a residual differential effect remained, and was reflected in the increased intake during the following 23-hr period. We do not, unfortunately, know how far into the 23-hr period this residual effect extended. Both food and water intake returned to normal during the following 24 hr.

Effects on body weight. Figure 3 shows that the increase in food intake during the 24-hr period immediately following adrenergic stimulation is clearly reflected in the animal's body weight 24 hr after stimulation. This increase in weight is significantly greater (P < .001) than that of a control period preceding adrenergic stimulation. No abnormal changes in body weight were recorded after cholinergic stimulation. The excessive water intake after the injection of carbachol appears to be fully compensated by increased diuretic action. Control data indicate that neither the abnormal weight gain nor the excessive food intake causing it were compensated within the week following stimulation.

Motivational qualities of the stimulation effects. To investigate the motivational properties of the behavior evoked by chemical stimulation, 11 animals were preoperatively trained to press one bar for food and another for water on different reinforcement schedules, with variable intervals averaging one reward per 30 sec. The test situation required a spatial discrimination between the two bars that were mounted side by side on one end of an operant conditioning apparatus.

The results of the postoperative tests are summarized in Fig. 4. The injection of norepinephrine in satiated animals resulted in a highly significant (P < .001) increase in the rate of bar-pressing for food without reliably raising the level of performance on the water bar above the prestimulation control level.

Cholinergic stimulation, on the other hand, resulted in a reliable (P < .001) increment of performance on the water bar without significantly increasing the number of bar presses for food.

Effects of chemical stimulation on normal hunger and thirst. To secure evidence on the possible central interaction between the two drives, norepinephrine and carbachol were injected in 24-hr food- or water-deprived animals. Figure 5 summarizes the results of the complete design.

Rats which had been fasted for 24 hr and made "thirsty" by the injection of carbachol ate little or no food during a 30-min observation period without water, beginning 10 min after stimulation. Only 3 of the 11 animals tested consumed any food at all after cholinergic stimulation. The difference between the control intake and that following the injection of carbachol was highly significant (P < .001).

Similarly, animals deprived of water for 24 hr drank little or no water when made "hungry" by the injection of norepinephrine. Although 8 of the 11 animals tested drank some water after adrenergic stimulation, the average water intake was significantly smaller (P < .01) than that of a control test. Only water was available in both tests.

When "24-hr-hungry" rats were made more hungry by the injection of norepinephrine, they ate significantly more (P < .001) food than they did during the control period. When "24-hr-thirsty" animals were made more thirsty by the injection of carbachol, they drank significantly more water (P < .001) than they did during the control period.

Control for vasomotor effects. Both adrenergic and cholinergic substances are known to have pronounced effects on the vascular system which might excite or inhibit neighboring neural tissue. To control for this factor, sodium nitrite and barium chloride, powerful vasodilator agents, and posterior pituitary extract, a vasoconstrictor, were applied locally to all animals in a counterbalanced sequence in quantities comparable with those used with the adrenergic and cholinergic drugs.

That the epinephrine-induced eating effect observed in the present experiments is probably independent of the vascular effects of the adrenergic agents is shown by the failure of posterior pituitary extract to elicit eating or drinking in any one of the 11 animals tested. All animals showed a slight tendency toward hypoactivity and decreased reactivity to sensory stimulation, which was, however, in no instance severe enough to account for the observed failure of the drug to affect food intake.

The cholinergic drinking effect similarly does not appear to be based on the vasodilation which is incident to the injection of acetylcholine or carbachol, since control injections of sodium nitrite and barium chloride failed to replicate the characteristically selective effects on water intake. A pronounced increase in general activity, as well as mild motor seizures similar to those observed after an overdose of carbachol, were observed in some animals after both injections. During the period of hyperactivity both eating and drinking appeared briefly in 3 of the 11 animals tested. This effect was statistically unreliable and significantly smaller (P < .001) than those of adrenergic and cholinergic stimulations, and failed to be specific to either eating or drinking.

Control for local pH changes. Changes in the local acid-base composition of the stimulated region could conceivably exert significant and differential effects on the surrounding neural tissue.

To control for this factor, the pH values of all substances used in the present studies were established, both in supersaturated solution and in brain homogenate. Adrenergic and cholinergic substances of varying pH values were effective in eliciting eating and drinking, respectively, whereas control substances of identical or similar pH values consistently failed to evoke comparable results. Although the adrenergic agents generally tended
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FIG. 7. Localization of 47 implant placements (33 positive, 14 negative). Coordinate system is based on the de Groot (21, 22) atlas of the rat brain. Filled circles indicate consistently good effects of both adrenergic and cholinergic stimulation, white stars inside filled circles indicate consistently excellent effects of both adrenergic and cholinergic stimulation; white stars represent predominantly cholinergic effects; black stars represent predominantly adrenergic placements; black triangles indicate negative placements.

to be more acid than the cholinergic ones, dopamine (3-(3,4-dihydroxyphenyl)-ethylamine), which mimicked the adrenergic eating effect, showed a pH almost identical with that of carbachol. Physostigmine sulfate, which had no selective effect on food intake, showed a pH fully as acid as some of the adrenergic agents.

HISTOLOGICAL VERIFICATION OF INTENDED PLACEMENTS

A coordinate system, based on the de Groot (21, 22) atlas of the rat brain, schematically summarizes in Fig. 7 the results of the histological examination of a total of 47 placements (33 positive and 14 negative). This distribution includes all 36 animals used in the present study as well as 13 additional rats (8 positive and 5 negative cases).

Inspection of Fig. 7 indicates no clear anatomical separation of the neural elements which regulate feeding and drinking behavior, respectively. Although five animals showed a consistently above-average effect of cholinergic stimulation in combination with a below-average effect of adrenergic stimulation, in no instance did either effect occur alone, and the correlation (omitting all negative instances) between the adrenergic and cholinergic effects was positive and highly significant ($r = .51; P < .01$). The correlations between successive tests of the same effect ranged from .77 to .92 for the adrenergic eating effect, and from .67 to .90 for replica-
tions of the cholinergic drinking effect. The mean correlations of .86 and .83, respectively, were highly significant ($P < .01$).

The anterior-posterior distribution of the positive instances appears to be fairly restricted, most active points being concentrated in a plane corresponding to $A = 5.4$ to 5.8, with only a few positive instances as far anterior as $A = 6.6$. Only two modestly positive cases ($A = 5.0$ and $A = 7.0$) fall outside of this range and may represent the extreme limits of effective spread, since both animals showed greater than average latencies for both effects.

The vertical distribution of positive points, on the other hand, is less well defined, ranging from thalamic and subthalamic placements just ventral to the mammillothalamic tract ($H = - .5$) to the subfornical region and subthalamic placements just ventral to the mamillary bodies.

The lateral dimension extends from approximately $L = - .7$ to $L = 1.9$, but is not so wide as this range would lead one to expect, since positive points in the dorsal portion of this distribution seem to be concentrated closer to the midline, showing negative instances as far medially as $L = 1.2$; ventral (perifornical) placements show excellent positive effects as far laterally as $L = 1.9$.

Although many of the positive points correspond well to the “lateral hypothalamic feeding area” described in the literature (4-6), many of the more medial and dorsal placements are considerably removed from this area and correspond more closely to the “drinking area” described by Anderson and McCann (15-17).

An indication of the extent of spread or diffusion of the injected chemicals can be obtained from a study of the distribution of positive and negative points in Fig. 7. It is recognized that these placements were taken from different animals; nevertheless, the extremely close proximity of positive and negative cases indicates that the effective diffusion was relatively small.

**DISCUSSION AND CONCLUSIONS**

The results of the present series of studies demonstrate clearly that the placement of adrenergic substances into a circumscribed region of the diencephalon elicits specific changes in one type of motivated behavior (food intake) while the placement of cholinergic agents into the same area of the hypothalamus evokes pronounced changes in a different type of motivated activity (water intake).

The behavior which is elicited by the injection of these chemicals appears to have many of the specific motivational properties of normal hunger and thirst, since satiated animals will work to obtain food or water following the injection of the appropriate substance, in spite of the fact that their efforts are only infrequently rewarded.

The motivational effects which these two groups of chemicals elicit from the same region of the midbrain appear to be, at least to some extent, antagonistic. Adrenergic substances elicit food intake in satiated animals but inhibit drinking in thirsty rats, whereas cholinergic substances evoke vigorous drinking in satiated animals but inhibit food intake in the hungry animal.

These results suggest that the well-known tendency of thirst to inhibit eating may, at least partially, be a central neural effect, perhaps analogous to reciprocal inhibition, as suggested by Miller (12), rather than a purely peripheral effect of dehydration such as dryness of the mouth and throat.

These inhibitory effects appear to be highly specific, since hungry animals apparently were made even more hungry by the injection of norepinephrine, and thirsty animals consumed significantly more water after the injection of carbachol.

The principal question to be raised with regard to the observed effects of chemical stimulation concerns the neural and/or chemical mechanisms responsible for the elicitation of the pronounced behavioral changes.

In view of the fact that both acetylcholine and norepinephrine have well-documented neurohumoral functions in the peripheral nervous system, it is tempting to consider the observed central effects of these drugs as due to a similar transmitter action. Before this notion can be entertained, however, a number of problems and alternate possibilities must be considered.

Considerable evidence has been accumulated suggesting a neurohumoral action of acetylcholine in the central nervous system. The existence of a comparable adrenergic mechanism, however, has not yet been satisfactorily demonstrated. These catecholamines apparently do not cross the blood-brain barrier in detectable amounts, so that the systemic administration of these drugs cannot be expected to have a direct effect on central neural elements. The few studies in which adrenergic solutions have been injected directly into the brain have not provided adequate control over diffusion, so that only a very gross localization of the observed effects has been possible.

The use of crystalline chemicals in the present series of experiments has reduced the problem of uncontrolled spread considerably, although the exact extent of effective diffusion can only be estimated on the basis of the anatomical distribution of positive and negative placements. This analysis depends necessarily on a composite picture, based on histological data from many animals. It nevertheless appears clear that the effective spread must be quite limited, since both positive and negative placements occur in very close proximity.

The fact that none of the placements attempted in any of the experiments showed either the cholinergic or the adrenergic effect alone suggests further that the effective spread of carbachol and norepinephrine was not, in fact, significantly different. Placements very closely adjacent to points from which both highly positive eating and drinking effects could be obtained were entirely negative with respect to both drugs.

Since the crystalline drugs were tamped into the inner cannula and, since neural tissue in direct contact with any foreign body, such as the metal cannula, almost invariably shows some necrosis and recession, it appears unlikely that any active neural elements were in direct contact with the stimulating substance before a considerable amount of dilution had taken place. It is neverthe
It does not seem likely that the observed effects of the smaller doses of these substances on food and water intake could be due to the inactivation or abnormal functioning of these neural elements since: a) almost identical effects have been elicited from this portion of the midbrain with minimal-current electrical stimulation; b) the behavior elicited by the injection of these chemicals appeared in all respects "normal"; and c) EEG recordings taken from the tip of the implant failed to show any gross changes in the electrical activity of the affected region, such as one might expect if seizure activity were induced by the introduction of the chemicals. It should parenthetically be noted that the injection of chemicals in solution does not, per se, guarantee that physiological concentrations will be active at the site of stimulation, because the rates of destruction and diffusion of the different chemicals remain largely unknown.

Assuming then that the observed effects are due to the excitation or inhibition of neural elements within a reasonable distance from the implant tip, and that this neural activity is within normal limits, the contention remains to be supported that this activation is, in fact, due to the neurohumoral properties of the adrenergic and cholinergic substances rather than to one of the many side effects which these chemicals are known to produce in peripheral structures.

In the following discussion of the control experiments which were performed in an attempt to obtain evidence on this question, it should be borne in mind that we are concerned with the effects of drugs on neural tissue which is probably not in direct contact with the crystalline chemical itself, but is sufficiently removed from the point of application to provide physiological concentrations of the two neurohumors used in this study.

While differential solubility and diffusion of some of the control substances cannot be ruled out, so that initially "comparable" amounts or osmotic pressures may, in fact, not result in comparable effects at the point of effective stimulation, this fact is at least partially controlled by the use of a wide range of dosages for each drug implanted in these experiments. While it is not intended to prove the null hypothesis, i.e., demonstrate beyond any doubt that a particular substance could not, under any circumstances, produce effects comparable to those observed after adrenergic or cholinergic stimulation, an attempt was made to rule out this possibility for a sufficiently wide range of dosages to make such an interpretation highly unlikely.

The first of our control experiments was concerned with the possibility of nonspecific neural activation similar to that produced by the topical application of a variety of chemical agents to the surface of the cortex.

The drug chosen for this purpose was strychnine sulfate, which has been used extensively as a tool in physiological neuronography (23, 24) and has been shown to evoke extremely violent rage reactions, similar to those seen following electrical stimulation, when injected into the media! hypothalamus (24a). Strychnine appears to act primarily at synaptic junctions and activates cell bodies or dendrites with which it is in contact without having a noticeable effect on adjacent fibers (21). The mechanism of strychnine action has been variously explained and may involve a selective blockade of inhibitory synapses (25, 26).

In the present study no overt behavioral effects of the injection of strychnine were observed, and no effect on subsequent food or water intake was recorded for either the standard 1-hr poststimulation period or the following 23-hr period. Although these results argue strongly against a general activation factor, they should not be interpreted as negative evidence as far as the cholinergic nature of the acetylcholine stimulation effect is concerned, since it appears extremely unlikely that the activating action of strychnine is in any way related to the inactivation of cholinesterase which Nachmansohn (27) has demonstrated in vitro, because atropine has been shown to exert no influence on strychnine action (28).

The second question to be raised concerns the effects of osmotic stimulation which occur as a by-product of the injection of hypertonic solutions. This problem is particularly relevant in the present context, since Larsson (9) has reported pronounced hyperphagia in satiated animals after microinjections of hypertonic salt and sugar solutions into an area just caudal to the optic chiasma, and similar results have recently been reported by Epstein (10). Anderson and McCann (13, 16) have reported excessive drinking as well as increased food intake in goats after microinjections of hypertonic saline into the same general area.

Since the specific osmotic pressure changes produced by crystalline chemicals are a function of their solubility and rate of diffusion, it was not possible to duplicate the specific osmotic effects of carbachol and norepinephrine at the site of stimulation. It was found, however, that the placement of various dosages of pure NaCl, designed to cause maximal osmotic stimulation, failed to duplicate the specific effects of the adrenergic and cholinergic agents. Furthermore, none of the control substances which were used in our experiments produced any consistent effects on food and water intake, in spite of the fact that their osmotic effects undoubtedly varied considerably.

While it is possible and, in view of earlier experiments, in fact probable, that osmotic pressure changes may cause a general stimulation effect or rise in excitability, it appears clear that this factor cannot account for the selective effectiveness of norepinephrine and carbachol at this particular site, unless one assumes a nonmonotonic
relationship between osmolarity and excitation of the neural elements controlling food and water intake. As far as the results of the present control experiment are concerned, the observed increase in general activity may itself explain what little effect on feeding and drinking behavior occurred.

A further problem, which could lead to an erroneous interpretation of the observed effects of the adrenergic and cholinergic agents, concerns the pronounced vaso motor effects which these substances exert. Such vascular changes might themselves excite neighboring neural tissue and hence give rise to the observed behavioral phenomena.

The local application of acetylcholine and carbachol causes pronounced vasodilatation and a consequent fall in blood pressure. Whereas epinephrine appears to exert only a slight cerebral vasoconstrictor effect, the constrictor action of norepinephrine on cerebral vessels is quite strong, and reduces cerebral blood flow under conditions in which cardiac output is only negligibly affected.

To control for this factor, sodium nitrite and barium chloride, powerful vasoconstrictor agents, and posterior pituitary extract, a vasoconstrictor, were used. These agents appeared particularly suited for the present purpose, since their vasomotor effects are exerted directly on the vascular contractile elements and are neither antagonized by adrenergic nor cholinergic blockade, nor prevented by vascular denervation.

Since a wide range of dosages of these vasomotor agents failed to duplicate the specific effects of adrenergic and cholinergic stimulation on food and water intake, respectively, the vascular side effects of these neurohumors do not appear to be responsible for the main effects of our study. It is interesting to note, however, that the pronounced changes in general activity, which overdoses of norepinephrine and carbachol induced, were duplicated by the corresponding control substance.

Changes in the local acid-base composition similarly do not seem to account for the observed selective effects of adrenergic and cholinergic stimulation. In order to control for this factor, the pH values of all substances used in our experiments were established, both in supersaturated solution and in brain homogenate. Because of possible differential diffusion rates and solubility factors, these values may not correspond directly to the changes occurring at the site of effective stimulation. Since the range of pH values of the control substances extended a considerable distance both above and below the values of the adrenergic and cholinergic agents, it seems unlikely, however, that this factor could be responsible for the main effects of the experiment.

The results of the present series of experiments can best be summarized by the postulation of two anatomically overlapping systems of neural elements at the level of the hypothalamus, which participate in the regulation of food and water intake, and appear to be selectively sensitive to adrenergic and cholinergic stimulation, respectively. The differential excitation and inhibition of a single neural mechanism appears unlikely, in spite of the fact that all placements showed both effects, since the relative magnitude of the eating and drinking effects, though constant in a given animal, varied considerably between subjects.

The results of our control experiments on the effects of nonspecific activation, osmotic stimulation, vasomotor effects, and local changes of the acid-base composition of the stimulated region support the notion that the observed effects may be due to a neurohumoral action of the cholinergic and adrenergic substances. Further experiments, using specific antagonists of acetylcholine and norepinephrine, are in progress to obtain further evidence on this point.

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REFERENCES