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THEORETICAL REVIEW

Specificity of Chemical Stimulation of the Rat Brain and Other Related Issues in the Interpretation of Chemical Stimulation Data

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SINGER, G. AND R. B. MONTGOMERY. *Specificity of chemical stimulation of the rat brain and other related issues in the interpretation of chemical stimulation data.* PHARMAC. BIOCHEM. BEHAV. 1(2) 211–221, 1973.--Data from studies using direct chemical stimulation of the rat brain to investigate eating and drinking behavior have given rise to the hypothesis of chemically coded neural control or behavior circuits, often in close spatial, anatomical proximity. These studies and others are reviewed. The questions of the anatomical and chemical specificity of direct stimulation of the brain, and the relationship between chemically elicited behavior and deprivation induced behavior is examined.

Chemical stimulation Eating Drinking behavior Neural control circuits Chemical specificity Anatomical specificity Adrenergic Cholinergic

PRELIMINARY reports showed that an adrenergic substance injected into the lateral hypothalamus of the rat via a permanently implanted cannula elicits eating in food satiated animals, and the injection of a cholinergic substance through the same cannula elicits drinking in water satiated animals [19, 20, 21]. Subsequent confirmation of this work and extension to other brain sites led to the postulation of chemically coded behavior control circuits in close spatial anatomical proximity [13, 14, 48]. The implication is one of chemical as well as anatomical specificity.

Recent reports in the literature have questioned the evidence for both the chemical and anatomical specificity of brain circuits, as well as the relationship between chemically induced and deprivation induced drinking and eating behavior.

The purpose of this paper is to review the evidence in regard to both specificity and the interpretation of chemical stimulation data. Specificity will be examined with regard to anatomical and chemical factors first, and the direct and indirect evidence for the behavioral and physiological equivalence of natural deprivation states and central chemical stimulation will be discussed. Modifications to the model of chemically coded neural behavior control circuits arising from the examination of recent experimental data will be suggested.

ANATOMICAL SPECIFICITY

Routtenberg [66,68] has led criticism of the anatomical specificity of the effects of direct chemical stimulation of the brain (CSB). He initially proposed [66], and later reported having demonstrated [68], that an alternative interpretation of the data on cholinergic elicitation of drinking in the rat was that the injected biochemicals diffused through the brain tissue, and into the ventricle. The behavioral effects of the biochemicals might then be due to modification of the chemical milieu of the ventricle, or stimulation of receptors on the wall of the ventricle, or both. It was contended that the ventricular involvement hypothesis better explained some of the data, (such as the elicitation of drinking by cholinergic stimulation of the fornix, a structure composed of neural axons) and the apparent redundancy of the complex thirst circuit proposed by others [15,39].

Three types of evidence will be considered in an evaluation of the ventricular involvement hypothesis: firstly, studies involving direct ventricular injections; secondly, studies of the diffusion patterns of centrally applied chemicals; and, thirdly, evidence of the anatomical distribution of chemically responsive sites.

Direct Use of the Ventricular Route

In reply to Routtenberg, it was reported that direct

intraventricular injections of crystalline carbachol (1–3 μg) in the rat produces catatonia, tremors, or bizarre motor behavior, but not significant drinking [16]. Drinking could only be elicited by intraventricular injections of either mixtures of carbachol with either eserine or norepinephrine, or very low doses of carbachol, and then with latencies of 10 or more min, compared to the 3 min usually found in CSB studies. The ventricular involvement hypothesis implied that a centrally applied neurochemical diffuses through brain tissue, into the ventricle, there either to have its effect, or be transported by the CSF and diffuse back into brain tissue, finally reaching and activating a nonventricular site. Fisher and Levitt attempted injections at nonresponsive sites close to the ventricle, to allow this postulated mechanism a chance to function. None of the animals so tested gave significant drinking responses.

Other investigators have injected cholinergic, aminergic, and related compounds into the lateral cerebral ventricle in the rat [59,61]. In 335 experiments with 48 rats [61] acetylcholine and eserine evoked hyperthermia, but no eating or drinking. The catecholamines, dopamine, noradrenaline and adrenaline elicited dose dependent eating, but no drinking. The chronic infusion of carbachol under five dose schedules, over 26 days, failed to produce significant increases in water or food intake [59].

Finally, solutions of carbachol of varying concentrations, have been used to show that it is possible to elicit drinking in the rat with cholinergic stimulation of the ventricles [42]. However, as previously reported [16], this phenomenon showed markedly different dose response relationships from those in CSB studies involving nonventricular sites. There was also a considerably greater latency to commence drinking than in nonventricular CSB studies. The differential dose requirements and the greater latencies for ventricular drinking make it unlikely that this mechanism would do more than contribute to cholinergically elicited drinking.

While it is clear, then, that biochemicals injected in the central ventricles can diffuse into brain tissue, and can have marked and consistent behavioral and physiological effects [12,61], the above data suggest that the effects of neurochemicals applied intraventricularly are either different, or show markedly different latencies and dose response relationships, from the effects of the same substances when applied locally to a specific, nonventricular brain site. It seems, then, that the ventricular hypothesis is an inadequate explanation of the behavioral effects of direct CSB and is inconsistent with the differences in behavioral parameters found between CSB and ventricular stimulation. Routtenberg, in his recent review in which he still argues in favour of the ventricular involvement hypothesis [67], does not account for these differences between the effects of CSB and ventricular stimulation.

Diffusion Patterns of Centrally Applied Chemicals

Injections were made of four dyes of unequal molecular weights, in volumes of 0.5, 1.0, 2.0, 3.0 and 4.0 μl, into the thalamus or hypothalamus of anaesthetised rats [57]. The brain was rapidly frozen *in situ* 10–25 min after injection, and the area of dye penetration determined by sectioning tissue. The extent of diffusion generally bore a direct relationship to injected volume, and it was recommended that the volume of injections into the rat brain be restricted to 0.5 μl, which gave an average spread of 1.04 mm with these dyes. The more typically used volume of injection in the rat has been 1.0 μl, which gave an average diffusion pattern of 1.9 mm in this study. Criticism was made [68], however, of the extrapolation of these findings with dyes to the structurally different neurochemicals.

In support of the ventricular involvement hypothesis [68], histochemical fluorescence was used to trace the movement of neurochemicals applied to the caudate nucleus and septal area in the rat. Although it was concluded that the results (1) "support the view that the ventricle transports chemicals applied to brain tissue", and (2) "are clearly relevent to discussions of widespread behavioral effects of neurochemicals applied to the brain", the validity and generality of these conclusions have been questioned [53]. It was pointed out that clear results were obtained with only one neurochemical, dopamine, of the three tested. Significant results were unobtainable with noradrenaline, and the technique was unsuitable for carbachol. Yet the majority of the studies of the behavioral effects of CSB have employed noradrenaline and carbachol, not dopamine. Thus Routtenberg *et al.* rendered themselves liable to the same criticism that they had levelled at others, viz. extrapolation of their findings without consideration of possible differences in diffusion patterns resulting from structural differences of the chemicals.

Further, Routtenberg *et al.* used crystalline doses of approximately 10 μg, 2–20 times larger than the usual crystalline dose, and 10–1000 times greater than the usual dissolved dose. Further still, in typical CSB studies optimum behavioral effects are found with, and therefore greatest use has been made of, the lower third of these dose ranges. It was therefore argued that Routtenberg *et al.* had failed to employ appropriate experimental procedures to demonstrate their postulated mechanism of ventricular involvement for the neurochemicals most directly concerned. Although Routtenberg [67] cites some aspects of this criticism [53] of his report, he has ignored the serious questions of his conclusions being based on data from only dopamine, not noradrenaline or carbachol, and agrees with the criticism of his use of much greater (crystalline) dose levels.

Routtenberg [66] had earlier concentrated part of his discussion on the difficulty of interpreting the evidence for blocking cholinergically elicited drinking by contralaterally applied atropine, a difficulty in interpretation apparently shared by others (see Discussion of Paper, in [58]). Recently, tritiated atropine has been administered in crystalline doses of 1.0–5.0 μg to the hypothalamus of rats [26]. The atropine remained strictly localised in a sphere, 1.0–1.8 mm in dia., during the first three min after injection. A similar distribution was found after one hr, and at intermediate times radioactivity was only slightly elevated, estimated to reflect concentrations 2,000–10,000 times below behaviorally effective doses, at distances up to several millimetres from the implantation site. Variations in dosage altered activity within the sphere, but did not significantly increase the diameter. This was confirmed by microelectrode recordings which showed no reaction to even large doses in cells 1.5 mm from the cannula tip. Although these results have clearly different implications from Routtenberg's, the authors emphasise that their "observations apply only to small quantities of atropine and perhaps closely related chemical substances which are permitted to dissolve in the tissue fluids, by a technique which does not induce mechanical forces due to the

injection of liquids under pressure", ([26], p. 1412).

However, the injection of solutions of tritriated or ^{14}C-labelled serotonin, norepinephrine, or acetylcholine in 1.0 μl volumes into the lateral hypothalamus [60] produced generally identical results to the dye diffusion studies described above [57]. Radioactive material was found in structures some distance from the microinjection site, but at concentrations which would have no apparent pharmacological action, thus supporting previous findings [26]. In particular, acetycholine injected 1.5 mm lateral to the midline apparently does not exert its action by way of the intraventricular route, since virtually no radioactivity was detected in the artificial cerebrospinal fluid collected after perfusion from the lateral through the fourth ventricle. These findings are directly contrary to the ventricular involvement hypothesis [66], but again are not dealt with in Routtenberg's recent review [67].

Grossman (e.g. [19]) has consistently chosen crystalline chemical stimuli, in preference to solutions, citing Maclean [43] as evidence for the proposed reduction in spread of chemicals centrally applied in crystalline form as against solutions. However, examination of Maclean's [43] original reports reveals several crucial differences between his procedure and that typically reported for CSB studies using solutions as stimuli.

The volume of Maclean's injections is estimated as 0.01 to 0.02 ml. This is up to 40 times greater than the usual injected volume (0.5–1.0 μl). The concentration of MacLean's solutions is 2.5% or 10%. This is up to 5000 times greater than the usual concentrations ($1-72 \times 10^{-4}$ M) of stimulus solutions. Maclean's absolute dose is therefore up to 1000 times larger than those used even in CSB studies employing crystals (1–5 μg). Further, Maclean's method involves an outer cannula (or needle guide) which only penetrates the skull. The actual penetration of brain tissue is made at the time of injection, by the needle inserted through this guide, to a predetermined depth; Maclean limited his activities to only one such injection at each site. This procedure contrasts with that of typical CSB studies, in which the outer cannula usually protrudes well into the brain tissue (e.g. [8]), often right to the intended site of stimulation (e.g. [69]), and is used as a needle guide for repeated injections.

In addition to these procedural differences, Maclean's evidence for spread of centrally applied solutions is indirect, based on a similarity between the behavioral responses following stimulation of subventricular structures and those following direct intraventricular injection. Even if one accepts Maclean's conclusion that this behavioral similarity is due to leaking of the injected solution back along the needle (perhaps not unlikely in his studies in view of the very large volume, concentrations, and doses involved), it is relevant to note that Maclean [43] neither claims, nor offers evidence for any wider dispersion through brain tissue of dissolved chemicals than of crystalline chemicals, nor is there to our knowledge any other evidence to suggest this.

More recently, Routtenberg [67] has suggested that the ventricular involvement hypothesis should be broadened to include the possible involvement of the vasculature in diffusion of locally applied chemicals. This possibility, currently only supported by evidence for transport of microinjected crystalline ^{3}H-atropine by the cerebral vasculature, remains to be fully investigated.

In summary, while it again appears necessary to be cautious in extrapolating findings from one chemical to others, there seems to be little direct evidence for widespread diffusion or transport of the neurochemicals used in behavioral studies. However, neither dye diffusion nor autoradiographic investigation can provide definitive evidence about effective diffusion, that is, the diffusion patterns of behaviorally effective concentrations, since either the test drug may metabolise and a labelled metabolite may diffuse widely, or the drug may diffuse to other receptor sites but not in adequate concentrations. Further approaches to the problem may consider the use of both autoradiographic investigations coupled with dose level behavioral studies.

Anatomical Distribution of Chemically Responsive Sites

Generally, workers in this field informally report a difficulty in consistently making implants into responsive loci, a difficulty which is hard to reconcile with the notion of widely diffuse effects from microinjections. For instance, the use of crystalline doses of 1.0–3.0 μg, has given nonresponsive loci within 0.25 mm of responsive loci, for neurochemicals applied by an extendible cannula, which was lowered in 0.25 mm steps until a behavioral response was elicited [8]. Another estimate of the extent of effective spread of crystalline microinjections was given as approximately 0.5–1.0 mm, based on the anatomical distribution of positive and negative placements [24], while it has been pointed out that many studies have provided evidence against the proposal that positive results correlated highly with closeness of injection site to the ventricle [16].

The application of neurochemical solutions via very fine cannulas (modified 27-gauge needles), gives distances between effective and ineffective sites between animals of as little as 100 μm [3]. These data again support the view that diffusion of behaviorally effective doses of centrally applied neurochemicals is limited.

CONCLUSION

With some reservations regarding the possible individuality of diffusion patterns of different biochemicals and the currently early stage of investigations of this phenomenon, the weight of the evidence considered in the above three sections seems clearly to favour an anatomical localisation of the major effects of centrally applied neurochemicals. In contrast there is little direct evidence for a widespread diffusion or transport of these neurochemicals in concentrations at all likely to be behaviorally effective. However, additional experimental controls seem indicated such as measuring latencies, and dose level characteristics of CSB, to differentiate anatomically localised effects from ventricularly induced responses, as well as further direct studies of diffusion patterns.

CHEMICAL SPECIFICITY

The second question raised by the evidence for a neural circuits model of behavior is that of chemical specificity. In order to establish the behavioral specificity of the effects of CSB, it seems necessary to demonstrate the following: (1) the behavioral effects are not simply due to a general arousal or depression of behavior; (2) biochemical manipulations of the endogenous neurochemicals should produce effects consonant with the existence of metabolic cycles known to exist for neurotransmitters in the periphery; and (3) the behavioral effects are not due to nonneurochemical

factors such as introduction of chloride ions, changes in osmotic pressure or pH.

General vs. Specific Arousal or Depression

There has been a long-standing popular view, with surprisingly little evidence, that the basic function of the central adrenergic neural network is the activation of behavior, whereas that of the central cholinergic network is inhibition of behavior (e.g. [6]). Although this view has been strongly criticised [74] the related question for CSB studies is whether the behavioral responses are only symptomatic of general behavioral arousal or depression, the actual nature of the response depending on those available to the animal in the experimental situation. When both food and water are made available to animals, consistently specific effects have been reported, an adrenergic elicitation of eating and a cholinergic elicitation of drinking [19,20]. This finding has been subsequently confirmed many times, including studies using solutions of neurochemicals as stimuli (e.g. [48]).

Further, there is a well documented antagonism between the adrenergic and cholinergic behavioral effects; adrenergic stimulation both increases food intake and decreases water intake in the deprived rat [20,22]. The converse effect is found with cholinergic stimulation. In this characteristic, central chemical stimulation mimics the interaction of deprivation induced hunger and thirst. This interaction has been investigated and shown to be a local effect, at least in the rat amygdala [73], some metabolic aspects of which were recently elucidated [72]. It has been suggested [74] that this direct neurohumoral interaction between activated behavior control circuits may be one of the important motivational integrative mechanisms.

There is some difficulty in interpreting reports of cholinergic elicitation of eating or sleeping after hypothalamic stimulation in rat, and adrenergic elicitation of drinking [61]. As pointed out, the regions stimulated, the methods of stimulation, and even perhaps the strain of animals used may all contribute to variability of results within species.

It is important that studies are precise in their report of locus of stimulation. In proposing a chemical coding of behavior, it is emphasised that the coding is as much a function of CNS locus as it is of the neurochemicals [74]. This has been shown by the demonstration of the elicitation of either maternal behavior or male sexual behavior following injection of testosterone into the hypothalamus of the male or female adult rat, by varying only the locus of stimulation within the lateral hypothalamus [13]. More recently, a cholinergic mechanism for killing behavior has been demonstrated in the rat hypothalamus [76].

Thus it can be seen that the model to be developed here is based on both anatomical and chemical coding of behavior, and it is suggested that some of the present inconsistencies in the evidence may be explicable in terms of hidden anatomical differences between studies.

Neurotransmitter Metabolism

If the effects of CSB are genuinely due to a neurohumoral involvement of the exogenous neurochemicals, it should be possible to manipulate other aspects of the metabolic cycles of the postulated endogenous neurotransmitters in a predictable way. In fact, such controls have been employed in earlier studies [19, 20, 21]. Injections of atropine (a known cholinergic blocking agent) completely eliminated cholinergically elicited drinking, while having little effect on eating; and injections of ethomoxane (an adrenergic blocking agent) reduced adrenergically elicited eating, but had little effect on drinking. Similar, but less complete, effects occurred on natural thirst or hunger.

Similarly, injections of eserine (an anticholinesterase) elicited drinking [48], while injections of dopamine (precursor of noradrenaline) and dimethylaminoethanol (precursor of acetylcholine) elicited eating and drinking respectively [21]. In accord with the proposed mode of action, the magnitude of effects of the two precursors was less, while the latencies were greater, than for noradrenaline or carbachol. More recently it has been shown that hypothalamic injections of eserine as well as diisopropyl fluorophosphate (DFP), both inhibitors of cholinesterase, will cause water intake in water satiated animals [83]. The effects of DFP last longer than the eserine effect, which is in accordance with the known irreversibility of DFP as an inhibitor of cholinesterase: a similar eserine effect is found in deprived animals [49]. These data strengthen the inference from earlier studies that cholinergic synaptic transmission is involved in drinking in the rat.

The injection of desmethylimipramine into the rat hypothalamus 20 min before a similar injection of norepinephrine, has been reported to augment the adrenergically elicited eating response [4]. It was concluded that this was evidence for the proposition that exogenous norepinephrine has its effect on the endogenous adrenergic pathways via the presynaptic reuptake mechanism. Similarly neither serotonin nor dopamine elicited eating in food satiated rats but microinjections of desmethylimipramine (DMI), which blocks the reuptake of norepinephrine into the presynaptic terminal, potentiated at least 8 fold the action of injected norepinephrine [75]. We have shown that intrahypothalamic injection of DMI alone did not increase the food intake of food satiated animals but caused food deprived animals to eat significantly more [54,55]. These findings are strong support for the notion that norepinephrine is released in the hypothalamus during hunger, since it appears that its action is potentiated when presynaptic uptake of norepinephrine is blocked, and that therefore an adrenergic hypothalamic system is involved in the eating behavior of the rat.

Even more convincing is recent evidence employing 6-hydroxydopamine (6-OHDA) [11]. 6-OHDA is known to specifically affect catecholaminergic neurones, apparently by being absorbed into the neural endings by the synaptic uptake mechanism. In lower doses, 6-OHDA results in a temporary depletion of norepinephrine; in higher doses, it causes the permanent destruction of the adrenergic nerve ending, presumably due to the toxic by-products of its auto-oxidation [70,80]. Observations show an initial increase in eating after injection of 6-OHDA, but then a diminution of response [11]. This has been interpreted as being due to an initial release of norepinephrine from the presynaptic endings, caused by the uptake of 6-OHDA, and then a gradual depletion of the available norepinephrine, due to the effects of 6-OHDA on the adrenergic neurones. Eating induced by 6-OHDA was abolished by pretreatment with DMI. This study is thus further evidence for the endogenous involvement of norepinephrine in the hunger circuit in the rat.

Finally, cannulas have been used as chemotrodes to show that the electrical activity in chemically stimulated

brain sites remained normal after such stimulation, except when an overdose, producing gross behavioral malfunctions, had occurred [20]. In summary, then, these data support the specificity of the action of centrally applied neurochemicals.

Nonneurohumoral Factors

Original studies also employed several controls to rule out the possibility that the observed behavioral effects might be due to other than neurohumoral characteristics of the injection procedure [19, 20, 21]. Comparable amounts of crystalline NaCl, were injected to control for a general tonic effect, and strychnine to control for a general neural excitatory effect. Neither manipulation had any effect on the observed consummatory behavior.

Two additional controls are possible in studies employing solutions as stimuli. Generally, the stimulus solutions are made isotonic with the CSF, by additional of NaCl, so as to rule out osmotic effects. This procedure has the added advantage of allowing use of a placebo condition, in which 0.9% NaCl is injected (e.g. [69]). Secondly, the pH factor of solutions can be checked, and if a marked deviation should occur it can be corrected (e.g. [52]). The immediate mechanical pressure possibly produced by injection of a solution into the brain does not appear to have any systematic effect on behavior, since the studies using solutions have generally replicated the results of studies using crystals, in which presumably no such pressure changes occur.

CONCLUSION

The weight of the evidence reviewed above seems clearly to favour the existence of chemical specificity in the behavioral effects of centrally applied neurochemicals, providing due regard is given to the precise locus of stimulation. Further, it appears reasonable to conclude that this chemical specificity reflects the involvement of the exogenous neurochemicals in the endogenous metabolic cycles. As has previously been pointed out, the actual nature of the neurophysiological activity produced and the ultrastructural site of action of the injected chemicals are not known [67]. There is as yet no direct experimental evidence to show that the effect is confined to the synapse. For instance, it has been shown that the transmitter substances used in CSB can lead to both hyper- and depolarisation when applied to the axons of different neurons [41]. However, the work involving 6-OHDA would strongly support the hypothesis that presynaptic mechanisms are involved in the chemically elicited eating response, and studies employing microelectrodes in conjunction with CSB, or iontophoresis, have shown increased unit firing at the site of application of cholinergic materials [67]. All of these data are suggestive circumstantial evidence for the identification of the endogenous neurotransmitters in terms of the behavioral effects of the injected chemicals. But it may prove impossible to gather definitive data in this regard, since stimulation techniques sufficiently fine to support reliable physiological inferences, such as iontophoresis, have not been shown to have meaningful behavioral effects. Perhaps the most fruitful approach to this problem will be to continue to produce supportive evidence, particularly by exploiting the converging operation of measuring neurochemical changes as the dependent variables following manipulations of environmental or behavioral factors.

INTERPRETATION OF CHEMICAL STIMULATION DATA

The central question for the theory of chemically coded behavior control circuits in close spatial anatomical proximity, is the extent to which chemically elicited behavior is identical to similar behavior elicited through environmental stimulation such as deprivation. Further questions arise from the relationship of chemically elicited behavior to similar behavior produced through ablation or electrical stimulation.

In this section the direct evidence for the equivalence of central chemical stimulation and naturally occurring deprivation states will be discussed first. Indirect evidence concerned with the functional similarity between chemically and naturally elicited behaviors, and with regard to other physiological properties of the organism, will be followed by a brief discussion of the consequences, for the interpretation of chemical stimulation data, of the discovery of switching behavior during electrical stimulation [81].

Equivalence of Central Stimulation and Deprivation Induced Behavior States

Atropine sulphate injected into limbic and diencephalic brain sites where cholinergic injections elicit drinking, failed to block water deprivation induced thirst [16]. This was in contrast to an earlier report [39], and to another report [37] which showed that drinking induced through central cholinergic stimulation with either carbachol or eserine is almost completely blocked by simultaneous injection of the anticholinergic drug, atropine, to other limbic and diencephalic cholinergically responsive sites, regardless of whether they are ipsilateral, contralateral, homologous or nonhomologous. These findings threw some doubt on the earlier proposed identity of the substrate activated by brain stimulation and those active during natural thirst. A number of alternatives have been proposed [16] to explain these discrepant findings: (1) that the centrally activated cholinergic system is sufficient to induce drinking, but not necessary for drinking to occur during water deprivation, i.e. under the latter organismic condition several neural systems may be active; (2) the cholinergic component may form a reverbatory circuit where bidirectional blockade is effective; and, (3) the state of the substrate of identical circuits for these two forms of inducing water intake may be different in sated and deprived animals. Another way of conceptualising the discrepancy of atropine block is that the circuits for centrally induced drinking and natural thirst are either (1) chemically different, or (2) anatomically different, or (3) both. Alternatively, it is possible that they are chemically and anatomically identical but that the present experimental data in the case of natural thirst are based on different quantitative relationships.

An attempt was made [2] to explore the quantitative relationships by using methyl atropine nitrate, the quarternary ammonium form of atropine with more potent antimuscarinic effects as a blocking agent, while others [31] have varied the time of water deprivation using a constant dose of atropine. The findings of both studies

show a reduction in water intake from anticholinergic blockade but, although this reduction was significant in both series of experiments, it was far from complete and not nearly as effective as the earlier reported central blockade. Data have been provided on a range of cholinolytic drugs administered intraperitoneally including atropine in its tertiary and quarternary forms [28] and a dose dependent reduction in water intake was found. Since drinking under conditions of natural thirst is presumably dependent on the condition of a number of intero- and exteroceptors, which may or may not be represented in a central drinking circuit, the effect of atropine blockade on an interoceptive variable, salt aroused (hypertonic) drinking has been studied (2,38). These results again show incomplete blockage of water intake. In our own laboratory we have shown that food availability, an exteroceptive variable, can enhance the blocking effect of atropine. It seems therefore that the blocking action may have central and peripheral components and that it is also dependent on states of the organism such as plasma hypotonicity and food availability.

Since noncholinergic factors may also be involved in the natural thirst circuit we have recently explored the blocking action of the adrenergic transmitter, norepinephrine [20] on both centrally induced and natural thirst. Findings from our laboratory [72] show that, with bilateral placements in the lateral hypothalamus, injections of norepinephrine in concentrations equimolar to simultaneously injected carbachol, block carbachol induced drinking completely, which is similar to the results with atropine already reported [16]. Further, we have found that natural and salt induced thirst are blocked more efficiently by norepinephrine than by atropine. Similarly, the most effective blocker of hunger seems to be carbachol. In fact, whereas norepinephrine induced hunger is blocked by both phentolamine, an alpha-blocker, and carbachol, deprivation induced hunger could be affected only by carbachol [72]. Systematic dose level studies as well as a study of the two drugs in combination are still in progress. These results are in accord with earlier reported work [21,22].

Although these data seem to indicate, in confirmation of the findings of electrical stimulation and ablation studies, that anatomically the lateral hypothalamus plays a central role in drinking behavior, the chemical substrate involved seems more complex than expected from the earlier CSB studies. It raises the question of the mechanism of the norepinephrine blockade. At least two explanations seem plausible, either a direct blocking of cholinergic fibres similar to the norepinephrine elicited hyperpolarizing response in sympathetic ganglion cells [41]; or alternatively, the blockade may result from an interneuronally mediated reciprocal inhibition. Indirect evidence for the latter mechanism comes from experiments involving small doses of norepinephrine electrophoretically applied, which can increase the activity of some single units and decrease that of other units depending on their anatomical location [63]. This indicates there are at least two norepinephrine sensitive systems, which may account for an inhibitory effect on a cholinergic drinking circuit by norepinephrine at least partially independent of an adrenergic eating circuit. Alternatively, circuits consisting of both adrenergic and cholinergic fibres, as found in the peripheral nervous system, [30] are a possible explanation of this interaction.

The earlier studies [20,21] seemed to indicate an adrenergic feeding system and a cholinergic drinking system. The drinking system was shown to be muscarinic [77] and consequently the blocking studies discussed earlier in this section were concerned with the muscarinic blocker, atropine. Although earlier work on the feeding system had also shown extensions into other limbic and diencephalic structures [3,8], all of them adrenergically sensitive, recent developments seem to have taken a different turn which has some bearing on the equivalence of natural hunger and centrally induced eating. It has been suggested [33] that alpha and beta adrenergic systems act antagonistically, in that the alpha hunger system elicits feeding and beta satiety system blocks it. Results from studies using cannulas implanted in the midhypothalamic regions, where the alpha and beta adrenergic synapses for the two systems are purported to meet, seem to indicate that the combination of a beta agonist and an alpha antagonist not only led to complete blocking, but produced supersatiety. The main objections to this simple system, which meets all the requirements of a chemically coded feeding system, come from the suggestion [44,45] that the beta system acts to suppress feeding in response to unattractive tastes, whereas the alpha blockade is analogous to the finickiness found after ventromedial lesions. The difference in these two points of view is confounded by two factors. The alpha and beta classification refers to the effect of these chemicals on peripheral adrenergic receptors. However, the classification of an adrenergic compound as alpha or beta is far from clearcut. Thus norepinephrine and epinephrine are supposed to have both alpha and beta properties, and amphetamine may have alpha and beta properties through the release of norepinephrine or act as a beta agonist independently of its effect on norepinephrine. Thus any experimental results using these compounds can be interpreted in more than one way. In addition to this difference in interpretation based on the dual or even multiple classification of compounds, there is a difference in the experimental procedure, i.e. the use of dry food pellets [33] against animals fed on milk [44,45]; this introduces a confounding variable which is the amount of water content in the food. Because milk consists primarily of water, and ontogenetically acts as a source of both food and water, it was felt that the interpretation of milk licking as eating behavior was more equivocal than has been supposed. In a study designed to investigate this possibility [51] ingestive behavior was observed in rats following hypothalamic CSB with norepinephrine, carbachol or placebo in a cafeteria situation in which water, milk, wet mash, powdered food and lab chow cubes were available. Cholinergic CBS elicited significant intakes of water and milk only, and water was significantly preferred to milk. Adrenergic CSB elicited significant intake of wet mash only. These data support the suggestion that the rats have ingested the milk primarily as a source of water [34], and cast serious doubt on the validity of the alpha-adrenergic satiety hypothesis [44,45].

The concept of a simple muscarinic cholinergic drinking circuit has also been questioned by a number of more recent studies which have shown the involvement of angiotensin and beta-adrenergic agonists in drinking behavior. Evidence has been presented for hypothalamic beta-adrenergic elicitation of drinking, as well as the suggestion that the central cholinergic system mimics the hypothalamic beta-adrenergic system and opposes an alpha-adrenergic system [35]. However, in the septal area, a

comparison of the drinking responses to a beta-adrenergic agonist, angiotensin, and carbachol, showed that the magnitude of the response to carbachol was by far the largest [14].

In our own laboratory, we were unable to elicit drinking with isoprenaline in the hypothalamus in a site where both carbachol and angiotensin led to copious drinking. In fact, isoprenaline blocked drinking in water deprived rats and eating in food deprived rats. Prior injection of the beta-adrenergic blocker propranolol eliminated the blocking effect of isoprenaline on hunger, but not on thirst [71]. It has been proposed that positive results to central injections of beta-agonists may be due to diffusion to the periphery [14]. Peripherally administered beta-agonists produce copious drinking in the rat [32], which is accompanied by a signficant increase in plasma renin activity [64]; acute nephrectomy abolishes the drinking response to these drugs [47]. Thus, there are findings that the renin-angiotensin system might be involved in the drinking response to peripherally administered beta-agonists.

Evidence for a consistent drinking response following central administration of angiotensin is much stronger [10,18]. Widespread sensitivity to angiotensin has been found in the septal area, the thalamic region, preoptic region and in the hypothalamus. Current hypotheses suggest that the central mechanism of angiotensin induced drinking is independent of cholinergic mechanisms since the former is not blocked by atropine [18], but haloperidol, a dopaminergic antagonist does markedly reduce angiotensin drinking [17]. In the preoptic region, angiotensin induced drinking is reported to be independent of the cholinoceptive system, but depends on the integrity of catecholamine neurons [17]. It is further proposed that cholinergic pathways are involved in the regulation of osmotic thirst, while angiotensin is involved in drinking induced by hypovolemia [18]. This is similar to other hypotheses of a two-thirst system [27].

As yet it is difficult to see what role these possible other systems may play, but it is not inconceivable that input and output from the eating and drinking circuits are coded in a different way. Alternatively, these other drugs may also have their behavioral effects via the muscarinic cholinergic and alpha-adrenergic systems, since the beta-agonists, for instance, could be inhibiting the alpha-adrenergic system and thus disinhibiting the cholinergic system. Such a mechanism would have the reported effect of increased drinking. In any case, the evidence for a major role of alpha-adrenergic and muscarinic cholinergic transmitters in the central control of eating and drinking seems inescapable. So far the data presented here with regard to both the chemically coded drinking and eating circuits leave the issue of the relationship between deprivation induced behavior and chemically induced behavior unresolved. In the next section data relating to the functional similarity of behavior resulting from these two different methods of elicitation will be discussed.

Indirect Evidence for the Similarity of Chemically Elicited and Deprivation Induced Behavior States

Studies of the functional equivalence of chemical and deprivation induced behavior states have been concerned with quantitative and qualitative similarities in behavior, with the motivating properties of the behavior, and with other physiological correlates.

Quantitative and Qualitative Similarities

The relationship between the dose level of cholinergic and adrenergic stimuli and drinking and eating behavior has been examined [50]. It can be argued that, as in studies of the relationship of duration of water and food deprivation, a threshold has to be reached before a behavioral response different from base line consummatory behavior occurs; and that there is an increase in the magnitude of the consummatory response with an increase in the dose level; and that there is a maximum response. The functional relationship between water intake and dose level of cholinergic stimulation in the hypothalamus [50] and in the amygdala [69] show that the maximum response to carbachol does not occur with a maximum dose level but tends to drop again with an increase in dose level once a maximum response is reached. This lack of equivalence is interpreted by the experimenters as the result of other cholinergic systems being activated by larger doses of carbachol and that competing responses are elicited. Similarly the eating curve [50] is also different in showing peaks at two widely different concentrations of norepinephrine. However, a norepinephrine dose response curve for food intake has been obtained with ventricular injections [61], which is more similar to the carbachol drinking curve [50] with a distinct falling off of the response at the highest dose level. It is relevant to note that abnormal electrical activity in the brain, recorded via chemotrodes [20], only follows injection of doses sufficiently large to elicit the gross motor responses associated with a dropping off in the consummatory response.

Levitt, White and Sanders [40] show a similar carbachol dose level response curve for drinking, to that of Miller *et al*, for the lower concentrations which they tested in five different areas of the limbic-diencephalic system. Of further interest are the experiments [69] showing the quantitative relations between the intensity of both kinds of stimulation, i.e. dose level of carbachol under four levels of water deprivation. These experiments are indicative of a limited channel capacity [69] of the drinking system which can be reached through a number of combinations in different proportions of the two eliciting stimuli (cholinergic stimulation and duration of water deprivation) but that such combinations do not exceed the maximum response which is reached by the longest period of deprivation. Various other time relationships, such as latency and duration of drinking, are similar for chemically and deprivation induced drinking [65]. These results relating to duration of drinking have been confirmed [36].

Studies of the qualitative aspects of chemical stimulation have been concerned with changes in consummatory behavior as a result of the palatability of the proffered food or drinking fluids. Carbachol elicited drinking preferences lead to fluid intakes similar to the "well known preference-aversion curves for saline solutions produced by rats made thirsty by water deprivation" [79], and it is concluded that carbachol produces motivated behavior and not just reflexive drinking.

A greater preference for saccharine treated food and a lesser preference for quinine treated food for norepinephrine induced eating, as compared with deprivation induced eating has been reported [5,7]. This is in accordance with other hypotheses [45] although as indicated earlier it is doubted whether this hypothesis has been definitively tested [51].

It seems that there are differences, as well as similarities, in the consummatory behavior elicited by central chemical stimulation when compared with that following deprivation states. The theory of chemically coded drive circuits may well turn out to be more complex, as already suggested [46], and it is possible that present techniques of placement and stimulation do not as yet allow the separation of interacting chemical systems in close spatial proximity. A further confounding factor may be environmental differences, such as feeding schedules and diets, which may change the input from the interoceptors and exteroceptors into the central areas and thus differentially change the sensitivity to the chemical substances injected.

Regardless of the quantitative and qualitative similarities discussed earlier the question has been asked whether chemical stimulation has motivational properties similar to the deprivation state. Food and water satiated rats cholinergically stimulated in the hypothalamus will press a lever to obtain water but will not respond to an adjacent lever to obtain food [24]. The same animals showed a complete reversal of this pattern following adrenergic stimulation. This is in accordance with findings which showed a relationship between the rate of bar-pressing for water and dose level of carbachol in satiated animals [28]. However, in contrast to this, it has been reported that adrenergically stimulated food satiated rats will not bar-press for food, although they will eat freely available food [7]. Yet there seems to be a strong indication that, given the proper conditions, centrally induced stimulation will make an animal learn and work similarly to the specific motivational properties of deprivation states.

Physiological Correlates

Changes in plasma volume and osmolarity which occur as a result of water deprivation have not been found in thirst produced by carbachol injections. This has been interpreted as showing that chemically induced drinking is the result of direct activation of central neural structures, and is not secondary to systemic alterations of body fluid [79]. Reports of increased urine osmolarities suggest increased ADH secretion [48], and would indicate that the central neural structures activated by chemical stimulation are not controlling a reflex consummatory response but the overall water metabolism mechanism. Indirect support for this is also provided by experiments involving peripheral adrenergic agonists which elicit drinking and simultaneously inhibit urine flow and a contrary effect for antagonists; both of these exhibiting clearcut dose response relationships [32]. Further studies of other physiological concomitants of central chemical stimulation would be helpful for a more precise definition of its relationship to deprivation states.

A new technique, the push-pull cannula, which allows the withdrawal of CSF fluid from a living animal, provides a different approach to the investigation of chemical coding of brain circuits. Thus it is now possible to use biochemical events as a dependent variable, while the environmental events which lead to different organismic states can be varied under the experimenter's control. Such a push-pull cannula has been used to couple the hypothalami of two monkeys and showed that temperature changes induced in one subject (the donor), involving his whole body, led to opposite temperature changes in the other animal (the recipient), through the exchange of CSF drawn from the hypothalamus of the first animal [58]. This technique has been extended from the bioassay stage to a biochemical assay [78], by showing that CSF obtained from a limbic system pleasure area, adjacent to the stimulating electrode during electrical self-stimulation, showed higher norepinephrine activity when compared to control. Thus, the way is open to study hypothalamic CSF, from deprived and nondeprived rats, for transmitter activity relevant to their deprivation state. This could prove a valuable tool in further research into the relationship between deprivation and the endogenous biochemical states, and may throw some light on the relationship between natural deprivation and chemically induced behaviors.

Electrical Stimulation

Although the relationship between electrical brain stimulation, ablation and chemical stimulation has been recently discussed [81] there are many outstanding issues which need to be clarified for the explanation of chemical stimulation. One point of view which may be taken is that the nonchemical methods yield different results in many cases because chemical stimulation results in a more specific selection of anatomically adjacent circuits which are chemically distinct. Thus ablation techniques, however fine they are, can only separate and lead to the removal of anatomical units, regardless of the complexity of neurochemical coding within them, and a similar argument applied to electrical stimulation which is not only chemically indifferent, but bidirectional as well. Recent work shows that when a single stimulating electrode is used repeatedly, this will lead to a switch in behavior, e.g. from drinking to eating. This has not been shown to occur with regard to chemical stimulation [1]. There are, of course, many factors in Biedeman *et al.*'s [1] study relating to the time course of this stimulation which are not equivalent to those in Valenstein's, and therefore further work is needed in this area. An explanation of Valenstein's findings by Wayner [82] may have some bearing on research into chemically induced behavior. He suggests that there is a general arousal of the hypothalamus during drive and that organisms become more receptive to external stimuli relevant to drive, and that switching is a form of adjunctive behavior similar to schedule-induced polydipsia. This could mean that the activity induced through chemical stimulation may have both a general arousal activity as well as a selective activity, the latter being not unlike the hypothesis discussed in an earlier section [44,45]. Alternatively, the extent of the general arousal found in electrical stimulation may be the result of the summation of a number of simultaneously stimulated but chemically different systems.

CONCLUSIONS

Anatomical and chemical specificity of the effects of chemical stimulation of CNS structures are supported by indirect and direct evidence. Ventricular involvement in the transport of injected chemicals cannot account for a number of the behavioral parameters observed after CSB. It is, however, possible that ventricular involvement is a factor in CSB at some loci, and more careful experimental control, as well as observation of behavioral parameters such as latency, are advisable.

The results of CSB with chemical substances involved in or related to transmitter metabolism, other than the postulated transmitter substances themselves, suggests that

the observed effects are the result of the involvement of the exogenous chemicals in endogenous neurotransmitter cycles. However, direct evidence that this is due to synaptic action is currently lacking. The possibility that an action of CSB on nerve axons is responsible for the observed behavioral effects rather than an action at the synapse cannot be excluded, although recent pharmacological evidence using 6-OHDA [11] and DMI [54,55] provides strong indirect evidence of synaptic involvement. There are more similarities than differences between the behavioral responses observed after CSB and those after deprivation. The differences may be explicable in terms of the direct nature of the intervention by CSB in cerebral processes, rather than by any differences in the chemical substrate. Other factors, such as the influence of palatability of the test food, as well as more careful quantitative differentiation of the responses, may have to be taken into account in future studies, and may help to clarify present differences.

An example of this approach is Oatley's model of a drinking circuit [62], which specifies the involvement of various dehydration factors and takes account of their relative quantitative involvement. Other evidence strongly supports the proposition that cholinergic CSB activates not only drinking behavior but the mechanism for general water metabolism [79]. It seems possible, however, that the conceptualisation of a distinct adrenergic eating circuit, often parallel and proximal to a distinct cholinergic drinking circuit, may turn out to be an oversimplification. The data on the reciprocal blocking interaction of thirst and hunger seem to indicate that this interaction occurs at many of the common points in these circuits.

It is now possible to suggest an alternative to, or rather a development of, the models of neurochemical control of behavior proposed by earlier investigators [13, 24, 48]. We will discuss this model in the context of thirst and hunger, since the neurochemical substrates of these have been most extensively studied, but it is suggested that the principles elucidated may well prove applicable to the regulation and integration of other similar behaviors.

It is proposed that water deprivation produces both peripheral and central sensory inputs which increase the level of activity in a central cholinergic circuit. There are several such sensory inputs [62] and the central circuit is complex [13], and symmetrically present and functioning in both halves of the brain. Thus water deprivation stimulates a considerable amount of central neural activity, in diffuse but biochemically identifiable pathways.

There are two basic outcomes possible from this stimulation. First, it is proposed that this increased cholinergic activity interacts with the levels of activity in adjacent, neurochemically different circuits, at all synaptic points where these other circuits are proximal. This interaction is proposed to be one of mutual inhibition, probably of a local nature [73], although it is not yet known whether the mechanism of this interaction is one of (1) diffusion of transmitter into adjacent, neurochemically different synapses (e.g. spread of acetylcholine into noradrenergic synapses, where it has an inhibitory effect); or (2) interneural mediation (e.g. acetylcholine release acts to stimulate both the next neurons in a cholinergic pathway and smaller interneurons which have inhibitory connections with noradrenergic neurons); or (3) some similar mechanism(s) within neurochemically mixed fibres [30].

Secondly, it is the end result of this mutually inhibitory interaction which decides which drive circuit is currently dominant in central neural activity, and thus which behavior pattern will be elicited. For instance, if cholinergic activity is sufficiently intense, presumably because the sensory inputs from the effects of water deprivation are sufficiently great, it will both inhibit other, antagonistic circuits and thus the incompatible behavioral sequences mediated by those circuits, and produce water seeking and ingesting behavior. Eventually, peripheral sensory input from the effects of water-ingestion activates an inhibitory mechanism (perhaps only a reduction in the total sensory input into the central cholinergic circuit), which has been shown to be of only short-term effectiveness. The final inhibition of thirst is brought about by a lowering of central cholinergic activity, due to both a reduction in central sensory input, as the ingested water is metabolised, and to the inhibitory actions of other, still activated circuits, such as the noradrenergic circuit.

There are a number of advantages to this model. It is in accord with the known biological principles, as seen in analogously competitive neural systems. It accounts for the differential effectiveness of centrally applied atropine and norepinephrine as blockers of deprivation induced thirst. In this model, microinjected atropine produces a local blocking effect on the cholinergic circuit, at the point of application, but leaves the rest of the circuit intact. This local block is apparently sufficient to nullify the activity resulting from microinjected carbachol, but not the activity resulting from the multiple sensory inputs in water deprivation. Microinjected norepinephrine, on the other hand, not only produces a local blocking effect on the cholinergic circuit at the point of application, but also activates the noradrenergic circuit so that it exerts its inhibitory effect on the cholinergic circuit throughout the CNS, at all points where the two circuits interact. Thus norepinephrine, as has been empirically demonstrated, is a much more effective blocker of thirst than is atropine. A converse set of relationships is proposed to mediate the differential effectiveness of phentolamine and carbachol as blockers of hunger.

Finally, this model is able to fill the gap seen by Grossman [25] in his theory of the physiological bases of drive, when he was unable to suggest what might be the long-term satiety mechanisms. In this model, neural inhibitory competition between continually active drive circuits serves to keep in check the activity levels in the respective circuits, and thus acts as a long-term satiety mechanism. The model is thus able to account for the apparent adaptiveness and purposiveness of behavior, since it is the organism's most impelling present physiological need which will produce the greatest sensory input to the appropriate behavior circuit thus both producing the appropriate behavior and preventing the occurrence of incompatible responses. It also obviates the earlier proposal of a division of drives into homeostatic and nonhomeostatic [25]. The differences seen between hunger and sex, for example, in terms of susceptibility to satiety and deprivation, are explicable in terms of the different natures of the essential drive producing sensory inputs. Hunger is primarily elicited by internal stimuli, the bases for which (glucose utilization) are time dependent. Sexual behavior, on the other hand, is primarily elicited by external stimuli (given an appropriate internal milieu), and the occurrence of suitable external stimuli is not time dependent.

While it is true that parts of this model are currently speculative, it is compatible with the known data, and explicates some of the apparent confusions in those data.

REFERENCES

1. Beideman, L. R. and R. Goldstein. Specificity of carbachol in the elicitation of drinking. *Psychonom. Sci.* **20:** 5. 1970.
2. Block, N. L. and A. E. Fisher. Anticholinergic central blockade of salt-aroused and deprivation-induced drinking. *Physiol. Behav.* **5:** 525–527, 1970.
3. Booth, D. A. Localization of the adrenergic feeding system in the rat diencephalon. *Science* **158:** 515–516, 1967.
4. Booth, D. A. Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. *J. Pharmac. exp. Ther.* **160:** 336–348, 1968.
5. Booth, D. A. and D. Quartermain. Taste sensitivity of eating elicited by chemical stimulation of the rat hypothalamus. *Psychonom. Sci.* **3:** 525–526, 1965.
6. Broverman, D. M., E. L. Klaiber, Y. Kobayashi and W. Vogel. Roles of activation and inhibition in sex differences in cognitive abilities. *Psychol. Review* **75:** 25–50, 1968.
7. Coons, E. E. and D. Quartermain. Motivational depression associated with norepinephrine induced eating from the hypothalamus: resemblance to the ventromedial hyperphagic syndrome. *Physiol. Behav.* **5:** 687–692, 1970.
8. Coury, J. N. Neural correlates of food and water intake in the rat. *Science* **156:** 1763–1764, 1967.
9. Epstein, A. N., J. T. Fitzsimons and Barbara J. Simons. Drinking caused by the intracranial injection of angiotensin into the rat. *Proc. physiol. Soc.* **200:** 98–100, 1969.
10. Epstein, A. N., J. T. Fitzsimons and Barbara J. Rolls (nee Simons). Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol.* **210:** 457–474, 1970.
11. Evetts, K. D., J. T. Fitzsimons and Paulette E. Setler. Eating caused by release of endogenous noradrenaline after injection of 6-hydroxydopamine into the diencephalon of the rat. *J. Physiol.* **216:** 68–69, 1971.
12. Feldberg, W. *A pharmacological Approach to the Brain from its Inner and Outer Surface.* London: Edward Arnold, 1963.
13. Fisher, A. E. Chemical stimulation of the brain. *Scient. Am.* **210:** 60–69, 1964.
14. Fisher, A. E. Drinking induced by brain injection of carbachol, angiotensin and isoproterenol: Probable sites of action and differential blockade by atropine. Paper presented I.C.R.F.W.I. Cambridge, 1971.
15. Fisher, A. E. and J. N. Coury. Cholinergic tracing of a central neural circuit underlying the thirst drive. *Science* **138:** 691–693, 1962.
16. Fisher, A. E. and R. A. Levitt. Reply to Routtenberg - Drinking induced by carbachol: Thirst circuit or ventricular modification? *Science* **157:** 839–841, 1967.
17. Fitzsimons, J. T. and P. E. Setler. Catecholaminergic mechanisms in angiotensin-induced drinking. *J. Physiol.* **218:** 43–44, 1971.
18. Giardina, A. R. and A. E. Fisher. Effect of atropine on drinking induced by carbachol, angiotensin and isoproterenol. *Physiol. Behav.* **7:** 653–655, 1971.
19. Grossman, S. P. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science* **132:** 301–302, 1960.
20. Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am. J. Physiol.*, **202:** 872–882, 1962.
21. Grossman, S. P. Effects of adrenergic and cholinergic blocking agents on hypothalamic mechanisms. *Am. J. Physiol.* **202:** 1230–1236, 1962.
22. Grossman, S. P. Behavioural effects of chemical stimulation of the ventral amygdala. *J. comp. physiol. Psychol.* **57:** 29, 1964.
23. Grossman, S. P. Effect of chemical stimulation of the septal area on motivation. *J. comp. physiol. Psychol.* **58:** 194, 1964.
24. Grossman, S. P. Behavioural effects of direct chemical stimulation of central nervous system structures. *Int. J. Neuropharm.* **3:** 45–58, 1964.
25. Grossman, S. P. *A. Textbook of Physiological Psychology.* New York: Wiley, 1967.
26. Grossman, S. P. and W. G. Stumpf. Intracranial drug implants: An autoradiogrphaic analysis of diffusion. *Science* **166:** 1410–1412, 1969.
27. Houpt, K. A. and A. E. Epstein. The complete dependence of beta-adrenergic drinking on the renal dipsogen. *Physiol. Behav.* **7:** 897–902, 1971.
28. Khavari, K. A. and R. W. Russell. Acquisition, retention and extinction under conditions of water deprivation and of central cholinergic stimulation. *J. comp. physiol. Psychol.* **61:** 339–345, 1966.
29. Khavari, K. A. and R. W. Russell. Depression of drinking by tertiary and quaternary cholinolytic drugs. *Physiol. Behav.* **4:** 461–463, 1969.
30. Koelle, G. B. *Handbook of Experimental Pharmacology.* Heidelberg: Springer Verlag, 1963.
31. Krikstone, B. J. and R. A. Levitt. Interactions between water deprivation and chemical brain stimulation. *J. comp. physiol. Psychol.* **71:** 334–340, 1970.
32. Lehr, D., J. Mallow and M. Krukowski. Copious drinking and simultaneous inhibition of urine flow elicited by BETA-adrenergic stimulation and contrary effect of ALPHA-adrenergic stimulation. *J. Pharmac exp. Ther.* **158:** 150–163, 1967.
33. Leibowitz, S. Hypothalamic beta-adrenergic "satiety" system antagonizes an alpha-adrenergic "hunger" system in the rat. *Nature* **226:** 963–964, 1970.
34. Leibowitz, S. Hypothalamic alpha- and beta-adrenergic systems regulate both thirst and hunger in the rat. *Proc. natn. Acad. Sci.* **68:** 332–334, 1971.
35. Leibowitz, S. F. Pharmacological studies of hypothalamic systems mediating hunger and thirst. Paper presented I.C.R.F.W.I. Cambridge, 1971.
36. Levitt, R. A. Temporal decay of the blockade of carbachol drinking by atropine. *Physiol. Behav.* **5:** 627–628, 1970.
37. Levitt, R. A. and R. P. Boley. Drinking elicited by injection of eserine or carbachol into rat brain. *Physiol. Behav.* **5:** 693–695, 1970.
38. Levitt, R. A. and P. B. Buerger. Interactions between cholinergic mechanisms and the salt arousal drinking. *Learn. Motiv.* **1:** 297–303, 1970.
39. Levitt, R. A. and A. E. Fisher. Anticholinergic blockade of centrally induced thirst. *Science* **154:** 520–522, 1966.
40. Levitt, R. A., C. S. White and D. M. Sanders. Dose-response analysis of carbachol-elicited drinking in the rat limbic system. *J. comp. physiol. Psychol.* **72:** 345–350, 1970.
41. Libet, B. and H. Kobayashi. Generation of adrenergic and cholinergic potentials in sympathetic ganglion cells. *Science* **164:** 1530–1532, 1969.
42. Lovett, D. and G. Singer. Ventricular modification of drinking and eating behaviour. *Physiol. Behav.* **6:** 23–26, 1971.
43. Maclean, P. D. Cranial drugs diffuse quite some distance under pressure. *Archs Neurol.* **78:** 113, 1957.
44. Margules, D. L. Alpha-adrenergic receptors in hypothalamus for the supression of feeding behaviour by satiety. *J. comp. physiol. Psychol.* **73:** 1–12, 1970.
45. Margules, D. L. Beta-adrenergic receptors in the hypothalamus for learned and unlearned taste aversions. *J. comp. physiol. Psychol.* **73:** 13–21, 1970.
46. Margules, D. L. and L. Stein. Cholinergic synapses in the ventromedial hypothalamus for the suppression of operant behaviour by punishment and satiety. *J. comp. physiol. Psychol.* **67:** 327–335, 1969.
47. Meyer, D. K., B. Peskar and G. Hertting. Hemmung des durch blutdrucksenkende pharmaka bei ratten ausgelösten trinkens durch nephrektomie. *Experientia* **27:** 65–66, 1971.
48. Miller, N. E. Chemical coding of behaviour in the brain. *Science* **148:** 328–338, 1965.

49. Miller, N. E. and Chun-Wuei Chien. Drinking elicited by injecting eserine into preoptic area of rat brain. *Communs. behav. Biol.* **1**: 61–63, 1968.
50. Miller, N. E., K. S. Gottesman and N. Emery. Dose response to carbachol and norepinephrine in rat hypothalamus. *Am. J. Physiol.* **206**: 1384–1388, 1964.
51. Montgomery, R. B. and S. M. Armstrong. Cafeteria behaviour in rat after hypothalamic cholinergic and adrenergic stimulation, (in preparation)
52. Montgomery, R. B. and G. Singer. Lack of effect on drinking of stimulation of the ventral amygdala of rat with GABA. *J. comp. physiol. Psychol.* **69**: 623–627, 1969.
53. Montgomery, R. B. and G. Singer. Histochemical fluorescence as an index of spread of centrally applied neurochemicals. *Science* **165**: 1031–1032, 1969.
54. Montgomery, R. B., G. Singer, A. T. Purcell, J. Narbeth and A. G. Bolt. Central control of hunger in the rat. *Nature* **223**: 1278–1279, 1969.
55. Montgomery, R. B., G. Singer, A. T. Purcell, J. Narbeth and A. G. Bolt. The effects of intrahypothalamic injections of desmethylimipramine on food and water intake of the rat. *Psychopharmacologia* **19**: 81–86, 1971.
56. Myers, R. D. Modification of drinking patterns by chronic intracranial infusion. *Thirst* - 1st Int. Symposium on Thirst in the Regulation of Body Water, edited by M. J. Wayner. New York: Pergamon Press, 1964, pp. 533–549.
57. Myers, R. D. Injections of solutions into cerebral tissue; relation between volume and diffusion. *Physiol. Behav.* **1**: 171–174, 1966.
58. Myers, R. D. Chemical mechanisms in the hypothalamus mediating eating and drinking in the monkey. *Ann. N.Y. Acad Sci.* **157**: 918–931, 1969.
59. Myers, R. D. and T. J. Cicero. Are the cerebral ventricles involved in thirst produced by a cholinergic substance? *Psychonom. Sci.* **10**: 93–94, 1968.
60. Myers, R. D., M. Tytell, A. Kawa and T. A. Rudy. Microinjection of ^{3}H-Acetylcholine, ^{14}C-Serotonin and ^{3}H-norepinephrine into the hypothalamus of the rat: diffusion into tissue and ventricles. *Physiol. Behav.* **7**: 743–751, 1971.
61. Myers, R. D. and T. L. Yaksh. Feeding and temperature responses in the unrestrained rat after injections of cholinergic and aminergic substances into the cerebral ventricles. *Physiol. Behav.* **3**: 314–325, 1968.
62. Oatley, K. A control model of the physiological basis of thirst. *Med. Biol. Engng* **5**: 225–237, 1967.
63. Oomura, Y., Y. Ono, H. Ooyama and M. J. Wayner. Glucose and osmosensitive neurones of the rat hypothalamus. *Nature* **222**: 282–284, 1969.
64. Peskar, B., D. K. Meyer, V. Tauchmann and G. Hertting. Influence of isoproterenol, hydralazine and phentolamine on the renin activity of plasma and renal cortex of rats. *Eur. J. Pharmac.* **9**: 394–396, 1970.
65. Quartermain, D. and N. E. Miller. Sensory feedback in time response of drinking elicited by carbachol in preoptic area of rat. *J. comp. physiol. Psychol.* **62**: 350–353, 1966.
66. Routtenberg, A. Drinking induced by carbachol thirst circuit or ventricular modification. *Science* **157**: 838–839, 1967.
67. Routtenberg, A. Intracranial chemical injection and behavior: A critical review. *Behav. Biol.* **7**: 601–641, 1972.
68. Routtenberg, A., J. Sladek and W. Bondareff. Histochemical fluorescence after application of neurochemicals to caudate nucleus and septal area *in vivo*. *Science* **161**: 272–274, 1968.
69. Russell, R. W., G. Singer, F. Flanagan, M. Stone and J. W. Russell. Quantitative relations in amygdaloid modulation of drinking. *Physiol. Behav.* **3**: 871–875, 1968.
70. Saner, A. and H. Thoenen. On the molecular mechanism of action of 6-hydroxydopamine. *Molec. Pharmac.*, (in press).
71. Singer, G. and S. Armstrong. Cholinergic and β adrenergic compounds in the control of drinking behaviour, (in preparation)
72. Singer, G. and J. Kelly. Cholinergic and adrenergic interaction in the hypothalamic control of drinking and eating behaviour. *Physiol. Behav.* **8**: 885–890, 1972.
73. Singer, G. and R. B. Montgomery. Neurohumoral interaction in the rat amygdala after central chemical stimulation. *Science* **160**: 1017–1018, 1968.
74. Singer, G. and R. B. Montgomery. Comment on roles of activation and inhibition in sex differences in congnitive abilities. *Psychol. Rev.* **76**: 325–327, 1969.
75. Slangen, J. L. and N. E. Miller. Pharmacological tests for the function of hypothalamic norepinephrine in eating behaviour. *Physiol. Behav.* **4**: 543–552, 1969.
76. Smith, D. E., M. B. King and B. G. Hoebel. Lateral hypothalamic control of killing: evidence for a cholinoceptive mechanism. *Science* **167**: 900–901, 1970.
77. Stein, L. and J. Seifter. Muscarinic synapses in the hypothalamus. *Am. J. Physiol.* **202**: 751–756, 1962.
78. Stein, L. and C. D. Wise. Release of hypothalamic norepinephrine by rewarding electrical stimulation in the unanesthetized rat. *Fed. Proc.* **26**: 651, 1967.
79. Stricker, E. M. and N. E. Miller. Saline preference and body fluid analyses in rats after intrahypothalamic injections of carbachol. *Physiol. Behav.* **3**: 471–475, 1968.
80. Ungerstedt, U. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In: *6-hydroxydopamine and catecholamine neurons*, edited by T. Malmfors and H. Thoenen. Amsterdam-London: North-Holland Publishing Company, 1971, pp. 101–127.
81. Valenstein, E. S. Behaviour elicited by hypothalamic stimulation. *Brain Behav. Evolut.* **2**: 295–316, 1969.
82. Wayner, M. J. Motor control functions of the lateral hypothalamus and adjunctive behaviour. *Physiol. Behav.* **5**: 1319–1325, 1970.
83. Winson, J. and N. E. Miller. Comparison of drinking elicited by eserine or DFP injected into preoptic area of rat brain. *J. comp. physiol. Psychol.* **73**: 233–237, 1970.