EFFECTS OF DRUGS ON DEEP BRAIN CENTERS[1,2] 6509

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The authors make no pretense that this review is either complete or unbiased and have referred to papers published before July 1968 only when necessary.

Methods for Studying Drug Effects upon Deep Brain Centers

Drugs may be given by diverse routes for this purpose.

Systemic injections are widely used, sometimes complemented with multiple depth recording (1, 2). This is so in spite of the blood-brain barrier, of the general impossibility of determining whether an observed effect is direct or reflex, and of the inherent indefinition as to localization of action.

Perfusion of the whole brain by arterial route is used when a crude approximation to whether a drug acts centrally or peripherally is desired (3). It reduces the likelihood of reflex effects, but it has the same general drawbacks mentioned above (4).

Injections into the cerebrospinal fluid (5, 6) at least have the advantage that they leave little doubt that the observed effect is central, and probably through an action on periventricular structures (5, 7). Leakage of the injected drug to the periphery may occur, however (8), and the method gives little information as to localization of sites of action.

Perfusion of localized areas of the brain is in fashion (9–15), and is a promising technique. The once popular use of "push-pull" cannulae is to be discouraged, because they cause tissue damage (16); in fact, any "microinjection" or cannulation technique does (see below). Thus the localized per-

[1] Abbreviations used: EEG (electroencephalogram, electroencephalographic); RNA (ribonucleic acid); ATP (adenosine triphosphate); ACh (acetylcholine); AChE (acetylcholinesterase); E (epinephrine); NE (norepinephrine); 5-HT (serotonin).

[2] Supported by a grant (No. 2389b, to I.I.) from the Consejo Nacional de Investigaciones Cientificas y Técnicas, Argentina.

fusion method is advisable only for structures that are superficial, or that may be surgically exposed, such as the hippocampus (13, 14), or that line the ventricles, such as the hypothalamus (15) or the hippocampus itself (9–12). In all cases, perfusion systems in which diffusion rather than hydrostatic pressure is the moving force are preferable (13, 14). In the hippocampus, for example, even the slightest pressure on its surface will severely alter the sign and nature of its evoked electrical activity (17). Localized perfusion by close intra-arterial or arteriolar injection has been described (18); it is technically complex without really avoiding the barrier and the reflex vs direct action dilemma.

The topical application of pieces of filter paper soaked in drug solutions is classical (19), and has been recently much used in the neocortex (20) and the hippocampus (21–27). Again, it cannot be applied to deep structures and it does not permit precise estimation of the concentrations attained by the drugs in the extracellular space around the pertinent receptors. If this method is used with care, mechanical effects are negligible and it may provide useful information as to whether or not a given drug exerts effects on a certain structure.

On the other hand methods that involve penetration of needles or cannulae through brain tissue, including microinjections, implantations of crystals or pellets, etc. are inadequate, because of major mechanical effects. These procedures kill nerve and glial cells, distort local geometrical arrangements that may be of extreme importance (17, 22, 25), modify characteristics of the extracellular space, etc. A typical example of mechanical or otherwise nonpharmacological effects of such methods is the recent report that an intrahippocampal injection of tetrodotoxin causes seizures (28). Topical application of this drug using the less harmful filter paper technique had shown that it has local anesthetic properties on the hippocampus (21), as it has elsewhere (29). It also blocks events related to seizures (21). The widespread choice of the hippocampus as a site for microinjections is unfortunate, since it is well known that it may respond to minute mechanical irritation with seizures (30). Furthermore, with this technique, it cannot be decided whether an observed effect is due to an agonistic or to a blocking (by excess concentration) action of a drug, or to mechanical or pharmacological interference with inhibitory or excitatory pathways, or with glial drainage functions (31, 32). In spite of these criticisms, however, it must be recognized that these techniques may be the only applicable ones in certain circumstances (22), and that they have given much (if always disputable) information on drug action at deep centers (see last two sections of this article). Recently, cannulation (33) and pellet-implantation (34) methods with some advantage over previous ones have been reported.

Microelectrophoretic application is the method of choice for studies of single unit receptor responsiveness (35–38). Other kinds of information may not, however, be derived from it. It has, as with other methods, the drawback that effective drug concentrations may not always be known, and

that a discrimination between agonistic and blocking or unspecific actions is often impossible. Furthermore, the spread of the ejected substance to distant areas is perhaps more than usually assumed (39), and thus the possibility of indirect effects on the unit studied exists.

The total RNA content of a given organ or structure is an indication of the degree of its activation by physiological or pharmacological agents (40, 41). This method has been widely used for endocrine and other organs (40, 41), and it has only recently been applied to the whole brain (40) and to regional studies (13, 41, 42). Another promising approach to the pharmacology of deep brain centers is the study of effects on self-stimulation with different electrode implantation sites (43). Results with both these techniques will be considered in the next section.

Although uninformative as to actions, the detection of labeled injected drugs by radioautography or other procedures is an extremely valuable approach to the determination of possible sites where they may exert effects (7, 44–47). It is still not as widely used as would be desirable. Its use would avoid much unnecessary theorization and experimentation on unlikely sites of drug action.

Central Sites of Action for Amphetamine and Nicotine

Both these drugs have several well known central effects in common: (*a*) cortical and hippocampal EEG alerting (48–50); (*b*) increased performance and retention of conditioned responses (51–54); (*c*) central catecholamine depletion (55–58) or increased turnover (63, 65, 76); (*d*) increased hippocampal RNA concentration, probably secondary to the EEG effect (41). Effects (*a*), (*b*), and (*c*) are shared, in general, by a number of amphetamine analogs, including, at least partly, the recently introduced fenfluramine (59–61).

There are, unfortunately, too few comparative studies of the actions of amphetamine and nicotine (22, 41, 51, 52, 62–70), and still fewer related to a search for common sites of action in the brain (22, 41, 67, 68).

The possibility that the central actions of these compounds are secondary to an adrenergic mechanism, and the subsequent investigation of possible sites of action, have been much more intensely explored for amphetamine than for nicotine (71–74). Amphetamine was reported to release brain catecholamines when injected intracisternally (75) or intraventricularly (57), as it does upon systemic injection (56), whereas nicotine has only been tested by the latter route (55). It is unfortunate that the few available studies of the effect of these drugs on central norepinephrine turnover have been performed in the whole brain (63, 65, 76). Amphetamine, in a low, single dose, increases this turnover (63, 65), whereas nicotine only did so (63) after chronic, intensive treatment (76). It is possible that the failure to observe this effect with a single dose of nicotine was a result of having used the whole brain, instead of extracting various regions (63).

There are at least two possible sites of action for the alerting, learning

enhancing action of both drugs: the midbrain reticular formation (2, 48–50), and the medial septal nucleus (22, 50), the latter at least for the hippocampal arousing effect. The reticular activating system is widely assumed to be adrenergic, and its presumable stimulation by amphetamine is often taken for granted as such (74). Evidence for both statements is, however, at best indirect (4, 47, 49). Nevertheless, post-trial reticular stimulation (77) has similar effects on learning to those of post-trial amphetamine or nicotine injections (51–53).

A topical application of amphetamine or nicotine (or eserine) to the medial septum causes an enhancement of hippocampal responses to commisural or subicular stimulation. Since neither of the latter pathways passes through the septum, this effect is probably due to stimulation of the septal units, whose axons reach the hippocampus and presumably cause there heterosynaptic potentiation (22), as occurs when they are stimulated electrically (17, 23, 25). This effect of amphetamine and nicotine is antagonized by atropine and may also be observed after intraperitoneal injection, but not after topical application on the hippocampus (22). In fact, neither drug has direct effects on the hippocampus except that large doses of nicotine cause local seizures (48). The indirect hippocampal actions of amphetamine and nicotine, however, are not necessarily just secondary to an effect on the medial septum with exclusion of other sites. First, there is evidence that both drugs cause hippocampal theta rhythm by an action exerted mainly at the reticular level (48–50; see also 2). Second, the effect of both on hippocampal RNA (41) is blocked by a pre-treatment with alpha-methyl-tyrosine (78). The medial septum cannot be an adrenergic station since its cells are depressed by iontophoretic E (79), and since the effect of amphetamine or nicotine on hippocampal RNA is probably secondary to the induction of theta rhythm (41, 42), the best alternative is again, the reticular formation.

The possibility of a dual site of action for amphetamine (be these sites the septum and the reticular formation or not) is strengthened by behavioral experiments. This compound was found to enhance performance of both pseudo- (66) or conditioned responses (51, 52); at the same time, it improves retention of the latter (51, 52). The first effect is potentiated by atropine (51, 66), whereas the latter is partially antagonized by this drug (51).

In behavioral terms, however, the similitude of the alerting effect of amphetamine and nicotine is not absolute; the latter has been reported actually to depress locomotor activity of mice (81). This observation might be the result of using a particularly high dose for those animals; a recent observation on intraventricular infusion of nicotine in cats indicates that at first there is behavioral, autonomic, and EEG alertness, which, upon accumulation of the drug in the ventricles, may be superseded by signs of depression (82).

Amphetamine and nicotine increase self-stimulation rates (68). The for-

mer is less effective when electrodes are in the posterior hypothalamus than when they are in the septum, anteromedial hypothalamus, or midbrain tegmentum (43).

With regard to other than the alerting, learning-improving effects of amphetamine, recent data point to a striatal site for the induction of stereotyped behavior: it causes, on one hand, a local DOPA accumulation (83), and, on the other, an increase of homo-vanillic acid in the neostriatum (84). The anorexogenic and hyperthermic effects of amphetamine are not correlated with the increase in homo-vanillic acid (84, 85); in fact, the hyperthermic effect would rather seem to be peripheral, as is that of L-DOPA (86), since it is mimicked by p-OH-amphetamine, an analog which is claimed to share the peripheral, but to lack the central actions of the parent compound (87). Behavioral effects of amphetamine have recently been correlated with brain levels (88).

The effects of chronic treatment with amphetamine or nicotine are less well known than those of acute administration. This is regrettable, since the chronic use of both drugs is a serious medical and social problem. However, the chronic effects of amphetamine have been much more studied than those of nicotine. It was found to lower the weight of rats (89), first to enhance and, after 8–10 days to depress, maze performance (54), to increase lever pressing for illumination change—a possible way to regulate motor activity —(89), and to have little, if any, effect on chronic avoidance training (89, 90). In mice, chronic amphetamine administration may cause a bizarre "psychotic" syndrome (91). In rats, chronic amphetamine, and possibly also nicotine, lower hippocampal RNA, while leaving unaltered that of several other brain regions (41); this is opposite to what is found after one injection, and constitutes, so far, the only suggestion of a central site of action for these compounds after chronic administration.

Central Sites of Action for Imipramine-Like Agents

This drug family includes imipramine, desimipramine, 3-chlor-imipramine, amitryptiline, and nortryptiline, all extensively used clinically or experimentally. Imipramine-like agents deplete central NE (92–94) and 5-HT (95, 96). With regard to NE, they block DOPA-beta-hydroxylase (97, 98) and the active uptake of the amines by the adrenergic neuronal membrane (92, 93, 99). With regard to 5-HT, they inhibit tryptophan-decarboxylase (100, 101) and depress 5-HT turnover (95, 102); however, the latter effect may be secondary to synthesis inhibition (103).

Some recent investigations on central sites of action of imipramine-like agents have suggested adrenergic and serotoninergic mechanisms. Imipramine, desimipramine, and amitryptiline were found to raise the threshold for hypothalamic-induced hissing in cats, whereas chlorpromazine or haloperidol lowered this threshold (104). The region of the hypothalamus which when stimulated evoked hissing is rich in both NE and 5-HT (35, 105).

Midbrain reticular units, which are claimed to be adrenoceptive (36–38), respond to injections of imipramine, desimipramine, and amitryptiline first with an increase of firing, then with a period of depression, and finally with a "rebound" phase of markedly increased activity. The first two periods show correlative behavioral and EEG changes (arousal, and then a lay-down attitude with slow waves); the third period is accompanied by an apparently normal EEG (106). A similar triphasic action has been observed by others for imipramine on chicken motor behavior (107). A blockade of the EEG arousal reaction to reticular formation stimulation has been reported (108).

Tricyclic compounds reverse the hypothermic effect of intraventricular NE probably because of an enhancement of the peripheral hyperthermic action of the NE leaking out from the ventricles and into the systemic circulation. In this experiment, nortryptiline was found to be without effect on ^{3}H-NE uptake, subcellular distribution or metabolism (8).

Labeled tricyclic compounds distribute themselves widely but selectively in the brain; the maximum uptake is in the hippocampus (44, 109–111), whereas the hypothalamus shows little uptake and the reticular formation practically none (44). This casts some doubt on the supposedly direct nature of the hypothalamic and reticular effects described above.

Field responses evoked in the dorsal hippocampus or in the amygdala by septal stimulation are not affected by systemic imipramine, desimipramine, amitryptiline, and nortryptiline (26, 112), but the hippocampal responses are enhanced by chlorimipramine (26). However, topical application to the hippocampus of pieces of filter paper soaked in 2% solutions of these drugs results in an enhancement of septally-evoked responses by imipramine and desimipramine (and chlorpromazine), but not by chlorimipramine (26). This is, at best, a confusing picture; perhaps dose ranges are important for these hippocampal effects, or extrahippocampal actions may play a role, at least in the case of chlorimipramine. The physiological significance of these responses is not well enough known to allow any inference in relation to the antidepressant or other actions of these compounds. Potentials evoked in the septum by hippocampal stimulation, or in the dorsal hippocampus by amygdaloid stimulation, are enhanced by tricyclic compounds (112).

Injections of Catecholamines into the Cerebrospinal Fluid

Catecholamines given intraventricularly usually are depressant (5). Recent observations slightly complicate, but generally support this concept. Rats that normally are of a tranquil nature have their locomotor activity stimulated by 10 μg NE given intraventricularly; 50 μg increased locomotion in all rats and 200 μg abolished it (113). Others have found that intraventricular E consistently depressed locomotor activity and gross behavior of rats, and prolonged hexobarbital sleeping time (114). Intraventricular NE injections cause recovery of self-stimulation performance of rats that

had been previously depressed by disulphiram or diethylthiocarbamate (115), whereas intraperitoneal E depresses both bar-pressing for food and self-stimulation in the same species (116). The latter observation is in agreement with a previous study of the action of several catecholamines, including E, on avoidance conditioning and extinction (117).

There is one report on intraventricular infusion of NE to rats, in which both an increase of activity in a free-field situation, and an enhancement of continuous avoidance was observed (117b). It is possible that the mode or rate of injection, or both, of catecholamines into the ventricles is of importance in determining the behavioral outcome of such a treatment (117b), as has been found to be the case with nicotine (82).

Intraventricular injections of NE antagonize the facilitation of leptazol seizures by reserpine, though not the effect of leptazol alone; noradnamine was, however, effective in both cases (118). These observations are of interest in view of the recent evidence that leptazol may act directly on the hippocampus (119), and of the observation that hippocampal perfusion with NE increases its seizure threshold (120).

The intraventricular injection of isoproterenol, ethylarterenol, E, NE, or dopamine, in that order of potency, or an intravenous or intraventricular injection of pyrogallol, have an analgesic action in rabbits as assessed by the tooth-pulp method (121). This may bear a relation to the reported general anesthetic effect of intraventricular catecholamine administration (5), and to the claims that the analgesic action of opiates (122-126) or of intraventricular bradykinin (127, 128) are caused by a central adrenergic mechanism. With regard to morphine, convincing though indirect evidence was advanced several years ago by Muñoz (122, 123), which was repeated more recently by others (125). Low doses of amphetamine potentiate, and high doses antagonize, codeine analgesia in rats (126); the former enhance brain NE turnover, whereas the latter cause NE depletion (63, 65).

It is uncertain whether the effects of intraventricular catecholamine injections are caused by actions on adrenergic mechanisms. The distribution of ^{3}H-NE in the brain after intraventricular administration is related to, but not identical with that of endogenous NE (57, 129), and the former is probably not confined to adrenergic neurons, although it can be released by amphetamine (57). The metabolism of ^{3}H-E taken up by the brain is different when it has been given systemically or intraventricularly; in the latter case, it is predominantly by conjugation (46).

The actual sites of action of intraventricularly injected catecholamines, are speculative at this time. The hippocampus has already been mentioned in relation to antiepileptic actions. Other possibilities are hypothalamic areas, which are normally rich in endogenous NE, or the periventricular grey matter, which takes up exogenous ^{3}H-NE, as do the substantia nigra and locus ceruleus (129), or tegmental (36–38) or raphe nucleus cells (130), which are highly responsive to iontophoretically applied NE.

Is There a Central Site of Action for Peripherally Injected Quaternary Compounds?

These are generally assumed not to cross the blood-brain barrier by virtue of their charge (52, 131, 132). There is, however, some recent evidence that might be interpreted as pointing to central actions of these compounds upon systemic injection. A close intra-arterial injection into spinal segments of d-tubocurarine facilitates, and one of decamethonium depresses, vasomotor and vesical responses of spinal origin; quaternary agents pharmacologically related to either compound share these effects; intrathecal ACh or other drugs influence these actions (18). Chlorisondamine, penta- hexa- or decamethonium, and mecamylamine, but not TEA, are able to block nicotine-induced convulsions in mice when given orally, subcutaneously, or intravenously; upon intraventricular injection, however, they are much more consistently effective (133). Nicotine convulsions are of hippocampal origin (49). Intraperitoneal hexamethonium facilitates retention of a learned response and interferes with the similar effects of nicotine or amphetamine, which are definitely central (52). Atropine and scopolamine are about as effective as their N-methylated derivatives in blocking drinking behavior of rats (132). Although this was taken as indicative of peripheral control of this behavior (132), there is much evidence that its regulation is central (see last section).

None of the above mentioned experiments gives any definite indication that quaternary compounds may act centrally; after all, any of the effects mentioned could be reflex. However, the brain concentration of ^{3}H-methylatropine given subcutaneously is only two to five times smaller than ^{3}H-atropine, and their distribution therein is similar, with a maximum at the cortex (7). Hexa- and decamethonium may reach significant concentrations, after systemic injection, in the brain of rats (134) or chickens (135), and incubated slices of cerebral tissue take up labeled quaternary compounds (136, 137). Four hours after a systemic injection of labeled hexa- or decamethonium, however, significant uptake is only found at the arachnoid and pia-arachnoid spaces (45, 138). However, only one determination at such a long interval, is no indication that the drugs had not penetrated and left the brain earlier (45).

The possibility that quaternary compounds may have direct central actions is thus not to be rejected dogmatically, but may deserve further investigation. A search for sites of action should probably start with areas believed to be cholinergic (see last two sections). Several quaternary compounds alter brain ACh and AChE levels: triethylcholine and hemicholinium in hemispheres, medulla-pons, and midbrain (139); hemicholinium, in addition, in the hippocampus (140); and gallamine and TEA in the whole brain (139).

Drug Effects in the Hippocampus, and the Potassium Theory

The extracellular space of the hippocampus is peculiarly small (141), and Green suggested that local firing would cause there very readily a $(K^+)o$ accumulation, which would explain the peculiarly low seizure threshold of that structure (30). The Green hypothesis was tested and subsequently elaborated by one of the present authors (I.I.) and his group at Córdoba (13, 14, 17, 21–25, 41), who developed it into a general theory of hippocampal function (14, 25, 142–145).

The potassium theory states that, upon afferent stimulation, hippocampal $(K^+)o$ builds up and has a number of consecutive and interrelated effects: first, a facilitation of one hippocampal evoked response by any preceding evoked response; then, frequency and post-tetanic potentiation; later on, seizures, and finally, spreading depression. The events up to post-tetanic potentiation are secondary to the known enhancing action of high $(K^+)o$ on synaptic function (29), and are accompanied by an increased net synthesis of RNA. Pre-ictal, ictal and post-ictal events are accompanied by stimulation of the sodium pump (see 153), and by a subsequent depression of RNA synthesis, which is known to be ATP-dependent (147).

The above described sequence of events was a factual observation (148), including, recently, the variations of RNA (42). The relation of all these events to $(K^+)o$ was demonstrated in the last two years, mostly by pharmacological means, and thus led to the establishment of the potassium theory.

It was found that a moderate increase of $(K^+)o$ by perfusion with high K^+, caused both an enhancement of hippocampal evoked responses (10, 14), and an increase of total hippocampal RNA (13); the optimum $(K^+)o$ level for both these effects was of 11 mEq/l (13, 14). A further increase of $(K^+)o$ caused a fall of RNA concentration (13), and pre-ictal events (10) followed later by seizures (9, 14). The seizure-inducing effect of K^+ was potentiated by ouabain, which presumably prevented the entrance of the excess $(K^+)o$ into cellular compartments (12). The critical $(K^+)o$ value at which seizures occur was determined, by extrapolation, to be 34 mEq/l in rats (14). Both the increase of hippocampal RNA (13) and the effects on synaptic function and seizures (14) were antagonized by 15 mEq/l Mg^{++} in the perfusing fluid. Mg^{++} would, in theory, not affect other actions of K^+ on neurons except that on transmitter release (13, 29).

Fornical stimulation has a similar effect on hippocampal RNA as high K^+ perfusion: low rates (up to 4/sec) increase RNA, high rates (16/sec) lower it (42). All this suggests a relation of neuronal activity evoked by orthodromic excitation to RNA synthesis (13, 42). Such a relation has been recently demonstrated in Aplysia ganglion cells (146).

Further evidence for the K^+ theory came from observations on the effect of the application to the hippocampus of pieces of filter paper soaked in

veratrine or TEA solutions. Veratrine increases, and TEA depresses, the K^+ outflow per spike at excitable tissues (29). Correspondingly, the former was found to enhance, and the latter to depress, hippocampal facilitation (25) and post-tetanic potentiation (24), and to increase and decrease, respectively, the number of repetitive stimuli needed to evoke pre-ictal events (21) or full-scale seizures (25). Topical diphenylhidantoin had effects similar to those of TEA (25).

The K^+ theory is not incompatible (14, 25) with other hypotheses on seizures (149–151), and furthermore, it fits with observations by others: the $(K^+)o$ increase indirectly measured both in the hippocampus and in the neocortex during afterdischarges (152), and the enhanced sodium pump activity of cortical tissue after pentylenetetrazol convulsions (153).

Since, because of the effect of Mg^{++} and of other findings (14), a synaptic mechanism appears to be involved in the ultimate triggering process of seizures by high $(K^+)o$, experiments were started recently to test the effect of substances that may be transmitters on the number of repetitive stimuli needed to unchain a hippocampal seizure. Perfusions with glutamate, 5-HT, of ACh reduce this number, and NE or gamma-amino-butyrate have an opposite action; all drugs were tested at concentrations of 10^{-5} M (120). These data fit with those of others that ascorbic acid, which increases whole brain (154) and hippocampal NE, and lowers hippocampal ACh, increases the seizure threshold of this region (155).

The K^+ theory, in connection with seizures, has been extended to foci of post-traumatic gliosis, in the belief that in such foci the normal K^+ draining function of glial cells (31) would be impaired (32). In view of this hypothetical role of glia, it is, however, surprising that no change detectable at the electron-microscope level was observed in hippocampal glia after extremely prolonged seizures (156). Glial swelling was reported in the cortex after pentylenetetrazol convulsions (153), but such swellings are considered artifacts by others (156).

Drug Effects on the Hippocampus, with Relation to Learning and to Cholinergic Mechanisms

A role of the hippocampus in learning is usually assumed, although each author proposes a different role for it in this process. This is, of course, remindful of Saxe's Six Men of Hindustan. Seemingly, much of the confusion arises from the excessive attention paid to experiments involving hippocampal injury or surgical isolation (157, 158), after which procedures learning may proceed. Brain lesions are not the counterpart of adequate physiological stimulation, as occurs, for instance, with endocrine glands. Vicarious circuits, irritation, gliosis, and the elimination of afferent or efferent pathways may all play important roles after injury to nervous tissue.

A typical feature of the hippocampus during acquisition is a prominent theta rhythm (30, 159). This rhythm accompanies the performance of learned (160) or otherwise voluntary responses (161), or the occurrence of

dreams, which are also based upon previous experience for that matter (162). Novel stimuli produce, rather, fast waves, but, on repetition, they bring about theta waves (163, 164). It has been proposed that this rhythm creates an optimum condition for hippocampal heterosynaptic interactions mediated by a $(K^+)o$ increase and RNA synthesis, for both of which statements there is evidence (13, 17, 22, 41, 42), and that this underlies its role in learning (143–145). Others have advanced the idea that the hippocampus participates in the decision by the animal to respond correctly or incorrectly by comparing the phase of its own theta waves with that of similar waves from nearby structures (159). Still others have proposed that hippocampal theta rhythm subserves an inhibitory function (163), which would be necessary for learning; this third hypothesis fits with others that consider this inhibitory mechanism to be cholinergic (131, 165–168).

The first hypothesis (a "positive" role of theta rhythm in learning) fits with the observations that amphetamine, nicotine, and eserine, which favor learning, cause hippocampal theta and hippocampal heterosynaptic potentiation (see the second section), and increase hippocampal RNA (41). An interference with theta rhythm by electrical stimulation (169), by spreading depression (170, 171), or by puromycin (172) impairs learning. Puromycin effects are blocked by the simultaneous injection of some cations, suggesting that the drug binds at anionic membrane sites (173).

Puromycin is a well known inhibitor of protein synthesis, and, even if its effect on learning is unrelated to this effect (172), it must not be forgotten that both RNA (41, 174) and protein synthesis (175, 176) occur in the hippocampus during learning—and, at least the former, not during pseudoconditioning (41)—at a higher rate than elsewhere (41, 176). In spite of puromycin, more pharmacological research in this field is clearly desirable.

The hypothesis of a hippocampal inhibitory cholinergic mechanism in learning has stimulated much work. Two aspects must be considered here: one, the existence of hippocampal cholinergic transmission; the other, the evidence in favor of such a behavioral cholinergic mechanism. The first point is still very obscure. Hippocampal pyramidal cells are excited by iontophoretic ACh or glutamate, and inhibited by NE or 5-HT, or gamma-amino-butyrate (36, 177). In principle, however, this is not a very rewarding picture, as most cerebral units, in particular neocortical ones, behave similarly in response to these agents (16, 36, 178). Recently, some neocortical cells have been observed to evade this pattern (179, 180). The hippocampus contains all of these substances (35, 105), and, in principle, any of them could be a transmitter. As regards the now well known recurrent inhibitory circuit, however (181), it was observed to be wholly unaffected by topical application on the alveus of atropine, nicotine, hexamethonium, nethalide, phentolamine, or strychnine, or by systemic injections of LSD or picrotoxin (23). With respect to excitatory inputs to pyramidal cells, all of these drugs, with the exception of LSD and strychnine, had variable and erratic effects: sometimes they enhanced, other times they depressed, and

most of the times they had no action, on potentials evoked by fornical, commisural, or subicular stimulation (23), which are the three main excitatory afferent pathways to the hippocampus (17, 30). Strychnine consistently enhanced, and LSD depressed these responses (23); the former effect could, of course, not be attributed to a blockade of inhibition; the latter could not be correlated with an anti-5-HT mechanism, for 5-HT also inhibits pyramidal cells (177). There is, however, evidence that LSD may stimulate 5-HT receptors (and turnover) at other central areas (182).

Hippocampal cholinergic mechanisms may exist in relation to seizures, however, as evidenced by the interaction of nicotine (49) and quaternary blockers on them (133), by the epileptogenic effect of perfused ACh (120), and by other assorted evidence as well (155, 183, 184), although it is not yet very clear what relation these observations have to neurotransmission (120). However, the arousing and the epileptogenic effects of nicotine are blocked by F1-6654, a compound devoid of peripheral anticholinergic or antinicotinic activity (185).

The effects of hemicholinium on hippocampal ACh and AChE (149), and, in particular, the subcellular distribution of labelled hemicholinium in the hippocampus, suggest local cholinergic transmission (186). Modification of hippocampal AChE by behavioral variables provides further suggestions (187, 188). In all, however, evidence for hippocampal cholinergic transmission is generally vague and mostly indirect. Further support for it comes from behavioral experiments, as will be seen.

The retention deficit caused by an electroconvulsive shock is reduced by scopolamine and enhanced by eserine, suggesting that the shock acts by inhibition and by a cholinergic mechanism (189); there is too much convincing evidence, however, that electroshocks act instead by interfering with memory consolidation (190–192). Intrahippocampal injections of carbachol depress alternation behavior (193) and increase errors and inter-trial responding in a training routine (167); surprisingly, the latter effect was opposite to those of similar treatments with either eserine or atropine (167). A similar depressant action of the latter two drugs was also observed upon intraventricular injection (131), where the point was raised that maybe eserine acted through a depolarization block by excess endogenous ACh. This problem, in fact, is always present with a drug such as eserine, and the choice of an adequate dose is difficult. Learning facilitation by eserine has been reported by many (188, 194, 195).

Choline-acetyl-transferase activity of the hippocampus has recently been studied by one of us (J.A.I.) and his group; it was found to be low in isolated or underfed rats; (i.e., kept at 80–85% of their weight) handling and proper feeding, respectively, restored normal levels; simultaneous AChE assays showed no changes (187).

As regards the general involvement of the hippocampus in learning, and the possibility that it may be a site of drug action in this connection (144),

it was recently reported that intrahippocampal post-trial pentylenetetrazol injections facilitate retention (119), as was known for systemic injections of this substance (191, 192).

A classification of psychoactive agents based upon their action on hippocampal electrical activity has been proposed (196).

Extrahippocampal Cholinergic Sites of Drug Action

No cerebral synapse has yet been found that satisfies the established criteria for determining the chemical nature of transmission across it (35, 105, 181). Therefore, as in the preceding section, the term "cholinergic" is not used properly. It is, however, commonly applied to areas whose cells are cholinoceptive (36–38, 197), or which contain ACh or AChE (80, 197), or which respond to local applications of cholinomimetic or anti-AChE substances. Radioisotope (198) or histochemical cholinesterase determinations (80, 199–203) are now preferred, though some still use biochemical assay of regional extracts (187, 188). ACh assays are nowadays done preferably by gas chromatography techniques (204–209), or by a combination of gas chromatography and mass spectrometry (210); some still use the old and generally reliable bioassay methods (211–213), or combine them with gas chromatography (214).

The hypothalamus.—The ACh content of hypothalamus and neural lobe of several species is similar; the latter structure, however, has much less AChE or cholineacetyl-transferase activity than the former, and, furthermore, the relative proportion of both enzymes is different at both sites (211). These results stress the importance of not trying to derive too much from single observations on any of the three variables. The AChE activity of the various hypothalamic nuclei is different (200, 201).

The placement of carbachol or neostigmine into the lateral hypothalamus induces muricide in rats normally not prone to it, whereas NE, 5-HT, Na salts, or amphetamine have no effect; methylatropine caused rats that were spontaneous mice killers to become nonkillers (215). This is, of course, a further suggestion of a central site of action for quaternary compounds (see a preceding section). Intrahypothalamic ACh causes, in rats, a hypothermia reversible by atropine (216). Pilocarpine also causes hypothermia in mice, which is blocked by scopolamine but not by methylscopolamine (217). An injection of carbachol into the posterior hypothalamus lowers blood pressure, and then causes a delayed rise; this effect is potentiated by eserine, mimicked by ACh or oxotremorine, and blocked by methylatropine; eserine alone, however, raises blood pressure (218).

Atropine pellets implanted in the midline, rostrally to the paraventricular nucleus, block the adrenocortical system (219).

An injection of carbachol into the third ventricle increases natriuresis, through an effect that appears to be local, since much larger doses are

needed when the injection is into the lateral ventricles; atropine blocks this effect (220). ACh injected into the third ventricle increases water intake at the expense of NaCl solution intake in rats that may choose between both, be they normal, thirsty, or Na-depleted; NE promotes instead NaCl intake; in the thirsty animals, eserine acted like ACh, whereas atropine decreased the water intake (221).

A localized ventricular perfusion with ACh releases NE from the hypothalamus (15) reminiscent of the Burn-Rand hypothesis for NE release at adrenergic nerves (222). An extension of this hypothesis to brain mechanisms was recently made by Campos (223).

Septum and amygdala.—Strong AChE activity has been observed in many brain nuclei, including the medial septum (80). This nucleus is the pacemaker for hippocampal theta activity (48, 50, 224) and drug action upon it—stimulation by cholinomimetic agents, including nicotine and eserine (though also by amphetamine), blockade by atropine (22, 48, 50)—suggests that it is a cholinergic relay in the reticulo-hippocampal pathway. In spite of its size, ACh assays in this nucleus are desirable.

The lateral septum is believed to be involved in a neural circuit that controls drinking behavior (225, 226), and that involves amygdaloid and hypothalamic stations, all of which appear to be excited by local ACh (see above) or carbachol, and blocked by atropine (225–227).

Atropine also inhibits the increased aggressiveness of rats treated with amitone (an anti-AChE) placed in the septum or in the baso-lateral amygdaloid nucleus (228).

Drugs that affect brain ACh or AChE.—The above references were included to point out the possibility of central hippocampal, hypothalamic, septal, or amygdaloid cholinergic sites of drug action. The list could be made larger by including all those areas that have high AChE activity (80, 202, 203, 229), or cholinoceptive units, such as the medial geniculate (230, 231) or the raphe nuclei (130), reticular (232), and many other neurons and pathways (36–38, 179, 197).

There are many drugs that have central actions and affect brain ACh or AChE levels. Quaternary agents have been briefly considered in a previous section. A few other drugs will be dealt with here, in the hope of stimulating research oriented towards a determination of their site of action, in relation to brain centers supposedly cholinergic.

One such drug is oxotremorine, and it has been much investigated in this connection. A few aspects of its pharmacology will be treated here. It was recently found to increase ACh more markedly in the diencephalon-midbrain region, caudate nucleus, substantia nigra, and globus pallidus than elsewhere, and to cause no ACh increase at all in the cortex or lower brain stem (212). It has effects on blood pressure when injected into the posterior hypothalamus (see above), which are shared by muscarine and methacholine, but not by pilocarpine or other cholinergic agents; this was taken as an indication of receptor specificity (233). Its tremor-inducing action may be

the result of a mechanism more complex than hitherto assumed, as it is depressed by reserpine, alpha-methyl-tyrosine, diethylthiocarbamate, phenoxybenzamine, or propranolol (234). This brings again into focus the extension by Campos of the Burn-Rand hypothesis (223).

Barbiturates have been known since 1946 to increase brain ACh (235). Recently, it was reported that eserine may reduce the latency to the onset of barbital depression (236).

Reserpine has been known since 1949 to increase ACh in the hypothalamus and to reduce it in the hippocampus (237). In whole brain extracts an increase of ACh after reserpinization has been reported in rats (238) and toads (239). In the rat hippocampus, an increase of choline-acetyl-transferase activity was observed (240). Reserpine has effects on the electrical activity of the hippocampus (108), and it may be oversimplifying to ascribe these effects, as most, if not all others which it has on EEG and on behavior, to an interference with NE or 5-HT mechanisms, as is usually done.

Inhibition of the tail-flick reflex by morphine has been claimed to involve cholinergic central mechanisms (242), and in the development of tolerance to this agent similar mechanisms have been proposed as well (241).

Investigations of drug action on central cholinergic mechanisms or presumable sites offer many perspectives; however, there is a great disproportion between efforts oriented in this sense, and those directed toward central NE or 5-HT mechanisms of drug action (243).

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