Chapter - I

Introduction

There is considerable evidence that drugs can impair and enhance the acquisition and retention of learned response. In research studying the effect of drugs on learning and memory the major conceptual task is that of distinguishing the behavioural effects of drugs that are due to influences on learning and memory from other effects that influence behaviour. The problem is complicated further by evidence that the processes underlying very recent or short term memory may be different from those underlying long term memory. Thus, drugs have different effects on short and long term memory processes. Drugs can be given at different times either before or after training in order to study the drugs effects on acquisition, memory storage and retention.

It is well documented that post training treatments can have enhancing as well as impairing effects on retention. Enhancement is readily produced with stimulant drugs, electrical stimulation of the brain, and pituitary and adrenergic hormones. The enhancing effects, like the impairing treatments, are most effective when administered shortly after training. Moreover, the effect of a particular treatment depends on many conditions, including
the dose or intensity of the treatment as well as the training conditions used. Generally, low doses of drugs and hormones and low-intensity brain stimulation impair retention. Since the same treatments may either impair or enhance memory depending on the experimental conditions, it seems appropriate to refer to the treatments as "memory modulating" treatments.

Innumerable experiments have been conducted during the last two decades to investigate the role of various endogenous biochemicals in memory storage. (Thompson, 1955 & chapouthier, 1973). Among these RNA and proteins are considered to be the most appropriate candidate for memory consolidation. Hyden (1950) was the first person to recognize that during the process of learning, quantitative and qualitative changes occur in the levels of brain RNA. Hyden and Egyhazi (1964) trained rats to reach for food by putting their paws into a narrow-tube arranged in such a way so that only the left or the right paw could successfully reach the food. They found that nearly all rats, approximately 92% had a clear preference of left or right paw for movement of this kind. During 9 days of training, the rats of one group were required to use their own non-preferred paws, while these in the other groups were allowed to use the preferred ones. Thus the first group had to learn an accustomed way of getting food, while the second group did not. In the first group, greater amount of RNA
per neuron and changed percentage of RNA base ratios was found when compared to the second group. Thus their findings suggest that quantitative changes occur during the information processing.

Zelman, Kabat & Jacobson (1963) also support the idea that RNA plays an important part in the memory formation. They injected the RNA extract of the trained animals to the naive animals. Statistically significant differences obtained between these recipients injected with the RNA-rich extract taken from conditioned donors and (a) those recipients injected with an RNA-rich from animals given unpaired trials of light and shock or trials of light only (or shock only) and (b) animals, injected with an equal amount of acquired water. All this suggest that acquired behavioural tendencies could be transferred from one organism to another by means of purely chemical extracts. It also suggests that RNA might be capable of mediating the type of transfer.

Even qualitative changes in RNA have been observed by numerous investigators. Hyden analyzed the RNA composition in terms of four consistent elements i.e. adenine (A), guanine (G), Cytosine (c), and uracil (u). Hyden and Egyhazi (1962, 1963) trained rats to climb a sloping tight rope wire to a platform, for food. A control group, without the elements of learning, was twirled around
passively for a period of time. A third group was not stimulated at all. All the groups were then sacrificed and RNA composition of cells in the lateral vestibular nucleus was analyzed. They found that the trained group had RNA that contained a greater percentage of adenine and a smaller percentage of uracil. The guanine and cytosine properties remained the same.

Qualitative change in RNA base ratio was also observed by Shashoua (1968) in goldfish. They found that uracil, cytosine ratio changed after learning of a swimming task.

Hyden (1962) has demonstrated a reciprocity between glial and neural chemical processes. Hyden and Egyhazi (1963) in a later work, suggested that the glial cells and glial RNA might be the substrate for short term memory. Hyden et al. (1969) suggested that an acid protein, named 5100 which is unique to brain and has been located in glial cell membranes and cytoplasm and in the nucleus of the neuron is involved in memory mediation. According to him, this substance moves from glial to neurons, since the loss of glial RNA in activity exactly balances the increase in neuronal RNA with the same base ratio. The glial cells might thus specify part of neuronal protein synthesis.

Since RNA synthesis is closely linked with the
synthesis of proteins, an increment in RNA would, but naturally lead to an increased synthesis of proteins. It has also been argued that the protein molecule is a more likely candidate for the engram (Halstead, 1947). Proteins are large and elaborate polypeptides composed of long chains of amino acids, joined by peptide bonds. There are hundreds of amino acid in one protein molecule and there seems to be no low governing their sequence, any sequence seems just as feasible as another. They are very delicate and reactive molecule, sensitive to minor fluctuations.

Initially, Hyden's 1970 research provided support for the role of proteins in memory storage. Hyden and Lange (1970, 1971) have reported that certain brain specific proteins are formed during the course of learning. They identified three such proteins to be increased in their levels. He labeled one such protein as 8-100 in the hippocampus. These results are consistent with those of other investigations (Beach and Emmens, 1969). Kohan, Krigman, Wilson and Glassman (1970) found an increased protein synthesis in general in the hippocampus (as well as other subcortical locations) during a learning task. Hyden and Lange (1970, 1971) showed that when an antiserum of 5-100 was injected into the ventricles of the rat's brain during the training sequence, further improvement in performance is halted. This indicated that some kind
of biochemical changes are going on in the brain.

The drugs which can affect memory can be categorized as stimulant type and depressent type and diverse other types also. Amphetamine is a stimulant drug which is responsible for increasing the arousal level.

Amphetamine was first synthesized by Edéleanu in 1887. The first studies on the pharmacological actions of amphetamine appear to be those of Piness et al. Which established that it was a long acting pressor agent, with bronchodilator properties. It was an analeptic, able to reverse barbiturate anesthesia. Strangely, its CNS stimulant action was not reported until 1933 and this was very rapidly followed by the first accounts of its abuse.

Amphetamine increases catecholaminergic transmission. Earlier studies suggested that amphetamine (AMPH) could indirectly increase activity at catecholamine receptors by promoting the release of the transmitter from the nerve ending into the synaptic cleft, by blocking the inactivation of released transmitter through inhibition of the reuptake process, or by increasing the availability of releasable transmitter through inhibition of monoamine oxidase. Each of these mechanisms has, at various times, enjoyed the status as the primary site of action of the drug. During the past ten years, a major
effort has been directed toward specifying their relative contribution to the facilitating effect of the drug on catecholaminergic transmission. An understanding of the contribution of each of these possible effects is essential prior to an accurate interpretation of the in vivo effects on catecholamine disposition.

Amphetamine decreases the retention and/or accumulation of 3H-catecholamine by neuronal tissue both in vivo and in vitro. Glowinski and Axelrod (1965) reported that a high dose (20mg/kg) of AMPH administered prior to the intraventricular infusion of 3H-norepinephrine decreased the accumulation of the amine by 70%. Similarly, Von Voigtlander and Moore (1973) described a dose dependent AMPH-induced increase of 3H-dopamine in cerebroventricular perfusate of Cat. The ability of AMPH to decrease the retention or accumulation of norepinephrine and dopamine in vitro in tissues slices or synaptosomes has been extensively documented. Several authors, however, have noted the difficulty in interpreting changes in retention of 3H-amines in terms of uptake blockade or direct release when both of these processes are occurring simultaneously. Thus, during uptake studies a releasing agent, by promoting the efflux of accumulated 3H-amine, would appear to inhibit uptake. Conversely, during release studies, an uptake blocker would prevent the reuptake of spontaneously released 3H-amine, thus appearing to promote release.
Raiteri et al. (1975) directly addressed the question of whether AMPH could release catecholamines from synaptosomes by utilizing a superfusion technique, during which drug induced or spontaneously released 3H-amine is constantly removed from contact with the tissue preparation, thereby eliminating reuptake as a confounding factor. Using this experimental design, those authors failed to observe an AMPH-induced release of norepinephrine from synaptosomes prepared from hypothalamus, pons-medulla, or cerebellum. In contrast, they obtained a substantial release of dopamine from striatal synaptosomes. Hunt et al. (1979) have confirmed AMPH-induced release of 3H-dopamine from striatal synaptosomes using the superfusion technique and it would appear that AMPH functions primarily as a release at dopaminergic nerve endings. Although some controversy continues regarding AMPH-induced release of norepinephrine, it appears that AMPH functions more potently as an inhibitor of uptake at noradrenergic nerve endings.

Arnold (1977) investigated that amphetamine induced release of endogenous norepinephrine (NE), and Dopamine (D) in isolated brain tissues. NE and D were measured using an enzymatic assay. When amphetamine was incubated with chapped rat brain tissue, release of both NE and D was observed. This release was temperature dependent and the time course of release was similar for both amines. A comparison of the releasing effects of D and L-amphetamine
showed that at concentrations higher than $10^{-5} \text{M}$, d-amphetamine was more effective in releasing both NE and D. However, the ability of amphetamine to induce release was only slightly decreased by omission of calcium from the medium. The results suggest that amphetamine induced release that occurs at least in part by a non exocytotic mechanism. When the release of endogenous NE was compared with the release of previously accumulated ($3H$) NE, the exogenous $3H$-amine was released at lower concentration of amphetamine than were required for release of endogenous NE. On the other hand, $1$ to $100\cdot10^{-5} \text{M}$ amphetanine released similar amounts of endogenous and ($3H$) dopamine whereas higher concentration of amphetamine released proportionately greater amounts of endogenous dopamine. These results suggest that amphetamine-induced release of exogenous and endogenous biogenic amines may not occur from the same intracellular compartment.

This observation is consistent with similar earlier reports of desipremine sensitive-AMPH induced release of norepinephrine and cocaine sensitive AMPH induced release of dopamine from tissue slices. Fisher and Cho (1979) have provided substential indirect evidence that AMPH-induced dopamine release is dependent on the process of AMPH transport. Thus, accelerative exchange diffusion appears a likely candidate by which can promote Catecholamine release.
The effects of amphetamine on neuronal activity in the various nuclei of the hypothalamus have not been extensively studied. Krebs et al. (1969) established that amphetamine, when infused intraarterially into rats, resulted in an increased spontaneous firing rate of a large sample of extracellularly recorded neurons in ventromedial hypothalamus, whereas an increase, decrease or no effect occurred in the lateral hypothalamus with approximately equal frequency.

Leonard and Susan (1971) studied some neurochemical effects of amphetamine, methylamphetamine and p-bromomethylamphetamine in the rat. (1) low doses of d-amphetamine increased nonadrenaline concentrations in the rat; doses greater than 5mg/kg, however, caused a decrease. Methylamphetamine also showed this dual effect, but a reduction in brain nonadrenaline concentration only occurred when doses greater than 10mg/kg were administered. p-bromomethylamphetamine did not significantly reduce brain non-adrenaline concentrations even at a dose of 60mg/kg. The order of potency in reducing the concentration of nonadrenaline correlated with the central Stimulant effects; D-amphetamine produced the greatest and p-bromomethylamphetamine the least increase in motor activity. (2) D-amphetamine and D-methylamphetamine potentiated the action of 4,α-dimethyl-m-tyramine (H77/77) in depleting brain nonadrenaline; the greatest potentiation was produced by D-amphetamine.
This suggests that the phenylethylamines may affect brain nonadrenaline concentrations by acting on the reserpine resistant uptake mechanism. (3) Differences were found in the effect of the three drugs on brain dopamine concentrations; d-amphetamine caused a decrease while p-bromomethylamphetamine caused an increase. Methylamphetamine had no effect on the concentration of dopamine. Only p-bromomethylamphetamine significantly reduced the depletion of brain dopamine concentrations caused by H77/77. (4) Methylamphetamine and p-bromomethylamphetamine reduced the concentration of 5-hydroxytryptamine (5-HT) in the brain; administration of the same dose of d-amphetamine did not change the concentration of 5-HT. (5) Changes in the blood and brain concentrations of tryosine and tryptophan and in the concentrations of 4-amino-n-butyric acid in the brain could not be correlated with the changes observed in the concentrations of biogenic amines in the brain.

Acute doses of d-amphetamine generally do not produce gnawing in the rat, whereas acute apomorphine produces strong gnawing behaviour. However, the use of rating scales was sufficient for Creese and Iversen to build on the earlier evidence of Randrup and Munkvad that striatal DA mediated amphetamine induced stereotypy in the rat. By using the neurotoxin 6-hydroxydopamine (6-OHDA) injected into the head of the caudate nucleus.
Creese and Iversen (1974) found that the sniffing and head movement elicited by high doses of amphetamine were antagonized by local depletion of DA in the striatum.

Weiss and Laties (1962) reported that amphetamine is often classified as a central stimulant, capable of decreasing fatigue and improving learning. It has been suggested that amphetamine produced improvement in learning may relate to its ability to decrease fatigue. But this phenomenon was criticised by Thompson (1973). Thompson utilized a modification of a procedure that was initially developed by Boren in 1963 for rhesus monkeys. Thompson's procedure consisting of the following paradigm in which pigeons repeatedly acquired a new response sequence. Pigeons pecked a key in a chamber containing three response keys; all keys were illuminated at the same time by one of four colours. For each session, the pigeon's task was to learn a new four response sequence by pecking the correct key in the presence of each colour. For example; a sequence might be the following: left key, center key, right key, center key, which response sequence was correct was signaled by the key light colour i.e. in the presence of yellow key lights, a left key response was correct; in the presence of green key lights, a right key response was correct; in the presence of red key lights, a center key response was correct; in the presence of white key lights, a right key response was correct. The sequence
was varied daily so that the acquisition of a sequence could be measured in a single session. The average number of errors it took each pigeon to learn a new sequence was referred to as the pigeon's steady-state learning behaviour. Drug effects were examined on this steady behaviour.

D-amphetamine increased the total number of errors in the session, as well as increasing the total session time, i.e. both learning and performance were impaired by amphetamine. While these results do not agree with previous reports that amphetamine improves learning.

Numerous experiments have shown that learning and memory processes are enhanced by amphetamine in a variety of learning tasks. A common explanation given for the enhancing effects of amphetamine on memory is that the drug potentiates memory storage by altering brain catecholamine functioning.

This view is consistent with Kety's hypothesis that catecholamines may act directly to modulate neuronal process involved in memory storage. There is a large body of literature supporting this notion. However, it is well known that amphetamine also has a variety of peripheral effects including some known to affect brain functioning. Amphetamine dramatically alters central
blood flow and oxygen utilization in the brain, and there are data which suggest that central catecholamines may not be involved in the response. Thus, the possibility exists that amphetamine may affect learning at least in part through influences on the peripheral sympathetic nervous system and the adrenal glands. There is some evidence to support the view that peripheral catecholamines may be involved in the modulation of memory processes.

However, recent findings of Martinez et al. (1980) suggest that the memory effect of amphetamine may involve the sympathetic division of the autonomic nervous system. Posttrial intracerebroventricular administration of a wide dose range of d-amphetamine was ineffective in altering retention of an inhibitory avoidance task. In contrast, peripheral post trial administration of d-amphetamine was effective in enhancing memory and peripheral sympathectomy by 6-OHDA (6-hydroxydopamine) potentiated the memory enhancing effects of peripherally administered d-amphetamine. However, since d-amphetamine readily crosses the blood brain barrier. It could not be determined whether the d-amphetamine produced some central action that contributed to the observed finding.

Amphetamines enhancement of retention of an inhibitory avoidance response may be related to adrenal medullary function. This suggestion was based on the
finding that peripheral administration of both amphetamine, which crosses freely into the brain and 4-OH amphetamine, which does not, enhanced retention of the response at similar doses (Martinez, 1980; Martinez and Vasquez, 1980). Further intracerebroventricular administration of a wide dose range of amphetamine following training did not alter retention of inhibitory avoidance responding. (Martinez, Jensen, 1980). These results suggested that both amphetamine and 4-OH amphetamine may have a primary site of action in the periphery.

In order to investigate the involvement of the peripheral adrenergic system in the amphetamine-induced enhancement of retention, further experiments were conducted with 6-hydroxydopamine to produce a chemical sympathectomy or with adrenal medullectomy. Chemical sympathectomy shifted the effective dose of both amphetamine and 4-OH amphetamine to lower doses; adrenal medullectomy abolished the enhancing action of both amphetamines (Martinez, 1980). A number of studies have reported that learning is either impaired or unaffected by amphetamine. These findings are somewhat surprising, since it might be expected that at least some improvement in performance would result from increased alterness or increased activity level. Recent evidence support this common sense expectation.
Keleman and Bovet (1961) found that small doses of amphetamine (.3-1.0mg/kgSc) facilitated rat's escape and avoidance learning. The rats were placed on a hotplate (60°C) and allowed to escape by jumping up into the rim of a cylinder. On the first trial, the latency of the jumping response was shorter in amphetamine - injected rats than in control rats. With additional trials given at 30-second interval, the latencies of the amphetamine animals remained well below those of control animals. Since amphetamine increases activity (Dews, 1953), this could be interpreted as indicating merely that amphetamine facilitated performance by increasing the probability of occurrence of correct response. However, with the lower dose (.3 mg/kg); amphetamine facilitated learning on the successive trials but did not affect the latency of the correct response on the first trial. Small doses of methamphetamine (.5 mg/kg) facilitate discrimination learning in hamsters (Rahmann, 1961; 1960). This is difficult to explain solely in terms of the effect of the drug on activity level, since accuracy of choice rather than latency of response was used as the learning measure. Learning was impaired by a dose (2.0 mg/kg) which produced a high activity level. Rensch and Rahmann (1960) suggest that the improvement in performance was due to increased
attention and motivation. The effects were not temporary, however, because retention of the amphetamine-injected subjects tested over a 3 month period was superior to that of controls.

With this we may now pass on to the next chapter related with review of pertinent literature.