EFFECTS OF ADRENOCORTICOTROPHIC HORMONE ON CYCLOHEXIMIDE INDUCED AMNESIA IN RAT*

by

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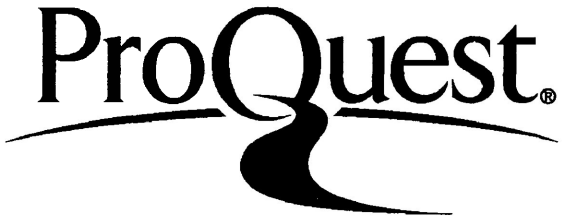

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Abstract

The effects of adrenocorticotrophic hormone (4 IU/kg ACTH) and cycloheximide (2.5 mg/ml/kg CXM) on retention of a one-way avoidance task were investigated in 100 day old rats of the SHS genetic line. There were 5 drug groups with 16 rats per group. The different drug groups were injected 30 minutes before training with either saline, CXM, ACTH, CXM plus ACTH, or CXM followed by ACTH immediately after training. Following the one-way avoidance training session the rats were tested for retention. Half of the rats from each drug group were tested 1 hour after training, and the other half were tested 5 hours after training. Retention was measured by extinction in terms of the number of avoidance responses and response latency. It was found that CXM impaired retention 5 hours after training; that ACTH did not influence retention either 1 or 5 hours after training; and that both pre and post training injections of ACTH counteracted the amnesic effect of CXM 5 hours after training. The results are discussed in terms of (a) how ACTH counteracted CXM's amnesic effect by stimulating the release of the neurotransmitter substances norepinephrine and dopamine, and thereby preventing neural blockage; and (b) how CXM's inhibitory effect on corticosteroid levels is dissociable from CXM's effect on retention.

Introduction

Learning new information, retrieving an old idea, or recalling a thought all require certain biochemical reactions to occur within the brain. Conversely, the inability to perform these cognitive memory tasks suggest certain biochemical dysfunctioning; and this suggests that relationships exist between certain biochemical processes and memory.

The experimental study of the biological basis of memory is complicated in many respects because one is never sure the experimental techniques of drug intervention, brain stimulation, ablation or lesioning, used to intervene on memory do not exert non-specific effects on other biochemical processes, on locomotor activity, or on behavioral responses. In addition, there are other aspects of the memory process to consider: does the experimental manipulation disrupt memory consolidation from short to long term memory? does it block recall? or does it inhibit the expression of memory by stimulating various defense mechanisms?

The present research investigated the biochemistry of memory by experimentally inducing amnesia in rats by injecting an amnesia-inducing drug. Much evidence indicates that

experimental amnesia results from either protein synthesis inhibition at time of learning, or from adrenergic depletion at time of initial memory processing (research reported later). Therefore, a protein synthesis inhibiting drug (cycloheximide) (Squire & Barondes, 1972) was used to induce amnesia of avoidance task in rats. In addition, an adrenergic stimulating drug (adrenocorticotrophic hormone, research reported later), was given in combination with cycloheximide to determine if the amnesia could be counteracted. If so, then the findings would suggest that the adrenergic system is involved in the memory process.

Research literature relating to the biology of memory is reported below. The review examines evidence implicating the role of protein synthesis in memory, the dissociation of drug induced non specific side-effects from amnesia, the involvement of adrenergic activity in relation to retention, the effects of peripheral changes on retention, and the interaction between the adrenergic system and the pituitary-adrenal system. And finally, the rationale of the present experiment is described.

Review of Literature

Mice injected with puromycin (PUR) - a protein synthesis inhibitor - 5 hours before training on an avoidance task

showed normal retention 15 minutes after training and impaired retention 3 hours after training (Barondes & Cohen, 1966). The authors suspected that because PUR inhibited protein synthesis this was the cause of poor retention. The fact that retention was not disrupted 25 minutes after training, but was impaired 3 hours after, suggests that protein systhesis may not be required for short term retention, but is needed for long term retention.

In other experiments, different protein synthesis inhibitors were used, and similar results were found. Acetoxycycloheximide (AXM) administered to mice 5 hours before training on an avoidance learning task caused no retention deficits 3 hours after training; however, there were deficits 6 hours after training which even lasted over the ensuing week (Barondes & Cohen, 1967). Cychoheximide (CXM) administered to mice 30 minutes prior to training impaired retention 3 hours after training (Squire & Barondes, 1972); and similarily, CXM administered to rats 30 minutes prior to training impaired retention up to 24 hours after training (Schmaltz & Delerm, 1974).

The results indicate that inhibition of protein synthesis disrupts long term retention in both mice and rats, and that this effect is found whether the drugs are administered

30 minutes or 5 hours before training. Interestingly, the drugs do not disrupt retention when given after training, which suggests protein synthesis is essential during acquisition (for review of drug induced amnesia, see Barraco & Stettner, 1976; Glassman, 1969; Squire & Davis, 1981).

One must be careful not to interpret the amnesic effect induced by these drugs solely on the premise that it is caused by protein synthesis inhibition. Other findings suggest that drug induced amnesia may originate from changes in neural-electric activity, locomotor activity, aversive conditioning, or the adrenergic system.

Flexner & Flexner (1966) found that the combined effect of AXM and PUR given one hour after training did not impair retention when tested 12 hours after training, even though the combined injection caused double the amount of protein synthesis inhibition. This is a contradictory finding, and it is possible that unique differences exist between PUR's effect and that of CXM. The effect of PUR differs from that of AXM and CXM in several ways. PUR causes an amnesic effect when injected after training, whereas CXM and AXM do not (Barraco & Stettner, 1976). PUR causes an abnormal amount of electrical activity within the brain,

(i.e. PUR induces epileptiform discharges within the brain, Cohen & Barondes, 1967), whereas CXM does not.

The question of whether amnesic inducing drugs cause behavioural changes that mimic amnesia, as opposed to actually changing biochemical memory processes, has been investigated. It is suggested that these drugs either inhibit locomotor activity, or create aversive associations to the test environment, and as such, make an animal's response rate appear as though there was memory blockage.

Experimentally testing whether an amnesic drug is exerting its effect on a particular biochemical system presumed to underlie amnesia, or if it's exerting non-specific side effects on locomotor activity, is important in order to correctly link cause and effect. Various experimental designs have been used which demonstrate the effect of CXM on locomotor activity, or on aversive conditioning, to be dissociable from CXM's amnesic effect.

Quartermain & Botwinick (1975), demonstrated that retention of an avoidance motivated, or food motivated, discrimination task was significantly lower for CXM treated animals, than it was for saline treated control animals. However, on reversal training the control group displayed significantly

less ability to learn the reverse task, in comparison to the CXM treated animals. Presumably, the CXM group were amnesic to the first task; whereas, the control group was experiencing proactive interference. Furthermore, these results suggest that drug effects on locomotor activity or aversion does not interact with learning.

Similarly, Nagelberg & Nagy (1977), found that pretesting injection of CXM into animals treated with saline at time of training did not affect retention; therefore, CXM's immediate effect on activity levels (i.e. hyperactivity, Squire & Barondes, 1972) did not interact with CXM's effect on retention.

The effect of CXM on activity levels is that it immediately causes hyperactivity, which then subsides to a normal level of activity at time of training, and then develops into depressed locomotor activity that lasts up to 24 hours past training (Day, Overstreet & Schiller, 1977; Squire & Barondes, 1972). Logically, one would predict the amnesic effect of CXM to be caused by the locomotor depression; however, this conclusion is false. Segal, Squire & Barondes (1971) demonstrated that CXM, and its derivative isoCXM both cause comparable locomotor depression, but isoCXM does not cause amnesia. And furthermore, Day, et.al. (1977)

have shown that CXM induced retention impairment for a conditioned avoidance response is improving 12 hours after training, whereas locomotor depression lasts up to 24 hours after training. Therefore, retention is improving despite there being significant locomotor depression.

In another experiment by Davis, Rosenzweig, Bennet & Squire (1980), they found that large doses of ansiomycin (ANI) (120 mg/kg) injected 5 hours prior to training caused significantly greater locomotor depression at time of training, than did a smaller dose of ANI (20 mg/kg) injected 20 minutes prior to training; and that the lower dose caused more significant amnesia.

The fact that CXM is used as either an agricultural fungicide or a rodent repellant (large doses) suggests that by using it one may induce debilitating aversive side effects which mimics amnesia. Experimental evidence counters this point, and demonstrates that CXM's effect on retention is distinct from its effect on aversive conditioning.

For instance, Schmaltz & Clement-Forestier (1977) demonstrated that the combination of CXM treatment and forced shock administered to rats while in the training apparatus caused significantly poorer learning when trained 1 day later,

but there was no effect on learning when trained 3 weeks later; hence, no aversive associations.

Similarly, Squire, Emanuel, Davis & Deutsch (1975) suggested that CXM's effect on aversive conditioning is separate from amnesic effects, because lithium chloride treatment causes aversive associations similar to CXM, but it does not affect memory.

Other possible artifacts of the experimentation that may interact with the amnesic effect are the effects of the drugs on learning, and the number of training trials given. Segal, Squire & Barondes (1972) found normal learning curves for both saline control groups and CXM groups. However, as the number of trials increased beyond 21, the effect of CXM became noticeable, and less acquisition resulted. In contrast, the effect of CXM in combination with a higher number of training trials was negligible, that is, retention was equal to that of the control group.

Separating the effects of protein synthesis inhibitors on retention from their non-specific side effects, is important because it suggests that protein synthesis is the biological process underlying the amnesic effect, as suggested by Squire & Barondes, (1972).

Apart from the interpretation that the amnesic effect of CXM results from protein synthesis inhibition, is the idea that CXM also exerts its effect by altering the level of catecholamines. By decreasing the level of the catecholamines norepinephrine (NE) and dopamine (DA) one effectively reduces the neurotransmitter substances essential for the processing or transmission of memory related neural impulses.

In one particular study, Flexner, Serota & Goodman (1973), demonstrated that CXM inhibited in vitro tyrosine hydroxylase activity, which is involved with the rate limiting step in the production of NE and DA. Hence, these authors argued that in addition to CXM's effect on protein synthesis, its effect on catecholamines is also responsible for amnesia. In contrast to this position are the findings by Squire, Kucenski & Barondes (1974), who found that CXM does inhibit tyrosine hydroxylase (11 - 13%) within 30 - 90 minutes after injection, however , more substantial inhibition of tyrosine hydroxylase by injection of alpha-methyl-para-tyrosine (α-MPT) did not cause amnesia. And these authors argue that the effects of CXM on the adrenergic system are not directly related to CXM's effect of retention. However, Quartermain & Botwinick (1975) did find that α-MPT caused retention deficits when administered 3 hours prior to training, similar to that induced by CXM.

Although these findings confront each other, there is an abundance of empirical evidence indicating the adrenergic system is involved in the memory process. Experiments have demonstrated that injection of catecholamines directly, catecholamine antagonists, or catecholamine agonists, all have an effect on retention.

Haycock, van Buskirk, Ryan & McGough (1977), found that immediate post training injections of either NE or DA enhanced retention of passive avoidance learning for step through latency and lick latency. Similar enhancing effects for retention have been found using the drugs corticosterone and hydrocortisone (Flood,Vidal, Bennet, Orme, Vasquez & Jarvik, 1978), and once again for NE and DA (Gold & van Buskirk, 1976b), and for ACTH (Bohus, 1971; Gold & van Buskirk, 1976a; Sands & Wright, 1979).

In many experiments it's been demonstrated that certain catecholamine antagonists disrupt retention, as does CXM, and these behavioural deficits can be counteracted by administering catecholamine agonists. For instance, CXM induced amnesia for a multiple trial appetative spatial discrimination task is reversed by administering the norE receptor stimulator clonindine prior to training, up to 1 hour after training, and up to 3 hours prior to retention testing.

(Quartermain, Freedman, Botwinick & Gutwein, 1977) Similarly, injection of amphetamine right after training also counteracted CXM induced amnesia when tested up to 72 hours later (Quinton & Bloom, 1975).

The combination of catecholamine antagonists and agonists is counteractive in the sense they offset each other's effect on retention. Hamburg & Kerr (1975) demontrated that the dopamine beta hydroxylase inhibitor DDC impaired retention when injected 30 minutes prior to training or testing; and its amnesic effect is reversed by injection of the NE precursor DOPS 1 hour prior to DDC injection. However, Bloom (1975) did not find d-amphetamine injections post training to reverse DDC induced amnesia.

Other experiments have demonstrated that the amnesic effect caused by injecting the dopamine beta hydroxylase inhibitor drug FLA-63 prior to training on a food motivated spatial discrimination task was counteracted by administration of the monoamino oxidase inhibitor drug pargyline 2 hours prior to retention test (Botwinick, Quartermain, Freedman & Hallock, 1977). And similarly, injection of l'tryptophan or corticosterone reversed CXM induced amnesia (Day, et.al., 1977).

An interesting finding in relation to the enhancing effect of ACTH on retention, is that it operates in an inverted-U dose response curve, and is time dependent. Injections of ACTH at .03 and .3 IU/rat immediately after training enhanced retention, whereas doses less than .03 or greater than 3.0 IU/rat impaired retention (Gold & van Buskirk, 1976 a,b). Similarly, Sands & Wright (1979) found that injections of ACTH in rats at a dosage 1 IU/kg disrupted retention; 1 IU/kg immediately after training enhanced retention; doses of 10 or 100 IU/kg did not significantly affect retention; and doses greater than 1000 IU/kg impaired retention. And furthermore, for ACTH to effectively enhance retention it has to be injected within 2 hours after training.

Biochemically, ACTH is thought to excite central nervous system (CNS) activity by promoting a greater interaction between neurotransmitters and appropriate neural receptors (Flood, et.al., 1978), and this excitation is thought to increase NE turnover by stimulating a higher metabolism of NE from DA (Dunn, 1980).

In addition to the biochemical effects of these protein synthesis inhibitor drugs on the central nervous system are their effects on the peripheral nervous system, (i.e. the pituitary-adrenal system). Amnesic drugs induce protein

synthesis inhibition within the adrenal glands, and thereby cause a reduction in the release of corticosteroids, according to Nakajima (1976). Nakajima (1975) suggested the amnesic effect of CXM was a result of its effect on the adrenal gland, because no retention deficits were found in CXM treated animals who had been adrenalectomized. Similarly, Flexner & Flexner (1970) found injection of PUR into adrenalectomized mice 1 hour after training did not impair retention; however, when these mice were adrenalectomized after training there were retention deficits. The role of the pituitary-adrenal system in the memory process is further supported by findings which show corticosterone antagonized CXM induced amnesia when given immediately after training (Nakajima, 1975), or when given 13 or 16 hours after training (Flexner & Flexner, 1971).

Nakajima (1976) believed a relationship existed between the level of corticosteroids and the degree of retention. That is, high levels of corticosteroids stimulate the corticoid receptors of the hippocampus, which causes an increase in adrenergic activity, and thereby promotes neural activity associated with retention. Whereas, low levels of corticosteroids understimulate the corticoid receptors of the hippocampus, which causes a decrease in adrenergic activity (i.e. neuronal blockage) and a negative effect on retention.

Biochemically, CXM reduces corticosteroid levels via its inhibitory effect on protein synthesis within the adrenal gland, and the decrease in corticosteroids stimulates a corresponding increase in ACTH. ACTH acts in a feedback cycle with corticosteroids, in which low levels of corticosteroids stimulate the release of ACTH, and the increased level of ACTH stimulates the adrenal gland to release corticosteroids. The feedback system continues until an equilibrium is established between ACTH and corticosteroids (Langley, 1971). The rise in ACTH caused by CXM induced depletion of corticosteroids does not stimulate an immediate rise in corticosteroid levels from the adrenal gland because the adrenal is unresponsive to ACTH as a result of the CXM treatment. For ACTH to counteract CXM's effect on the adrenal gland it must wait until the adrenal gland recovers from the effect of CXM. Once the adrenal gland recovers, it then responds to the high levels of ACTH, and thereafter the corticosteroid level is re-established. These changes stimulate adrenergic activity and promote transmission of neural memory impulses.

Other experiments demonstrate that the effects of CXM on the adrenal gland and the effects of CXM on retention are dissociable. Squire, St. John & Davis (1976) found the injection of aminoglutethimide (50mg/kg), depleted as much corticosterone as did CXM (120mg/kg), but it did not impair

retention of a discrimination task when tested 5 days later. And they also found post training injections of corticosterone (which was calibrated to offset the depletion caused by CXM) produced a more profound amnesic effect rather than being counteractive. Similarly, Dunn & Liebmann (1976) found injection of aminoglutethimide in mice 5 or 30 minutes prior to training did not cause amnesia for passive-avoidance training when tested 24 hours later. And neither did the drugs dexamethasone (an inhibitor of ACTH secretion, which block corticosteroid secretion during training) and cortexdone (which competes with both cerebral and peripheral corticosterone receptors) impair retention when tested 24 hours later.

Apart from the effects on retention caused by either changes within the central nervous system, or within the peripheral nervous system is evidence suggesting an interaction exists between norepinephrine and the pituitary-adrenal axis in relation to learning and retention. Ogren & Fuxe (1974) found the combined insult of adrenalectomy and 6-OHDA lesioning of the dorsal CA bundle of the hippocampus impaired learning and retention (1 week after training) of a conditioned avoidance response task. Whereas, no deficits in learning and retention were found in rats that were only lesioned or adrenalectomized. Similar results were found by Mason,

Roberts & Fibiger (1979), when retention testing took place 24 hours after training. Furthermore, this effect on learning and retention was antagonized by corticosterone injections (Orgen & Fuxe, 1977).

In another experiment, differences were found between the effects of the amnesic drug DDC and NE cortical lesioning plus adrenalectomy on acquisition and retention of an avoidance response task. In this experiment, rats were placed on a safe platform which was elevated from an electrically charged grid floor. Once the rat moved down onto the grid floor, it received shocks until it escaped back onto the safe platform. The findings demonstrated the rats with lesioning and adrenalectomy took longer to learn the task, and had greater retention deficits 24 hours after training; whereas, rats treated with only DDC learned the task normally, but displayed impaired retention 24 hours later (Roberts & Fibiger, 1977). These results are contradictory, because if DDC impaired retention as a result of inhibiting NE at time of retention test, then so should the 6-OHDA lesioning of NE (Ogren & Fuxe, 1974) cause similar retention deficits. However, this was not the case; possibly DDC has more of a non-specific effect (which has been suggested previously) and its effect differs significantly from 6-OHDA lesioning.

The major points brought out by experiments investigating the relation between protein synthesis inhibitors and amnesia are that the amnesic effects are more pronounced for long term retention than for short term retention, and that the impairment is temporary. The non-specific side effects of amnesic drugs on locomotor activity, and aversive conditioning are dissociable from the effect of these drugs on retention. The effect of these drugs on protein synthesis is not the only biochemical change, instead the effect of these drugs on adrenergic activity is also important. Adrenergic stimulation enhances retention, whereas, adrenergic depression impairs retention. Other findings suggest CXM inhibits protein synthesis within the adrenal gland, and that subsequently, this lowers corticosteroids and neurotransmitter levels, which in turn induces memory blockage. However, alternative findings suggest that either CXM's effect on the pituitary-adrenal system is dissociable from its effect on retention; or that an interaction exists between NE and the pituitary-adrenal axis in relation to learning and retention.

Rationale of the Experiment

The purpose of the present experiment was to determine whether ACTH would counteract CXM's amnesic effect.

ACTH was chosen for two reasons, First, ACTH enhances retention (Bohus, 1971; Gold & van Buskirk, 1976 a,b; King & de Wied, 1974; Sands & Wright, 1979) and this is thought to result from its facilitatory influence on the adrenergic system, i.e. ACTH increases the metabolism of NE and DA (Dunn & Gispen, referred from Dunn, 1980). Whereas, CXM impairs retention (Squire & Barondes, 1972) and this is thought to result from its inhibitory effect on adrenergic activity (Quartermain, Friedman, Botwinick & Gutwein, 1977). Therefore, if one can show that ACTH antagoizes CXM, then this would suggest that ACTH acted as an adrenergic stimulator.

Second, this particular combination of drugs allows one to test whether CXM actually affects retention as a result of its influence on protein synthesis within the adrenal gland, as hypothesised by Nakajima (1976).

Nakajima (1976) suggested that CXM has a peripheral effect, as opposed to a central effect on the nervous system. Inhibition of protein synthesis within the adrenal gland by CXM

decreases corticosteroid release, and this leads to less stimulation of the corticoid receptors of the hippocampus and subsequent reduction of the adrenergic neurotransmitter substances NE and DA. Preventing the release of NE and DA causes neural blockage of memory impulses and retention impairment. Once the effects of CXM expire and the adrenal gland recovers, then ACTH (which was elevated in response to low levels of corticosteroids) begins to stimulate the release of corticosteroids.

The part of Nakajima's hypothesis that is not clear, involves the question of why ACTH, which is released in response to CXM induced depletion of corticosteroids, does not directly stimulate the adrenergic system at the same time that CXM is inhibiting it, such counteraction should negate the amnesic effect of CXM. In this way, ACTH would be exerting its influence via the central nervous system, instead of through the pituitary-adrenal system. Therefore the argument of whether CXM causes amnesia as a result of its effect on the adrenal gland would be questionable.

To answer the question of whether CXM affects retention as a result of its effect on the pituitary-adrenal system, one would have to test the interaction of exogenous ACTH on CXM. If exogenous ACTH does not antagonize CXM, then this

would suggest that ACTH exerts its influence via the adrenal gland, and is without effect until the adrenal gland recovers from CXM treatment.

On the other hand, if the exogenous injection of ACTH does counteract CXM's amnesic effect, then this would suggest that ACTH had a central effect on the adrenergic system - independent of any effect on the adrenal gland. Therefore, one would not suspect CXM's effect on the corticosteroid levels to be the cause of the amnesia. If CXM did significantly inhibit the release of corticosteroids, then there would be a corresponding internal rise in ACTH, and together these substances would counteract each other's affect on the adrenergic system. Hence, there would be no effect on retention.

This present research may clarify the argument over the cause of CXM's amnesic effect. Nakajima argues that amnesia results from CXM's inhibitory effect on corticosteroid levels; whereas, Squire, St. John & Davis (1976) and Dunn & Liebmann (1976) argue that CXM's effect on corticosteroid levels is dissociable from its effect on retention.

Method

Subjects

Eighty 100 day old, experimentally naive rats (50 male and 30 female), from the SHS genetic line were used as subjects. This line is a genetically heterogeneous stock derived from a four-way cross among the selected lines (Roman high and low avoidance, and Maudsely reactive and non-reactive, see Satinder 1980 a, for details). The animals were bred and reared in the laboratory and weaned at 28 days of age. Before experimentation the animals were housed by groups of two of the same sex. During the course of the experiment the animals were coded and housed in individual cages on the same rack. The laboratory temperature was thermostatically controlled at 22$\pm$ 1^{o}C. Humidity level was maintained at 40%, and the fluorescent lighting was on a 12:12 hour light/dark cycle. Rats were tested in the light cycle.

Experimental Design

The experimental design was a 5 (drug groups) x 2 (time of testing) complete factorial, 8 animals in each factorial cell with a repeated measure (training and retention test).

The 5 drug groups are outlined in Table 1.

TABLE 1

Experimental Design

Group	Pre-training Treatment	No. at Training	Post-training Treatment	No. at Retention Test 1 hour	5 hours
1	NaCl	16	None	8	8
2	CXM	16	None	8	8
3	ACTH	16	None	8	8
4	CXM+ACTH	16	None	8	8
5	CXM	16	ACTH	8	8

Group 1 animals received physiological saline injections before training, and acted as the control group. Group 2 animals received CXM injections before training in order to test whether CXM would impair retention. Group 3 animals received ACTH injections before training in order to test whether this drug would enhance retention. Group 4 animals received both CXM and ACTH injections before training in order to test whether the facilitatory effects of ACTH on adrenergic activity would counteract the inhibitory effect of CXM on adrenergic activity, and thereby protect against CXM-induced amnesia. Group 5 animals received CXM injections before training and ACTH injections immediately after training in order to test whether ACTH's

effect on adrenergic activity would counteract CXM's inhibitory effect on the adrenergic system. In this group ACTH would be present only during initial memory processing, as opposed to being present in conjunction with CXM during acquisition as in group 4.

All drugs were administered interperitoneally, and the time of administration was the same for all animals according to drug conditions (see Appendix I for drug administration schedule). CXM was injected 30 minutes prior to training for two reasons. First, CXM is most effective as an amnesic agent when given between 10 minutes and 5 hours before training (Glassman,1969). Secondly, this time allowed for the effect of hyperactivity, which is associated with initial injection of CXM, to subside before training began (Segal, Squire & Barondes, 1971).

The dosage level of CXM was 2.5 mg/ml/kg (Schmaltz & Clement -Forestier,1977), and the dosage level of ACTH was 4 IU/kg. Each rat weighed approximately 250 grams, therefore the dosage level of ACTH was equal to the level used by Sands & Wright (1979) who found that 1 IU/rat ACTH enhanced retention in rats.

Retention was tested either 1 or 5 hours after training. Previous research shows that CXM impairs retention 3 to 24 hours after training, but not during the first 3 hours past training (Glassman, 1969).

Prior to either training or injection the rats were indivi-ually tested for unconditional escape response (UER) in order to determine the minimum but effective level of electric shock necessary to make each animal respond equally because the SHS genetic strain is a heterogeneous strain derived from genetic lines that are known to have differ-ent UERs (Satinder,1976). Therefore, these differences may have transferred to the SHS genetic strain, and thereby causing individual differences for UER.

The learning task used to test retention was a one-way jump-up paradigm in which animals avoided an electric shock, as unconditional stimulus (US) in response to an auditory conditioned stimulus (CS), by moving from an elec-trically charged grid floor on to a safe platform. A pilot study was carried out to determine the appropriate number of training trials needed to train the animal to learn the task at approximately 50% success level (i.e., 5 or more avoidance responses out of 10 trials). Retention was tested 1 or 5 hours after training by means of an extinction schedule

(details reported later). Extinction was used because the jump-up learning task is an easily learned task (Satinder, 1977), and for this reason it would be difficult to dissociate new learning from memory. Therefore, by using an extinction schedule confounding by new learning was avoided.

The behavioral measures used were ones most clearly associated with the learning task. The selection of the learning task was based on previous research showing that animals could learn the task relatively easily during one training session (Satinder & Petryshyn, 1974; Satinder, 1977). The response measures were as follows : number of avoidance responses, i.e., the number of times the animal responded to the auditory stimulus and avoided electric shock ; and total response latency, i.e., the total time taken by an animal to avoid the auditory stimulus and/or escape from the shock (total response latency was taken because normally response latency measure is taken either as an avoidance response or an escape response latency, since the extinction schedule was used in this research there was no shock given during retention test, hence there could be no escape response latency, so the total response latency was used).

Apparatus

The unconditional escape response (UER). The apparatus used to test UER was a circular Plexiglas runway 12 cm wide and 15 cm high with an outside circumference of 220 cm, which could be divided into 4 equal compartments by guillotine doors. The runway floor was constructed of 0.25 cm diameter stainless steel rods spaced 1 cm apart (center to center). A scramble shock could be delivered to the grids, and a digital clock was used to record response latencies (Satinder, 1980 b).

One-way jump-up learning. The jump-up apparatus was a Lafayette A-586 (85204) unidirectional avoidance system for rats. It consisted of a chamber 265 x 200 x 200 mm made from anodized aluminum and 6 mm thick clear Plexiglas , with 5 mm thick grid bars spaced 10 mm apart. On one side of the chamber was a platform 125 mm high, 200 mm wide, and 137 mm deep. The platform was elevated 80 mm from the floor level of the grid (Satinder and Petryshyn, 1974).

A sound source (speaker) was placed next to the jump-up learning apparatus, which was used to produce a 70-db, 9 KHz pure tone as CS against a background noise of 40 db (sound intensity was measured at the floor level above the standard reference level of .002 μbar by a General Radio sound level meter, Type 1551-C).

Procedure

Unconditional escape response (UER). For UER testing each animal was individually adapted to the circular runway for 1 minute period prior to receiving foot shock. Electric shock was administered until the animal escaped within 5 seconds by running a distance equivalent to a quarter length of the runway (all doors open) in either direction, and this was defined as UER. Each animal was given 10 trials of the ascending series by using the method of limits, with an intertrial interval of approximately 5 seconds. Shock intensities ranged between 0.27 to 0.97 ma (calibrated with the assumption that the animal contributed 47k resistance). The lowest number of ma necessary to elicit UER was used as the appropriate US during training (Satinder, 1977).

Learning: Training and retention test. Each experimental day was arranged in a specific pattern to allow for the synchronous training and testing of all animals individually (see Appendix I for experimentation schedule). Ten animals were trained and tested each day, and of these 10 two were randomly assigned to each of the five drug groups. Within each drug group, one of the animals was tested for retention 1 hour after training, and the other animal 5 hours after training.

All injections were given 30 minutes before training - except for animals of group 5 which received additional ACTH injections immediately after training. After the injection the animal was returned to its home cage, and 30 minutes later the animal was trained in the one-way jump-up learning apparatus (see Appendix 1 for training timetable).

During training the CS was presented for 10 seconds followed by 10 seconds of CS and US. The safe platform was exposed at the time CS started. To avoid the US, the animal had to jump onto the safe platform within the 10 seconds of CS. If the animal jumped then the CS was discontinued, and the animal was allowed to remain on the platform for the remaining duration of CS and intended US (i.e., 20 seconds minus response time). If the animal did not respond during CS, but it did escape in response to the US (in conjunction with the CS) then it was allowed to remain on the platform for the duration of the remaining US interval. After the CS and the US interval ended, the animal was pushed back on to the safe grid floor of the apparatus (provided the animal did escape) and the platform door was closed. The animal remained on the safe grid floor for the intertrial interval averaging 40 seconds, at which time the next training trial began. Ten training trials were given to each animal.

Immediately after training the animal was taken back to its home cage - except if the animal belonged to group 5, and in that case the rat was injected with ACTH and then returned to its home cage. An hour later, one of the two animals from each drug group was removed from its cage and placed in the one-way jump-up apparatus and tested for retention (see Appendix 1 for the timetable of testing times for those animals of the different drug groups tested 1 hour after training).

The extinction schedule used to test retention was identical to the training procedure except that the US (i.e., electric shock) was eliminated. The rat was presented with the sound source for a 20 second period during which time it was allowed to jump onto the safe platform. This extinction schedule tested whether the animals associated the conditioned tone with the electric shock, previously given, and as such avoided the grid floor by moving onto the safe platform in response to the CS tone.

After a period of 5 hours past training the second animal from each drug group was removed from its home cage and placed in the one-way jump-up apparatus and tested for retention. (See Appendix I for appropriate time table for testing). The same extinction schedule was used and the same two behavioral measures were recorded.

RESULTS AND DISCUSSION

Preliminary analysis of variance revealed no sex differences hence the data for both sexes were pooled for further analysis.

The mean number of avoidance responses (Figure 1) and the measure of response latency (Figure 2) are presented below for each of the two test groups (i.e. drug groups tested 1 hour, and tested 5 hours after training) within each of the 5 drug groups during both training and retention test.

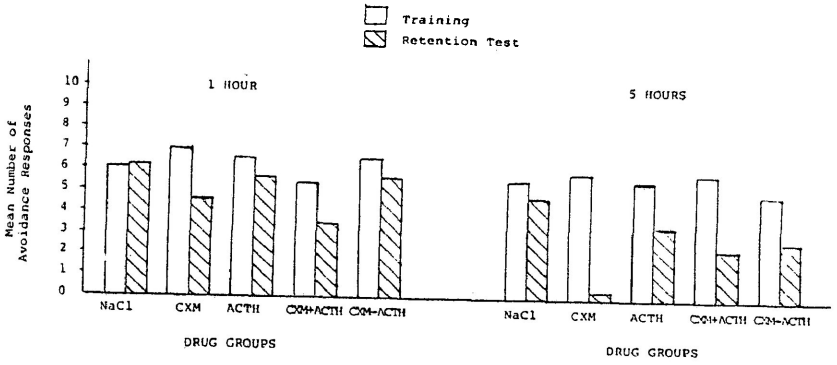

FIGURE 1: Mean number of avoidance responses for the drug groups tested 1 and 5 hours after training during both training and retention.

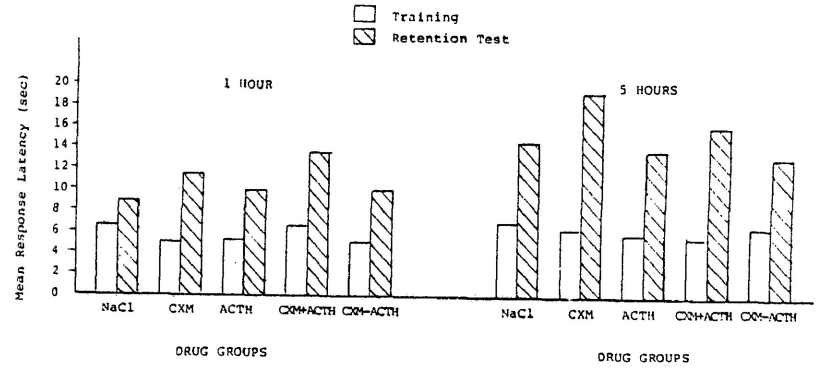

FIGURE 2: Mean response latency for the drug groups tested 1 and 5 hours after training during both training and retention.

A 5 (drug groups) x 2 (test group) x 2 (training and retention test) complete factorial analysis of variance revealed significant main effects for test groups (number of avoidance responses $F=6.7$, $df=1/70$, $P<.01$; response latency, $P<.01$). That is, groups tested 5 hours after training had significantly lower number of avoidance responses and higher response latencies than groups tested 1 hour after training. Also, the difference between training and retention test scores were significant (number of avoidance responses, $P<.01$; response latency, $P<.01$). That is, the animals responded with fewer avoidance responses and longer response latencies during retention test, than at the time of training.

During training, there were no significant differences among the five drug groups in either of the response measures. Similarily, during training differences between drug groups tested 1 hour and 5 hours after training were also non-significant. These findings suggest that the drug and test groups were homogeneous at time of training.

During retention test, there were no significant differences among the 5 drug groups when tested either 1 or 5 hours after training.

In retention test, within each of the drug groups the differences between groups tested 1 hour after vs 5 hours after training were significant in only the CXM drug group (number of avoidance responses $F = 7.2$, $df = 1/14$, $P < .01$; response latency, $p < .01$). These findings suggest CXM impaired retention 5 hours after training, which confirms other experimental findings that have also shown CXM's suppressive effect on long term retention (Schmaltz & Delerm, 1974; Squire & Barondes, 1972). However, ACTH was not found to enhance retention, which is in contrast to the findings by Gold & van Buskirk (1976 a,b) and by Sands & Wright (1979), who found that ACTH enhances retention. Why ACTH by itself did not influence retention in the present experiment may be partially explained by the fact that ACTH was given to Group 3 before training, whereas in the other experiments ACTH was given after training. In addition, the fact that the control

group showed no significant differences between testing 1 or 5 hours after training suggests that the time factor for retention test is not solely responsible for any difference found within other drug groups.

Changes in scores from training to retention test were not significantly different among drug groups tested 1 hour after training. But these changes were significantly different among groups tested 5 hours after training (number of avoidance responses $F=26.7$, $df=1/34$, $p<.01$; response latency, $P<.01$). In particular, these differences were evident in the CXM and the CXM+ACTH (in this latter group ACTH was given before training to CXM treated animals) drug groups on the number of avoidance responses ($\frac{F=5.4}{min}$, $df=1/6$, $P<.01$) and on the response latency ($P<.01$)

Interestingly, a test of interaction for the change from training to retention test (5 hours past training) between the CXM+ACTH group, and the CXM-ACTH group (in this drug group ACTH was injected into CXM treated rats immediately post training) revealed non-significant for both behavioural measures. The previously mentioned differences found in the within drug group analysis for the CXM+ACTH group can be accounted for by the fact that the rats in this drug group had higher response levels at time of training, than the rats

of the CXM-ACTH drug group, and this made the change from training to retention test appear significant, where in reality, it was not. Hence, the result of no interaction indicates ACTH was successful in reversing the amnesic effect of CXM, regardless of it being injected before or immediately after training.

There are three important findings revealed by the analysis. First, the results support previous findings which have demonstrated CXM's amnesic effect on long term retention, as opposed to short term retention (Schmaltz & Delerm, 1974; Squire & Barondes, 1972). Second, the effect of ACTH administered before training did not affect retention. Third, the effect of giving ACTH before training or immediately after training to CXM treated animals counteracted the amnesic effect of CXM on retention. This is demonstrated by the lack of significant change in the number of avoidance responses or response latency from training to retention test 5 hours after training. These results are interesting because they suggest that the stimulating effect of ACTH on the adrenergic system is important during acquisition of the avoidance task and important at the time of initial memory processing in order to successfully counteract CXM induced amnesia.

The present findings help to clarify whether CXM impairs

retention as a result of its inhibitory effect on corticosteroid levels, as suggested by Nakajima (1976). He believed that a causal relationship existed between corticosteroids and retention, i.e. low levels of corticosteroids cause retention deficits. His findings (1975) showed that CXM did not impair retention in adrenalectomized rats and therefore he concluded that the mechanism affected by CXM must reside within the adrenal gland. He hypothesized that CXM caused protein synthesis inhibition within the adrenal gland, and this caused a decrease in corticosteroid release, which in turn reduced stimulation of the adrenergic system, hence memory blockage. Further experimentation confirmed his beliefs as to the role of corticosteroids on retention. He found that the exogenous injection of corticosteroids counteracted CXM's suppressive effect on retention.

Arguments against Nakajima's theory arise from experimental findings that do not show a causal relationship between low corticosteroid levels and impaired retention. In specific, Squire, St. John & Davis (1976) reported that CXM induced amnesia could not be prevented even though corticosteroid levels were experimentally maintained; and similarly, Dunn & Liebmann (1976) found they could not induce a CXM like amnesia by using a drug that caused no protein synthesis inhibition, but did cause a decrease in corticosteroid re-

lease. Dunn (1981) argues that it is unlikely CXM's peripheral effect is the cause of amnesia (as proposed by Nakajima, 1975) because higher drug concentrations are needed when the administration is peripheral, as opposed to being central.

The results of the present research demonstrate that when ACTH was injected before or immediately after training to CXM treated animals the amnesic effect of CXM on retention was suppressed. The implication of this finding is that CXM does not affect retention when there are high levels of ACTH. And in turn, this suggests that CXM's effect on retention is independent of its effect on the pituitary-adrenal system.

The biological system disrupted by CXM is thought to be the adrenergic system because experiments have shown that a relationship exists between the level of neurotransmitter substances (NE and DA) and the effect on retention. Stimulation of the adrenergic system prevents CXM induced amnesia (Quartermain, et. al. 1977); and the inhibition of the adrenergic system produces a CXM like amnesia (Quartermain & Botwinick, 1975). Hence, CXM affects retention as a result of its suppressive effect on adrenergic activity. Based on this premise it is argued that ACTH acted in a similar manner as the other adrenergic stimulators, and thereby compensated for

CXM induced depletion of NE and DA. This interpretation is consistent with views put forward by Flood, et. al., (1978), Dunn & Gispen (1977; referred from Dunn, 1980) who suggest that ACTH directly stimulates neural activity.

If CXM does exert its amnesic effect via the pituitary-adrenal system, as suggested by Nakajima (1975), then one would expect the subsequent increase in the ACTH level, resulting from CXM's inhibition of corticosteroid release, to counteract the amnesia similar to the way exogenous ACTH antagonized CXM in the present research. This is obviously not the course of events because CXM does impair retention, therefore CXM must not significantly affect the pituitary-adrenal system.

In contrast to the above-mentioned suggestion that CXM's effect on the adrenal gland is dissociable from its effect on retention is evidence showing that a relation exists. Neither adrenalectomy, nor lesioning of cortical NA bundle disrupted retention itself; but the combined operations did (Ogren & Fuxe, 1974, 1977; Mason, Roberts & Fibiger, 1979). Thus suggesting CXM impairs retention by simultaneously affecting norepinephrine and the pituitary-adrenal axis. The relation of these findings to the present experiment is that ACTH antagonized CXM induced amnesia by disruptiong CXM's total effect, i.e., ACTH counteracted CXM's effect on NE, but

not its effect on the pituitary-adrenal axis. Accordingly, one would not expect the disrupted pituitary-adrenal axis to impair retention by itself. Therefore, the synergistic effect of NE inhibition and adrenal inhibition may be responsible for CXM induced amnesia.

One question that does arise is whether the dosage level of ACTH given was the appropriate dosage to completely antagonize CXM. Future experimentation may test whether varying the dose of ACTH affects CXM's amnesic effect any differently during training or retention test. Furthermore, the fact that ACTH reversed CXM induced amnesia when given before training, and when given immediately after training is contradictory to other experimental findings which show adrenergic stimulation (by giving amphetamine) immediately after training counteracts CXM, whereas pretraining injections do not (Quinton & Bloom, 1975; Bloom, 1975).

Stimulation of the adrenergic system as a result of high levels of ACTH aids the memory process against the influence of CXM. Gibbs & Ng (1977) suggest that there are three stages involved in the memory process - a short term phase, a labile phase, and a long term phase. Proper transition from the short term phase to the long term phase requires that the intermediary labile phase not be interrupted. This

labile phase is vulnerable, and as such, any changes in the biochemical system during this phase will disrupt it. However, the short term and the long term phases are more stable, and are not affected by the same biochemical changes. Therefore, depletion of the neurotransmitter substances by CXM causes neural blockage, and this interference disrupts the labile phase. As the neurotransmitter levels are restored, so is the recovery of the memory process. In the present research one can interpret the results by saying that the post training injections of ACTH adequately stimulated the adrenergic system, which in turn compensated for CXM induced depletion of NE and DA. This compensation prevented interference to the labile phase, and as such, allowed for normal memory processing.

In conclusion, the present research brings out three points. First, injection of ACTH 30 minutes before, or immediately after training counteracted CXM induced amnesia. Second, CXM's inhibitory effect on corticosteroid levels is dissociable from CXM's effect on retention. Third, normal levels of neurotransmitter substances must exist at time of initial memory processing to prevent CXM from interfering with the labile phase of the memory process.

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APPENDIX I

TIMETABLE OF EXPERIMENTATION

TIME	TREATMENT	TRAINING	RETENTION TEST	
			1 HOUR	5 HOURS
830	NaCl (1)			
845	CXM (2)			
900	ACTH (3)	NaCl (1)		
915	CXM+ACTH (4)	CXM (2)		
930	CXM (5)	ACTH (3)		
945	NaCl (6)	CXM+ACTH (4)		
1000	CXM (7)	CXM-ACTH (5)		
1015	ACTH (8)	NaCl (6)		
1030	CXM+ACTH (9)	CXM (7)		
1045	CXM (10)	ACTH (8)		
1100		CXM+ACTH (9)		
1115		CXM-ACTH (10)		
1130			NaCl (6)	
1145			CXM (7)	
1200			ACTH (8)	
1215			CXM+ACTH (9)	
1230			CXM-ACTH (10)	
200				NaCl (1)
215				CXM (2)
230				ACTH (3)
245				CXM+ACTH(4)
300				CXM-ACTH(5)

APPENDIX II

Table of Experimental Means

	Mean Number of Avoidance Responses				Mean Total Response Latency (sec)			
	1 hr test		5 hr test		1 hr test		5 hr test	
	Tr[1]	Re[2]	Tr	Re	Tr	Re	Tr	Re
NaCl	6.1	6.2	5.5	4.8	6.4	8.9	6.8	14.5
CXM	7.0	4.6	6.0	.4	5.0	11.5	6.2	19.4
ACTH	6.6	5.6	5.5	3.5	5.2	10.0	6.0	13.9
CXM+ACTH	5.4	3.4	6.0	2.4	6.6	13.7	5.8	16.2
CXM-ACTH	6.6	5.6	5.0	2.9	5.0	10.0	6.6	13.2

1 Tr = Training

2 Re = Retention Test