THE EFFECTS OF REDUCED CHOLINESTERASE ACTIVITY ON DISCRIMINATIVE BEHAVIOUR IN THE RAT.

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SUMMARY

1. The physiology of acetylcholine and cholinesterase (ChE) is briefly reviewed. Methods are described that enable brain ChE activity of the rat to be maintained at approximately 30% of normal.

2. The evidence on the relationship of ChE activity to behaviour is reviewed.

3. Reductions of cholinesterase activity are shown to reduce body weight, and the amounts eaten and drunk. When cholinesterase is allowed to recover, eating and drinking return to normal in 5-7 days, but the recovery of body weight is slower. When cholinesterase is chronically reduced, body weight and water intake remain depressed, but the effect on food intake is no greater than for the acute treatment.

4. The effects of ChE reduction on motor behaviour are investigated by analysis of a simple lever

pressing response. Fixed ratio lever pressing is slowed down by acute reduction of ChE. During chronic reduction the slowing becomes less marked and responding returns to near normal values. The slowing is due to an increase in the time spent away from the lever. Actual manipulation of the lever is not markedly affected.

5. Visual discrimination learning is shown to be impaired by chronically reduced ChE when the stimuli are complex patterns, but there is no effect of such treatment on simple brightness discrimination. Increasing the 'cost' of making errors by punishment with shock abolishes the effect of low ChE.

Reversal learning is not impaired by low ChE.

Several experiments in which animals are given opportunities to make 'unnecessary' unrewarded responses indicate that the ability to control responding is the function most affected by reduced ChE activity.

This effect on control of responding is attenuated both by increasing the cost of error responses, and by overtraining.

6. Various hypotheses to account for the results are discussed and it is concluded that there is little evidence of the acetylcholine-cholinesterase system being directly involved in learning, but that it does play some part in the control of response.

Statement.

This thesis contains no material which has been accepted for the award of any other degree, and to the best of my knowledge contains no material previously published or written by another person, except when due reference is made in the text.

Alan Richardson.

Acknowledgement.

My principal debt is to my supervisor, Dr. P.H. Glow, for his advice on all aspects of this research. I also want to thank everyone who worked in Dr. Glow's laboratory from 1963 to 1965 for their co-operation and assistance.

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and

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J. comp. physiol. Psychol., in press.

PART ONE.

INTRODUCTION.

1. PROBLEMS AND METHODS.
2. PHYSIOLOGY OF THE ACETYLCHOLINE SYSTEM.
3. EFFECTS OF DFP ON CHOLINESTERASE ACTIVITY.

1. PROBLEMS AND METHODS.

The general area of this investigation is the biochemical functioning of the brain in its control of behaviour. Within this area, the specific problem investigated is the significance of the acetylcholine - cholineacetylase - acetylcholinesterase system (ACh-S), for discriminative behaviour in the rat.

The functioning of the ACh-S was manipulated by irreversibly inhibiting the enzyme acetylcholinesterase (AChE) with a systemically injected drug - di-isopropyl fluorophosphate (DFP). The behavioural consequences were then observed in various instrumental learning and discrimination situations.

In this approach to the relationship between brain biochemistry and behaviour there are two broad problems. The first is that when drugs are introduced into the general circulation it is not possible to retain control over their site of action. The best that can be done is to measure the biochemical effects of the drug at different sites and attempt to correlate this data with any behavioural effects.

The second problem is that every behavioural situation is open to the influence of many variables, several of which may be affected by any one drug. This problem can be met in several ways. Drug effects on variables such as motivation can be measured independently and allowed for in interpretation; drug and non-drug groups can be experimentally equated, or tasks can be used that are insensitive to the variable under consideration.

The remainder of PART 1 deals with the biochemical effects of the drug treatments used, and subsequent parts with their effects on behaviour.

2. PHYSIOLOGY OF THE ACETYLCHOLINE SYSTEM.

Acetylcholine (ACh) was first synthesized in 1867, and investigation of its pharmacological properties began in the early 1900's. In 1921 Loewi showed that stimulation of the vagus nerve of a perfused frog's heart resulted in the release into the perfusion fluid of some substance that had an inhibiting effect upon a second frog's heart. This substance he called "Vagusstoff" (vagus substance). The "Vagusstoff" was identified as ACh by parallel

bio-assays. Later research showed that ACh was also present in perfusion fluids obtained from other parasympathetically innervated structures. However, even at the present time, ACh has not been chemically identified in perfusion fluids - the only evidence is still a similarity of the assays of perfusion fluids to those obtained using ACh, McLennan (1963).

From about 1930 to 1950 there was controversy as to whether synaptic transmission was due to a chemical "transmitter substance" or could be accounted for simply as the electrical stimulation of one nerve by another. One of the objections to the possibility of ACh being a transmitter was that it could not be destroyed quickly enough to account for the short duration of synaptic excitation. This objection was removed with the discovery in nervous tissue of the enzyme acetylcholinesterase (AChE). AChE has a high affinity for ACh, and hydrolyzes it to acetic acid and choline at a sufficiently rapid rate to make its proposed transmitter function quite possible. It was also found that brain contained other esterases capable of hydrolyzing ACh. These esterases differ from AChE in several ways, such as optimum pH, optimum substrate concentration,

substrate specificity etc. As yet their physiological importance is unknown. It has been estimated that less than 5% of the total cholinesterase activity of rat brain is in fact due to esterases other than AChE (Bennett et al 1958).

A second enzyme, this one involved in the synthesis of ACh, was found shortly after AChE. The enzyme, now known as cholineactylase (ChA) catalyzes the acetylation of choline to form ACh.

By 1950 - 1960 it is fair to say that the great majority of physiologists accepted that ACh, AChE and ChA functioned as a transmitter system in the peripheral nervous system (p.n.s.) and possibly also in parts of the central nervous system (c.n.s.).

The ACh - S has various functions in the peripheral nervous system. Goodman & Gilman (1955) state that all preganglionic nerve impulses, whether sympathetic or parasympathetic, stimulate ganglion cells through the intervention of acetylcholine. This statement is based on the known presence of ChA, ACh and AChE in ganglia, on evidence from perfusion studies, and on the effects of various drugs known to

interfere with the ACh - S. Beginning with the work of Loewi on the frog's heart, there is evidence of the same kind as above that ACh is also the transmitter at post ganglionic parasympathetic nerve endings. In addition to these pre and post ganglionic functions, there is similar evidence that ACh is the transmitter at the junction of motor nerves and skeletal muscles.

It can be seen that general cholinergic stimulation or inhibition of the peripheral nervous system will have widespread effects, some of which will tend to counteract others since both sympathetic and parasympathetic branches of the autonomic nervous system (a.n.s.) are affected together, Goodman and Gilman (1955).

Most of the evidence for the central action of ACh as a transmitter substance is indirect:-

(1) ACh is present in nervous tissue, together with the enzyme systems necessary for its synthesis and degradation.

(2) Drugs known to affect the ACh - S in the p.n.s. have similar effects in the c.n.s. These effects may

be abolished by drugs which are known to have an antagonistic action at peripheral sites. (For example, atropine abolishes the E.E.G. arousal produced by anticholinesterases, Himwich & Rinaldi (1955) and White (1962)).

(3) Changes in brain ACh content can be correlated with changes in the functional activity of the brain, Crossland (1953), and ACh can be detected leaking from the surfaces of the brain in amounts which are related to functional activity, Mitchell (1963).

(4) Probably the best evidence is furnished by iontophoretic injection of substances directly onto the nerve cell. ACh sensitive neurones have been found in medulla, visual and motor cortex, and cerebellum using this method. However, the effects of injected ACh vary. Neurones have been found which are facilitated, depressed and unaffected by ACh within the same area of the c.n.s.; Salmoiraghi and Bloom (1964). It is therefore likely that ACh is not the only transmitter substance in the c.n.s.

It follows from the nature of the ACh - S that there are three principal ways in which

its activity can be altered :-

(1) By altering the production, and/or release, of ACh from the pre-synaptic terminals. The synthesis of ACh can be inhibited by hemicholinium compounds, MacIntosh, Birks & Sastry (1956). Release of ACh from nerve endings is inhibited by botulinum toxin, Brooks (1956, 1954).

(2) Interfering with or assisting the action of ACh upon the post synaptic receptor. The main technique used here is administration of anticholinergic drugs - for example, atropine and scopolamine - or alternatively using drugs which mimic the actions of ACh. Since it is possible to construct active forms of these drugs which will and will not pass the blood-brain barrier, some check can be made of the 'peripheral' or 'central' location of any effects they may have.

(3) Prolonging the action of released ACh by inhibiting AChE. Inhibitors of AChE fall into two broad classes: reversible inhibitors and irreversible inhibitors. As with the anticholinergic and cholinomimetic drugs, there are members of both classes that will and will not pass the blood-brain barrier. For an inhibitor that acts both centrally and

peripherally, some of the latter effects may be controlled by an anticholinergic that does not get into the brain. In the case of inhibition with organophosphorus poisons it is possible to reactivate the peripheral enzyme by drugs that split the enzyme - inhibitor complex but are unable to enter the brain, Heath (1961).

In considering the behavioural effects of any of these manipulations it must be remembered that the selectivity of action which can be obtained is always extremely gross. The central/peripheral dichotomy completely ignores probable differences in importance of the alterations in specific central and peripheral structures.

3. EFFECTS OF DFP ON CHOLINESTERASE ACTIVITY.

This section is concerned with the biochemical effects of the standardized procedures used in the behavioural experiments. No attempt has been made to distinguish acetylcholinesterase from other

cholinesterases. The term cholinesterase (ChE) will be used in reference to total cholinesterase activity.

The general procedure was firstly to inhibit ChE with di-isopropyl fluorophosphate (DFP), and secondly, to reactivate inhibited ChE in the peripheral nervous system with a second drug, NN Trimethylene (1:3) - bis - (pyridinium 4 aldoxime) bromide (C434).

Two variants of this general method were used.

(1) An acute inhibition of ChE, followed by recovery of activity, as the enzyme was presumably resynthesized.

(2) Chronic inhibition, in which ChE activity was maintained at an approximately constant level by repeated injections of DFP.

DFP inhibits cholinesterases irreversibly, that is, there is little spontaneous reactivation. In vitro activity cannot be recovered by washing or by dialysis, Mazur & Bodansky (1946) and in vivo recovery is very slow, indicating that new enzyme may be synthesised, Koelle and Gilman (1946), Kewitz and Nachmansohn (1957). Since the

oil/water partition coefficient of DFP is high, it can pass from the blood stream to the brain quite readily, and therefore inhibits peripheral and central Ch E to approximately the same extent, Heath (1961, P.187).

The reactivator (C434) breaks the complex formed between DFP and the enzyme. It is capable, in small quantities, of protecting rats against fatal doses of anticholinesterases. The intramuscular LD_{50} of C_{434} in rats is 123 mgm/kgm; a dose of 20.5 mgm/kgm together with 4 mgm/kgm of atropine gave 95% protection against 2 x LD_{50} of the organophosphate Sarin, whereas no animal given atropine alone survived, Fleisher et al (1960a). In the same paper it was reported that 25 mgm/kgm C_{434} together with 4 mgm/kgm atropine raised the LD_{50}'s of Sarin and DFP., 12 and 23 times respectively, when reactivator and inhibitor were injected within 20 secs of each other.

In mice, one third of the intraperitoneal LD_{50} dose of C_{434} gave complete protection against 2 x an LD_{100} dose of DFP, and partial protection against 4 x an LD_{100} but there was no detectable reactivation of ChE in the brain, Hobbiger and

Saddler (1959).

The latter result has also been obtained in rats by Fleisher et al (1960b) who found that 25 mgm/kgm C_{434} had no effect upon the brain ChE of animals poisoned with 2 x LD_{50} of Sarin. This is due to the fact that C_{434} is relatively insoluble in oil, and will therefore be absorbed from the blood stream by the brain only poorly, Hobbiger & Saddler (1959).

In all the behavioural experiments the animals used were female rats, approximately 120 days old and 150 grams in weight. Similar animals were therefore used to determine the biochemical effects of the drug treatment in the experiments described below.

Experiment 1. Effects of DFP on cholinesterase activity of rat brain.

Methods.

(1) Acute effects: DFP dissolved in Arachis oil to a concentration of 10 mgm/ml was injected into the gastrocnemius muscle at a dosage of 1 mgm/kgm. The reactivator, C_{434} was made up in sterile distilled water to 50 mgm/ml and injected intraperitoneally at 50 mgm/kgm. Where appropriate the animals received control injections of water, equivalent in volume to those of DFP or C_{434}. They were killed at various times after injection by decapitation, and the brains were rapidly freed from the skull and stored at-28°C.

(2) Chronic effects: The procedure for animals in which ChE was chronically reduced by DFP was similar to that for the acute animals except that on every third day after the first injection, they received half of the original dosages.

Cholinesterase activity was assayed by both titrimetric and manometric methods, details of which have been reported in Glow, Rose and Richardson (1966). After acute treatment the assays were made on homogenates of whole brain, and after chronic on either whole brain or various

separate anatomical regions. The results were expressed as percentages of normal cholinesterase activities.

Results.

Figure 1 shows the ChE activity of whole brain at various times after an acute injection of DFP, and 2 days after each injection when treatment was continued chronically. Twenty-four hours after treatment with 1 mgm/kgm of DFP brain ChE was reduced to 30-35% of normal, but recovered over succeeding days in a negatively accelerated manner.

There were no differences between the animals injected with DFP + H_2O and those injected with DFP + C_{434}, (Table 1).

TABLE 1. Cholinesterase activity of brain after acute treatment with DFP + H_2O and DFP + C_{434}.

Days after injection	DFP + H_2O			DFP + C_{434}			Statistical Comparison		
	$\bar{X}$ (1)	SD	N	$\bar{X}$	SD	N	t	df.	Sig.
1	33.9	8.9	15	31.3	6.2	14	0.890	27	NS (2)
3	44.0	7.1	21	40.9	9.7	14	1.038	33	NS
6	50.0	6.4	30	53.6	10.6	10	1.249	38	NS
9	54.6	8.3	10	57.6	9.3	27	0.874	35	NS

(1) $\bar{X}$ = Mean % of normal ChE activity.

(2) NS = Not significant.

(Note: 'Significance' in the text will refer to the 5% level or better. In the tables differences which reach the 10% level will be shown, although these are not taken as significant. All differences which fail to reach the 10% level are labelled NS).

The ChE activity of whole brain in animals chronically treated with DFP remained approximately constant at about 30% of normal when measured on the second day after injection. A straight line was fitted to the data. The fit was significant ($t = 2.117$, df. = 57, $P < 0.05$.) The equation of the best fit line shown in Figure 1

was Y = 33.699 + (-0.141) X, where Y = % of normal ChE, and X = days after initial injection. Animals killed on day 10 - that is, 24 hours after injection - had a mean ChE activity of 15.8% of normal (N = 7, S.D. = 3.3).

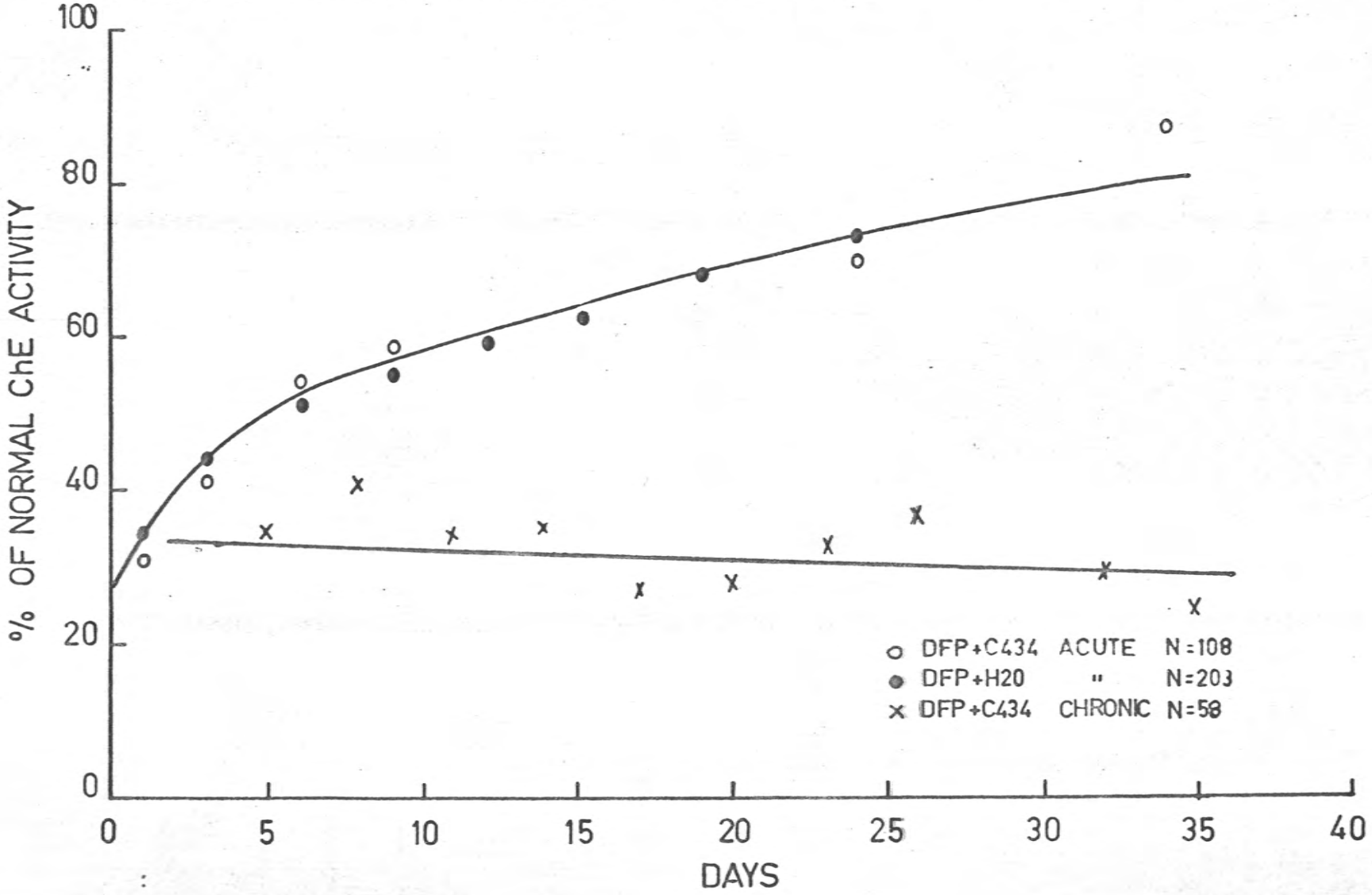
There was no effect of the reactivator on brain ChE in the chronic condition as shown by assays made seventeen days after the initial injection - that is, 2 days after the last 'booster' injection, (Table 2).

TABLE 2. Cholinesterase activity of brain after chronic treatment with DFP + H_2O and DFP + C_{434}.

Days after initial injection	DFP + H_2O			DFP + C_{434}			Statistical Comparison		
	$\bar{X}$ (1)	SD	N	$\bar{X}$	SD	N	t	df.	Sig.
17	28.6	3.9	6	28.4	2.7	6	0.0632	10	NS

(1) $\bar{X}$ = mean % of normal ChE activity.

FIGURE 1 TREATMENT WITH DFP AND BRAIN CHOLINESTERASE ACTIVITY



The effects of chronic treatment with DFP + C_{434} on nine separate areas of the brain were generally similar to the effects on whole - brain. In all cases ChE activity was maintained at an approximately constant level for up to 74 days. However, cortex, basal ganglia, hippocampus and thalamus tended to have lower values than colliculi, cerebellum, medulla, hypothalamus and spinal cord, (Table 3).

TABLE 3. Chronic treatment with DFP and ChE activity in various brain areas

BRAIN AREA		Days of Treatment														
		5	8	10	14	17	19	20	21	22	26	28	35	43	62	74
CORTEX 251*	Mean	27.8	21.2	20.3	26.6	17.5	26.2	26.5	21.8	15.1	24.7	32.9	25.9	19.4	21.4	18.0
	S.D.	9.3	9.1	7.0	6.1	7.8	3.6	0.0	4.3	1.1	0.0	14.7	5.8	3.9	8.3	3.5
	N.	6	5	9	10	10	7	1	5	4	1	7	9	3	5	4
BASAL GANGLIA 2117*	Mean	24.4	24.0	15.7	24.1	18.8	26.7	20.3	23.7	11.6	16.9	23.6	21.5	19.5	16.6	18.9
	S.D.	4.1	3.4	4.8	4.2	6.1	3.7	0.0	3.9	3.4	0.0	4.5	4.6	24.0	5.6	5.8
	N	6	5	9	10	10	7	1	5	4	1	7	8	3	5	4
HIPPO-CAMPUS 348*	Mean	27.9	24.0	14.4	22.1	17.9	28.7	18.6	24.9	13.8	18.1	27.0	28.5	20.0	20.8	28.6
	S.D.	5.2	3.4	4.1	8.1	7.1	3.1	0.0	8.3	3.0	0.0	12.6	6.2	3.6	6.2	11.6
	N.	6	5	8	11	8	5	1	4	4	1	7	7	3	4	4
THAL-AMUS 617*	Mean	29.1	26.7	16.5	28.2	18.6	26.3	24.7	25.3	11.3	31.2	23.8	25.5	20.7	20.0	27.7
	S.D.	4.7	4.7	3.4	4.1	7.5	3.4	0.0	3.0	1.7	0.0	10.0	11.6	5.1	5.5	4.4
	N.	5	4	10	11	9	6	1	4	4	1	4	4	3	4	3

TABLE 3 (Contd.)

BRAIN AREA		Days of Treatment														
		5	8	10	14	17	19	20	21	22	26	28	35	43	62	74
COLLI-CULI 583*	Mean	29.1	32.2	17.7	35.6	27.9	35.3	27.1	30.7	15.8	33.5	36.2	35.2	35.6	25.7	37.6
	S.D.	5.6	8.2	4.4	7.8	6.4	5.4	0.0	6.0	3.3	0.0	8.5	6.6	5.1	8.2	17.6
	N.	5	5	9	11	10	7	1	4	4	1	6	9	3	5	4
CERE-BELLUM 191*	Mean	51.5	44.0	21.4	52.3	32.5	59.3	49.3	45.2	25.7	40.7	46.2	46.5	43.1	42.1	40.3
	S.D.	21.4	4.4	6.7	7.2	8.9	12.8	0.0	4.8	1.9	0.0	18.1	9.3	5.5	5.9	7.0
	N.	6	5	9	11	10	7	1	5	4	1	6	8	3	5	4
MED-ULLA 440*	Mean	35.6	38.3	29.4	48.1	40.3	49.7	31.8	40.9	27.2	36.2	48.9	39.5	40.1	34.1	34.6
	S.D.	5.1	4.4	6.7	8.4	8.1	10.7	0.0	7.0	8.3	0.0	10.5	8.5	3.2	3.2	3.5
	N.	6	5	8	11	10	6	1	5	4	1	7	9	3	5	4
HYPO-THAL-AMUS 359*	Mean	52.0	46.4	28.9	40.5	46.5	57.2	37.5	32.6	29.8	40.5	59.0	35.1	38.0	34.8	-
	S.D.	4.0	1.8	11.2	5.0	5.8	12.4	0.0	0.0	3.1	0.0	16.9	5.1	6.8	11.7	-
	N.	2	2	5	8	4	2	1	1	4	1	2	3	2	2	-
CORD 367*	Mean	32.3	30.1	21.0	39.4	29.7	45.6	27.5	27.7	18.3	23.5	40.3	34.4	40.2	34.5	21.4
	S.D.	7.5	3.1	7.8	0.55	3.9	2.3	0.0	6.1	6.3	0.0	9.4	9.0	4.0	11.5	2.0
	N.	6	5	9	10	8	6	1	4	4	1	5	8	2	3	3

* Normal AChE activity in $\mu l.\ CO_2$ per 100 mg. per 30 min.

Discussion.

Recovery of ChE activity after an acute injection of DFP is rapid for the first five days and slows down thereafter. The rate varies among the nine sub-regions of the brain used in this experiment. It is slowest in the cortex, basal ganglia, hippocampus and thalamus; fastest in the cerebellum; and intermediate between these 2 in the colliculi, medulla, hypothalamus and spinal cord. (Glow, Rose & Richardson, 1966). The chronic injection schedule maintained ChE at levels below those found by Russell et al (1961) to be necessary to produce behavioural effects.

The lack of any reactivation of brain ChE by C_{434} is consistent with the published results of Hobbiger & Sadler (1959) and Fleisher et al (1960 b.) Although C_{434} reactivates inhibited ChE of gastrocnemius muscle in the acute condition, it does not do so in the chronic case. (Glow, Rose and Richardson, 1966). However, this does not necessarily mean it is ineffective in the chronic case, since Fleisher et al (1960 b.) found that it reversed DFP produced neuromuscular block before

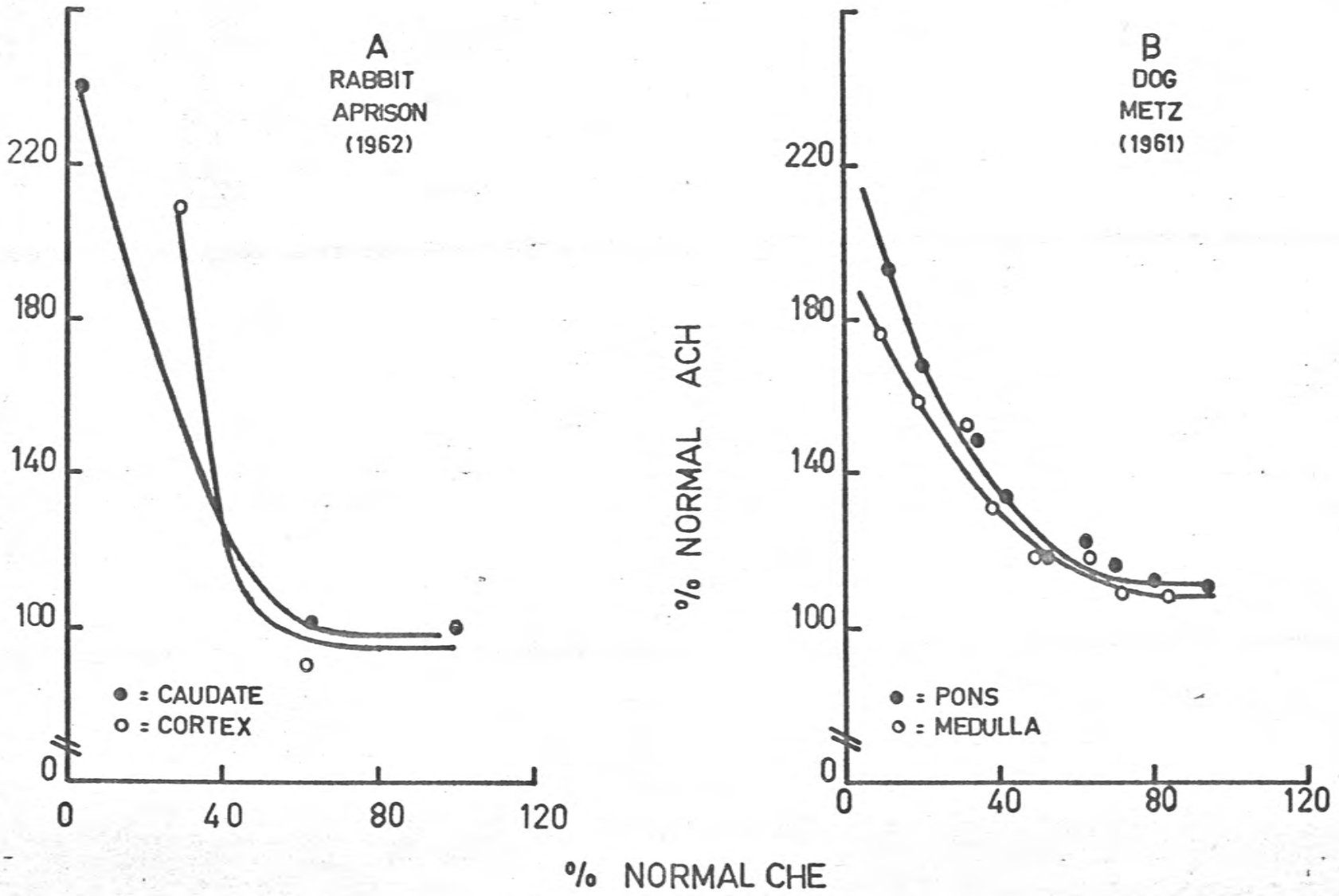
any increase in ChE activity could be found.

Russell, Watson & Frankenheuser (1961), Banks (1957) and White (1956), all found that reductions of ChE to 40% or less of normal were necessary to produce effects on behaviour. The injection procedures described above reduce ChE to as low as 15% of normal 24 hours after injection.

The level of 40% is approximately the degree of reduction necessary to produce large increases in brain ACh, as shown in Figure 2. (Both sets of data in Figure 2 are for assays made within one hour after injection of the inhibitor. In A, DFP was injected into the right carotid artery, and in B, T.E.P.P. was injected intraventricularly. The data in B was calculated from graphs showing ACh and ChE levels in relation to a respiratory reflex. The original observations were grouped to give average ACh - ChE relations.) Increased ACh in rat brain after organophosphorus anticholinesterases has been reported by Du Bois et al (1949) and Stewart (1952).

Whether the same relationship between ACh and ChE exists when ChE is chronically reduced is uncertain. When brain ChE of rabbits is reduced by DFP the initial increase in ACh is followed by a return to normal values, even though ChE remains as low as 20% of normal, Michaelis et al (1954). It is, therefore, possible that the relation shown in Figure 2 may only apply immediately after injection of the inhibitor.

FIGURE 2 RELATION OF ACH TO CHE LEVELS IN THE BRAIN



PART TWO.

REVIEW OF LITERATURE ON CHOLINESTERASE ACTIVITY IN RELATION TO BEHAVIOUR

1. NATURALLY OCCURRING DIFFERENCES IN BEHAVIOUR AND CORRELATED DIFFERENCES IN CHOLINESTERASE ACTIVITY.

2. EXPERIMENTAL MANIPULATIONS OF CHOLINESTERASE ACTIVITY AND BEHAVIOUR.

1. NATURALLY OCCURRING DIFFERENCES IN BEHAVIOUR AND CORRELATED DIFFERENCES IN CHOLINESTERASE ACTIVITY.

1. Performance on the Krech Hypothesis Apparatus and Cortical ChE.

The general method of correlating individual differences in behaviour with individual differences in brain chemistry has been extensively used for the rat by the Krech, Rosenzweig and Bennett group of the University of California. In assessing their results it should be remembered that any ChE activity values given are based on assays of tissue homogenates, and could therefore reflect morphological differences, rather than differences in the amount of ChE present at functional sites. Although this does not decrease the interest of any correlation obtained between ChE and behaviour, it does mean that such a correlation could be irrelevant to the general problem of the functioning of the ACh - S and its relation to behaviour.

The main behavioural technique used has been the Krech Hypothesis Apparatus (K.H.A.),

although some work, to be discussed later, has been done with standard maze learning techniques. The K.H.A. is a linear four unit maze, presenting a sequence of four choice points to the rat. At each choice point one alley is lighted, and one dark. One alley leads into the next unit of the maze, the other is blocked by a door. Curtains prevent the animal from seeing which alley is blocked. In the normal form of the apparatus neither light nor position is consistently associated with the open door. However, the behaviour of the animal in this situation is not random. The rat typically responds selectively to one cue; this selective 'testing' of one cue is termed a 'hypothesis'.

There are eight possible hypotheses :- Light, Dark, Light Perseverative (i.e. choosing a lighted alley when the immediately preceding response to light had been correct); Dark Perseverative, Left, Right, Left Perseverative and Right Perseverative. For naive animals, illumination tends to be the dominant cue; that is initially such animals will choose the lighted alley although they may change to a position hypothesis later;

Rosenzweig, Krech and Bennett (1958).

All animals are given ten days pre-training on a straight runway and at the same time are adapted to a feeding schedule. On test days 1 and 2, 6 trials are given. These 12 trials are regarded as one day in the data analysis. On days 3, 4, 5, 6 and 7, 12 trials are given. Each trial involves 4 choices. The sequence of lighted and dark alleys is balanced so that in the 12 trials any one cue is associated with the open door only 50% of the time. The same balanced pattern is repeated each day.

On each test day a measure of spatial - visual preference is calculated by determining for each of the spatial hypotheses and each of the visual hypotheses the percentage deviation from 50% - e.g., if over the 12 trials of one day an animal showed 80% choices of the lighted alley, then the light hypothesis would score + 30. These percentage deviations are then summed for the four spatial hypotheses and the four visual hypotheses. The difference between these two sums is the spatial - visual preference score.

Rosenzweig, Krech and Bennett (1958) have

reported that over days 1 - 3, odd/even trial correlations for total dark or total light choices were all equal to or above + 0.80. For the derived spatial - visual preference scores the odd/even trial correlation was + 0.74. This appears to indicate that both the animal's choice of light versus dark and its use of spatial or visual hypotheses are reliable measures. However, this may be partly due to the fact that initially all animals show illumination preferences. As training progresses the stability of the measures could decrease.

The results obtained will now be briefly reviewed.

The general basis of all the experiments reported has been that there is a relationship between functioning of the ACh-S and behaviour. Specifically, it has been held that the amount of ACh available at an animal's cortical synapses is directly related to its behaviour. ACh is extremely difficult to assay whereas ChE is not. It was assumed that the amount of ACh and ChE covary, and that ChE could be used as an index to the amount of ACh. Krech, Rosenzweig, Bennett and Krueckel (1954) found that

rats showing high spatial preference scores on the K.H.A. had higher ChE activity in visual, somaesthetic and motor cortex than animals with visual preferences. This was contrary to the group's initial expectations which were that spatial animals should have high ChE in the somaesthetic cortex, and visual animals high ChE in the visual cortex - Rosenzweig, Krech and Bennett (1960). The original theory was thus one relating ChE (and hence ACh) in a particular sensory area with a particular sensory preference. Due to the contrary evidence of this first experiment it was revised in the following way. The Spatial - Visual Preference Score was taken to be a measure of the adaptability of behaviour rather than simply a measure of sensory preference. It was argued that to obtain a spatial preference score an animal had to be able to ignore the dominant visual cue (initially most animals show visual preference scores). This ability to ignore the dominant cue was taken to indicate a higher level of adaptive behaviour. It therefore became possible to account for the results by arguing that higher overall ChE meant higher overall ACh, hence higher overall cortical synaptic efficiency, and hence a spatial

preference score - that is, more adaptive behaviour.

This change in definition of what the behavioural technique actually measures was made entirely on non-empirical grounds. In effect, it states that rate of adaptation to an unrewarded stimulus is directly related to learning ability and other measures of behavioural adaptability. This is not an unreasonable assumption but, nevertheless, it is not one which can be taken for granted, since the behaviour of an animal at an unrewarded light/dark choice point is complicated by several factors - e.g., alternation tendencies, position habits, exploratory behaviour, adaptation to motivational effects of light and dark. It could as easily be argued that very 'fearful' rats avoided the lighted alleys and that the results show a correlation between ChE and fearfulness. Further, it could be argued that the state of cortical arousal of the 'fearful' animal is continuously greater than that of normal animals, which produces (by enzyme induction) the higher level of ChE.

There is no point in continuing with further such speculative 'explanations'. They only serve to show that if performance on some test is to

be taken as an index of 'adaptiveness' then some effort should be made to demonstrate that the test does in fact measure the same kind of thing as established measures of adaptive behaviour. This could have been accomplished by correlating K.H.A. scores with scores on maze learning, extinction, avoidance learning etc.

More data on the K.H.A. was reported by Rosenzweig, Krech and Bennett (1958). In this experiment, chemical analysis was made at varying periods after training, and it was found that spatial and visual animals did not differ in enzyme activity until later than 110 days. Three possible reasons were given for this.

(1) ChE may not be an adequate index of ACh metabolism before 150 days.

(2) Since their assay did not distinguish acetylcholinesterase and other esterases it may be that the latter were more abundant in younger animals; therefore uniformly high, false estimates of ChE were obtained.

(3) The test may raise the ChE level of both groups to a common ceiling.

The first explanation is completely

arbitrary but capable of test. The second explains the fall in ChE with age but not the differential fall which is after all, the important question. The third is also arbitrary but testable. A fourth possibility not given by the authors is that since at the time of testing, there was no difference between spatial and visual animals, ChE (and hence ACh) is not a determinant of their different kinds of behaviour.

Performance on a modified version of the K.H.A. was reported by Krech, Rosenzweig and Bennett (1956). Two groups of animals were run. Group 1 had the lighted alley made progressively correct over the 7 days of testing. (As described earlier, in the normal form of the apparatus no cue is consistently associated with reward.) Group 2 had the left-hand alley made progressively more often correct. After chemical assay animals in each group were divided into high ChE and low ChE sub-groups. Animals in Group 1 with high ChE values made less choices of the lighted alley than animals with low ChE. The difference between the two groups in total number of responses to the lighted alley over the 5 days

of testing was statistically significant. For all animals the correlation of preference score for the specified cue, i.e., light, with ChE was - 0.62. This correlation was significant and held within the 2 strains of rats used, being - 0.84 for the S_1 and - 0.35 for the S_{13}; Rosenzweig, Krech and Bennett (1958).

These results were interpreted as showing that high ChE animals were more adaptive than low ChE animals in the sense that they can behave more readily in a manner which reflects environmental probabilities of reinforcement. Low ChE animals on the other hand, were more committed to the dominant stimulus; this led them to choose the lighted alley more frequently than was justified by the actual probabilities of reinforcement.

However, it is possible to interpret the difference between High and Low ChE Groups as simply one of sensory preference - not necessarily indicating any difference in 'adaptiveness' of behaviour - since there was no difference in the rates of adaptation to the situation. The difference in numher of choices to the lighted alley was present on day 1 and the two curves remained parallel to day 7.

A further point related to the meaning of the measures obtained from the K.H.A. is that in experiments with humans on probability matching, it is considered more 'adaptive' to choose the cue of greatest frequency, rather than to attempt to match the environmental distribution. This maximizes the number of 'hits' or correct choices. On this reasoning the low ChE animals which tended to choose the most frequently correct cue, i.e., light, most of the time, are more adaptive than the high ChE animals which choose the lighted alley less often.

In Group 2, total left-hand alley choices over 5 days was not different for the high and low ChE groups. The difference in slopes was statistically significant, and the correlation between individual slopes and ChE was - 0.50. On day 1 there was a correlation of + 0.45 between left choices and ChE. Prior to the crossing of the curves at days 4 - 5 the correlation of spatial preference score and ChE was + 0.44. After the crossover point the correlation was 0.00. (The within strains correlation for Spatial Preference/ChE before the crossover was + 0.34 for the S_1; + 0.43 for the S_3, Rosenzweig, Krech and Bennett (1958)).

From these results it was inferred that low ChE determines low initial left alley choices, but a more rapid assumption of dominance by the left-hand side cue. Low ChE animals are less able to tolerate ambiguity in the environment, while high ChE animals stay close to the actual probabilities of reinforcement, which is defined as the more adaptive type of behaviour. Since the low ChE animals begin with predominantly visual choices and later change to predominantly L.H.S. (spatial) choices the data from this group was assumed to refute the sensory preference explanation of the group differences and demonstrate that they are due to a general factor of 'attachment' to dominant stimulus rather than 'attachment' to specific stimulus.

These interpretations are founded on little evidence. Firstly, the difference between the slopes of the two groups was due mainly to the differences on the first day, after which the slopes differ very little. (The correlation of ChE and preference score was + 0.44 for the first two days and 0.00 for the last three.) If the difference on day 1 is interpreted as being simply a preference for one stimulus mode it could be asked why, in Group 1, the curves remain apart. If the visual animals

of Group 2 rapidly catch up to the spatial when L.H.S. is the correct cue, one might expect the spatial animals of Group 1 to rapidly catch up to the visual animals when Light is the correct cue. Such an expectation makes some implicit assumptions; firstly, that all sensory preferences are equal in strength (that is, it is no more difficult to extinguish a light preference than it is to extinguish a position habit) and secondly, that there is no difference in rate of acquisition of spatial and visual habits on the K.H.A.

The first assumption is probably justified on the data presented since the slope of the curve for visual preference (Low ChE) animals in Group 2 (left correct) is no different to the slope of the spatial animals (High ChE) in Group 1 (light correct).

The second assumption is not supported by the data. The visual (Low ChE) animals of Group 1 show a faster rate of increase in choices to the positive cue than the spatial (High ChE) animals of Group 2, where position becomes the positive cue. This difference between the rates of acquisition of spatial and position habits can be

given a speculative explanation, which fits the sensory preference theory, in the following way:-

(1) An animal with a natural visual preference finds the visual cue rewarded and tends to increase its visual choices rapidly.

(2) Similarly an animal with a natural spatial preference, on finding its preferred position choice rewarded, will continue to make position choices at an increasing rate. However, the position preference can be for either R.H.S. or L.H.S. Therefore, for some animals (those with R.H.S. position habits) reinforcement will not act to immediately strengthen an already strong response. In contrast, animals with visual preferences all tend to choose the lighted alley which is the cue to be reinforced. It is therefore to be expected that the Group 1 low ChE animals (visual) will show a faster rate of acquisition than the Group 2 high ChE (spatial) animals.

Since individual data was not presented, no check is possible on these speculations. However, the data obtained from the solvable K.H.A. can clearly be interpreted in other ways than those proposed by Krech, Rosenzweig and Bennett. In

particular, the possibility is not ruled out that the difference between high and low ChE groups was one of position versus light - going habits rather than a general factor of adaptiveness.

Further data on the K.H.A. was reported by Rosenzweig, Krech and Bennett (1958). 103 animals of three different strains were run on the K.H.A. and the results analyzed for the whole group, and for the three strains individually. The grouped data showed the same trends as before. Animals with spatial preferences had the same cortical ChE levels as visual animals immediately after testing but declined much less rapidly thereafter. The relationship also held within 3 different strains (S_1, S_3, S_{13}). Correlations of ChE with spatial preference score were S_1 : + 0.45, S_3 : + 0.22, S_{13}: - 0.04. The average of the three within strains correlations was + 0.19 using Fisher's R - Z transformation. However, the absolute ChE levels for the three groups differed. Visual S_1's had higher cortical ChE than spatial animals of the other two strains.

The S_1's tended to be both light-going and left-going. The S_3's were strongly light-going

and moderately left-going. The S_3's also showed more hypotheses and maintained them for longer than did the other strains.

This paper introduced no new interpretations of either the biochemical or the behavioural data. It was concluded that the S_3 animals which had lowest cholinesterase were more stereotyped, and less adaptive than the other strains. The within strains correlation of preference and ChE is important since it goes some way towards answering the criticism that the association of ChE and behaviour is an irrelevant strain difference.

A 'problemless' version of the K.H.A. was used by Pierce (1959). In this apparatus the alley blocking doors were all removed which meant that any choice was correct. Two strains of animals were run on this test and their behavioural scores correlated with brain ChE measurements.

The S_3 (low ChE) animals showed greater visual preferences than the S_{13}'s over a 5 day period, although initially both strains showed visual preferences. This supports the earlier data. The negative correlation of ChE with spatial preference is opposed to the positive correlations

obtained by the Krech, Rosenzweig and Bennett group. This result could perhaps be rationalized in accordance with the general position of Krech, Rosenzweig and Bennett, as follows.

If, as suggested earlier, the K.H.A. is thought to provide a measure of habituation to a stimulus, that is, it is an extinction situation; then the 'problemless' version can be seen as an acquisition situation. All animals have initial visual preferences. High ChE animals being more adaptive will acquire stronger habit strength for the light-going response than the low ChE animals, therefore, negative correlations are obtained. In the problem solving (extinction) K.H.A. the high ChE animals extinguish their light-going response more quickly and obtain spatial scores - hence the positive correlation.

(2) Performance on maze learning tests and cortical ChE.

All the data presented in this section is from Rosenzweig, Krech and Bennett (1960).

Animals of S_1 strain did better than those of the S_3 when tested on the Hebb-Williams, the Dashiell and the Lashley 111 mazes. As has been noted before, the S_1 animals have high ChE and show spatial hypotheses; the S_3 have lower cortical ChE and show visual hypotheses. These results therefore agree with the data and theory of previous experiments.

However, crossed S_1/S_3 animals showed negative correlations between ChE level and learning ability on the same three tasks. Descendants of an earlier S_1/S_3 cross (the S_{13} strain) were also found to show negative correlations between learning and ChE.

Some evidence of the same kind was obtained from two paired strains bred for high and low cortical ChE, the Roderick - Castle and Roderick - Dempster high and low strains (RCH/RCL and RDH/RDL). The RCH animals performed worse than their RCL counterparts on the Dashiell and

Lashley 111 mazes, but there was no significant difference on the Hebb- Williams. The Hebb-Williams was the only task which at all differentiated the two RD strains, but the comparison reached only the 10% level of significance.

These negative relationships between learning behaviour and cortical ChE caused Rosenzweig et al to make a major theoretical change. It was argued that ACh and ChE were under separate genetic control, and hence ChE could not be taken as an index of ACh available at synapses. The amount of ACh available would be jointly determined by the level of ChE and the level of ACh. The actual levels of ACh and ChE for the three pairs of strains (S_1/S_3, RCH/RCL and RDH/RDL) were determined, and the strain with the greater ratio of ACh to ChE was in fact found to be the strain with the greater learning ability.

However, this leaves unexplained the differences within strains on different tests of adaptiveness. It is possible that differences in task difficulty could lead to zero relationships at one end of the scale, and positive relationships at the other end; but this could not explain the positive, zero and negative correlations of ChE and the

author's criterion of adaptive behaviour, for the S_{13} strain, on the unsolvable K.H.A., progressively solvable K.H.A., and maze learning respectively. Nor could it explain why the RCH/RCL strains showed no behavioural difference on the Hebb-Williams, while this was the only technique which did differentiate the RDH/RDL strains. In addition, the difference between ACh/ChE ratios was greatest for the two RD strains, while these were the strains that showed the least behavioural difference (the one difference, that on the Hebb-Williams, was significant at only the 10% level.)

The data from the RD strains could in fact be used as evidence contrary to the hypothesis advanced by the authors.

Finally, the theory has consistently specified a relation between cortical ACh and behaviour. All the data on ACh/ChE ratios was derived from whole brain samples, and must therefore, for ChE at least, depend mainly on subcortical structures since the bulk of the ChE activity is in the thalamus, colliculi and basal ganglia.

(3) Summary and conclusions.

The results can be summarized as follows:-

(1) There is a strain difference (S_1 - S_3) in hypothesis behaviour, which is correlated with a difference in cortical ChE. This correlation also holds <u>within</u> strains.

(2) There is similarly a difference in maze learning behaviour of the S_1 and S_3 strains. Two strains bred for high and low ChE (RCH - RCL) showed differences in behaviour. Two other strains bred for the same traits (RDH - RDL) showed little or no difference in behaviour. No within strain relationships have been reported for the maze learning data.

Although, in several places the Krech, Rosenzweig and Bennett group have emphasized that they have only shown a correlation between ChE and behaviour, the underlying rationale of their experiments has always been that there is a <u>causal</u> relationship. Some inconsistencies in the claimed correlation between ChE and behaviour have already been pointed out. In addition to these, however, the fact that different strains of animals have been

used makes the demonstration of a causal relationship even more difficult. Selected strains could differ in characteristics which determined both behaviour and ChE. One such possibility - that ChE is merely a reflection of general protein metabolism - has been discussed by Bennett et al (1961) and discounted, since strains which differ in ChE do not differ in brain per cent protein.

On the behavioural side, it has been shown that little attention has been paid to whether different measures of adaptive behaviour do in fact measure the same thing. There has been no attempt to be more analytic as regards the behavioural measures and demonstrate a correlation with rate of acquisition of response, rate of extinction of response, etc.

The relationship of ChE and behaviour has been discussed in only the most general terms. Although it has been admitted that ChE differences may simply reflect differences in cell density, no attempt has been made to confirm or to reject this objection. Also, there has been no development of the idea that differences of

duration of transmitter action due to differences in ChE should affect behaviour. The physiological side of the theory has stayed at a fairly speculative level with little attempt to relate it to known relations of ACh and ChE at synapses.

The most important evidence against the differences in ChE and ACh observed by the Krech, Rosenzweig group being important determinants of behaviour is that obtained by the use of inhibitors of ChE. Reductions in whole brain ChE of 60% are needed before behavioural differences can be observed, Russell et al (1961). This is far in excess of the ChE differences found by Krech, Rosenzweig and Bennett.

To conclude, it appears that :-

(1) A general correlation of ACh/ChE with behaviour has not been established.

(2) The correlations which have been obtained do not, in any case, provide evidence of a causal relationship.

2. EXPERIMENTAL MANIPULATIONS OF ChE ACTIVITY AND BEHAVIOUR.

In the following section the studies reviewed will be grouped, as far as possible, according to the behavioural technique employed. It should be noted from the outset that type of task is not all that varied. Different species, different kinds of inhibitor, and different modes of administration have been used. Therefore, in most cases, comparison of different experiments is necessarily highly speculative.

(1) General Responsiveness.

Changes in general responsiveness to stimulation as a result of decreased levels of ChE have been reported. Metz (1958) found a linear relationship in dogs between percentage change in a respiratory reflex response to electrical stimulation, and percentage decrease in ChE in the parts of the medulla concerned with respiration. ChE was inhibited by injection of TEPP into the fourth ventricle. Facilitation of the reflex was greatest for a 20%

reduction in ChE, but still occurred down to a reduction of approximately 60%. Further decreases in ChE below this level actively inhibited the reflex. Metz attributed the facilitation to excitation by the extra ACh which accumulates at synapses when there is less ChE with which to break it down. This effect is most pronounced for a small decrease in ChE. As the ChE activity is decreased more and more, allowing progressively more ACh to accumulate, the degree of facilitation decreases also. When 40%, or less, of the original ChE remains, the accumulated ACh tends to actively block transmission and the reflex is inhibited. Assays of free ACh levels supported this interpretation. As ChE activity decreased free ACh levels increased, and the increase became most apparent at that point where the reflex was first inhibited, Metz (1961). A similar relation between free ACh and the respiratory reflex was obtained when the synthesis of ACh was inhibited by a hemicholinium compound, Metz (1962).

James and Ginsburg (1948) have studied the effects of inhibiting ChE upon conditioning in dogs. In a shock-avoidance conditioning situation there were marked individual differences in behaviour.

Some subjects, which the authors called "inhibited", were very relaxed and generally lethargic in the conditioning harness. They showed little responsiveness to shock and their avoidance responses were both poorly sustained, and unaccompanied by marked changes in heart rate or respiration. This was in contrast to "excited" subjects which were very responsive to shock, and did show changes in heart-rate etc. Normally, these patterns of behaviour did not change; they were persistently characteristic of a given animal. However, it was found that inhibition of ChE by prostigmine methyl sulphate could change an "inhibited" type into an "excited" one. It is possible to interpret this result in the same way as those of Metz described previously. A decreased level of ChE may allow more ACh to accumulate at post-synaptic membranes, producing increased response to stimulation. However, since prostigmine methyl sulphate has a quaternary ammonium ion it is unlikely to pass the blood-brain barrier, except in very small amounts. Its effects were therefore probably due to interference with peripheral mechanisms.

(2) Motor behaviour.

Harvey et al (1947) have reported that intra-arterial injection of DFP caused a decrease in the grip strength of human subjects. Grob and Harvey (1949) have reported the same effect when TEPP was used as the inhibitor. This effect may be counteracted by the use of reactivators of phosphorylated ChE, Grob and Johns (1958). Similar changes in muscle strength probably occur in all muscular systems since ACh is the transmitter at all motor end-plates.

A marked alteration of motor behaviour in the rabbit due to ChE inhibition was demonstrated by Aprison, Nathan and Himwich (1954). Injection of DFP into the right common carotid artery resulted in "forced circling". However, the circling did not appear consistently; although the usual result was circling to the left, some circled right, and some showed no circling at all. In those animals that did circle there was always an asymmetrical reduction of ChE on the two sides of the brain, whereas in those that did not circle there was only a very slight inhibition of ChE, and no detectable asymmetry. Animals that circle to the right have higher ACh

concentrations in the left than in the right cortex, whereas those that circle left have higher ACh in the right. Furthermore, the rate of turning is correlated with the difference between the two sides, Aprison (1958).

In a similar experiment on rabbits, forced circling was again produced, but no abnormal electrical activity could be detected in the caudate nucleus or thalamus which would account for the circling, Harwood (1954). The circling behaviour persisted for 4 hours after injection, and the animals were prepared for recording half an hour after injection. It is not stated how long this preparation took but it is possible that there were electrical changes which had diminished by the time recordings were made. On the other hand it could be that the caudate and thalamic regions from which recordings were taken were not involved in the production of circling. However, this possibility seems unlikely. The caudate nucleus was the brain area most affected by DFP (9 - 10% of normal on the injected side) and White (1956) has shown that if DFP is injected directly into the caudate, circling will occur when ChE activity is still 20-40% of normal. In

White's experiment reductions in ChE to 40% of normal produced no circling, and at 10-15% of normal the circling behaviour became only transient.

Since spread of the DFP from the site of injection was small the caudate is definitely a potential factor in the production of circling. However, it is likely that asymmetrical stimulation or inhibition of any part of the motor pathway could contribute to circling behaviour.

Forced circling also occurs as a result of intracarotid injection of eserine in guinea pigs, and can be inhibited by atropine and scopolamine, de Jonge and Funcke (1962).

(3) Instrumental Reward Conditioning.

In an experiment on the Stone multiple unit 'T' maze, using rats as subjects, Platt and Wickens (1957) found that if the animals were injected with 2 mgm/kgm of DFP., allowed to recover for 10 weeks, and then trained, they performed no worse than controls. Similarly if animals were trained, injected, left for

10 weeks, and retested, then they retained no less of the maze habit than controls. In 10 weeks the ChE activity would have returned to normal. This experiment therefore shows that there are no irreversible, long-term effects of an acute reduction of ChE upon learning ability; nor does the reduction of ChE in any way destroy an already established memory trace.

Faster learning of the Lashley 111 Maze was reported by Stratton and Petrinovich (1963) as a result of post-trial injections of eserine (0.25 - 1.00 mgm/kgm). The degree of facilitation was not equivalent for two strains bred for rapid and slow maze learning respectively. In addition, at high dosages, maze learning was slowed by the eserine injections. The authors discuss their results in terms of a "neural consolidation" hypothesis. Some electrical 'trace' is assumed to remain in the brain after a correct trial, and to leave a permanent structural change if allowed to persist for long enough. Eserine would be expected to prolong the persistence of the 'trace' and thus accelerate learning. Evidence was presented that the effects of eserine on ChE would have ceased 24 hours later,

to counter the objection that the maze learning results were actually due to interference with the acquisition of information. Thiessen et al (1961) have suggested that post-trial facilitation may be an indirect effect of reduced locomotor activity. Any such reduction, they claim, should decrease the amount of post-trial interference. However, this explanation does not seem applicable to the experiment of Stratton and Petrinovich (1963) since the largest dose of eserine, which should have the largest effect on locomotor behaviour (see Part 6, Experiment 1.) actually had less facilitating effect on learning than the lower doses.

Eserine (0.3 mgm/kgm) has also been used in rats learning a water version of the Dashiell maze, Consalvi (1961). Some evidence was obtained that the performance of animals given eserine was inferior to that of controls, but the study is of little value since the learning period consisted only of a fixed block of 15 trials, all on one day, and no attempt was made to train the animals to a performance criterion. Any animal which swam about a lot would therefore have a high error score, regardless of its actual progress in learning the maze.

When trained at solving Hebb-Williams maze problems, rats in which ChE had been reduced by "Systox" to less than 40% of normal made more errors than controls, Banks (1957). As in the previous experiment, the animals were not run to a criterion of performance but simply given a fixed number of trials on different problems. It has been suggested that the Hebb-Williams maze, when used in this way, actually measures exploratory behaviour as much as problem solving ability, Woods, Ruckelshaus and Bowling (1960). Any natural differences between animals in motor activity, or any differences produced by reduced ChE activity would show up as high error scores. To add substance to this possibility, hyperexcitability and motor restlessness have actually been claimed to be a consequence of anticholinesterase poisoning, Goodman and Gilman (1955 p.460).

There has been a little work on the effects of reduced ChE upon discrimination. Platt (1951) found that when albino rats were injected with 1 mgm/kgm of DFP and trained on a black versus white discrimination, after delays of 19 and 119 days, they were not significantly different to controls. On the other hand, animals trained 42 days after injection

were better than controls. Platt interpreted this result as showing a differential effect of the three ChE levels. However, examination of the mean trials to criterion of the groups casts some doubt on this interpretation. The means for the DFP animals at days 19, 42 and 119 were 60, 42 and 45; and for the control animals 55, 59 and 39. The control 42 day group mean is equivalent to that for the two 19 day groups whereas the DFP group's mean is closer to those of the 119 day group.

There is a strong hint of a downward trend from day 19 to 119; for which the author advances increased age, handling and temperature changes as possible explanations. In view of this, the difference between the two 42 day groups could most parsimoniously be treated as a sampling effect.

The rate of learning by rats of a successive discrimination problem in which the cues were visual (black v. white) auditory, and tactile, was unaffected by 0.05 - 0.10 mgm/kgm of eserine injected before running, Whitehouse (1959).

Finally, Chow and John (1958) could find no effect of either intracerebral DFP or eserine, upon stable visual and spatial "hypotheses" in the

Krech Hypothesis Apparatus.

In a conventional Skinnerian situation, Glow and Rose (1965) showed that reduced ChE could produce resistance to the extinction of a conditioned lever pressing response. A single injection of DFP was used to inhibit ChE, which was allowed to resynthesize during the course of the experiment. However, it was found that if peripheral ChE was protected by a reactivator then this resistance to extinction did not occur. This was taken to show that the resistance to extinction found when both central and peripheral ChE were inhibited was in fact mainly a peripheral phenomenon.

Russell (1954, 1958, 1964) has also reported that chronic inhibition of ChE with "Systox" produced no significant effects on consummatory responses, locomotion, simple learning, operant conditioning and visual discrimination, nor any gross signs of peripheral sensory or motor inadequacy. However, these results are presented so briefly that it is impossible to judge their significance.

(4) Instrumental Avoidance Conditioning.

Russell, Watson and Frankenhauser (1961) found that chronic inhibition of ChE in rats by the organophosphate "Systox" did not affect the acquisition of a conditioned shock avoidance response, but significantly slowed down its extinction. They also found that there was a 'critical level' of ChE - no effect on behaviour being found unless brain ChE was less than 40% of normal. With respect to this study the following points should be noted:- (1) ChE was reduced throughout the body. The results could be due to peripheral factors. (2) Although the trend towards slower extinction in animals with low ChE was significant, the number of animals used was very low - a total of 24 divided into six groups of 4 each. In an experimental situation where performance is extremely homogeneous this would be unimportant, but avoidance conditioning is a situation which often produces wide variations between animals. In the authors' own data, two groups, in the acquisition phase of the experiment had ranges of 2 - 19, and 1 - 13, for the number of trials required to reach criterion. (3) The

criterion of extinction was three successive trials in which a response did not occur within 10 seconds after the CS. The response to be made was jumping onto a platform, and it was found that low ChE animals tended to 'oscillate' - that is, jump on and off the platform. In the discussion of the Hebb-Williams experiment of Banks (1957) it was noted that motor restlessness has been reported after anti-cholinesterase poisoning. It is possible that some such effect was responsible for the slower extinction of the reduced ChE animals, rather than any inability to acquire, or to utilize information from the environment.

There have been several reports of the effects of eserine upon performance of conditioned avoidance responses (C.A.R.). Pfeiffer and Jenny (1957) trained rats to climb a pole (CR) to a buzzer signal (CS). 0.25 mgm/kgm of eserine injected subcutaneously, inhibited the C.A.R. The effect of eserine could be abolished by atropine. Rats given neostigmine (s.c.) together with ACh (i-p) showed marked peripheral effects but no inhibition of the C.A.R. Neostigmine and ACh are both quaternary compounds and unlikely to enter the c.n.s.,

except in small amounts; the lack of any effect of this treatment therefore strongly suggests that the inhibition found after eserine was a central effect.

Similar results were obtained in cats by Funderburk and Case (1947). Eserine was found to abolish a conditioned leg flexion avoidance response, whereas neostigmine produced far greater peripheral symptoms but less effect on the C.A.R. In animals pretreated with atropine, eserine had no effect.

Eserine in doses of 0.16 mgm/kgm (i-p) had no significant effect on conditioned avoidance behaviour in rats, although there was an increase in the mean number of shocks taken, Goldberg et al (1964). In this study it was found that brain ChE 30 minutes after injection was 78% of normal; a degree of inhibition which would be expected to have little effect upon brain ACh. (See Part 1).

Bures et al (1962) reported that 1.0 mgm/kgm of eserine only partly affected the performance of an active C.A.R. but suppressed a one trial passive avoidance reaction at 0.2-1.0 mgm/kgm. When 4 trials were given on the passive avoidance

reaction, the eserine had no effect. Initial learning of the one trial passive avoidance reaction (P.A.R.) was also suppressed by eserine. Further data by Bures et al (1964) showed that consolidation of the memory trace, and later permanent memory in a P.A.R. were unaffected by eserine. The effect was only on the learning and retention phases.

The results of Experiment 1, Part 6, show that the above facts may not be what they seem. At the same dose levels and testing times, lever pressing rate in the Skinner box was greatly reduced. Since the P.A.R. measure was only the distribution of an animal's time between a small (shock) compartment and a larger one, it is possible that after injection the animal is so sick that it simply squats in the smallest, most enclosed space. This would explain the results on performance. The learning results could be quite plausibly attributed to a generally reduced environmental awareness due to the sickness that follows treatment.

(Although physostigmine did not alter the distribution of time between the two compartments in normal unshocked animals, this does not show that exploratory behaviour was actually unaffected. The measure of exploratory behaviour was the time spent in the small compartment in a 3 minute test session. In normal controls this time was 130 seconds - that is 72% of the total - leaving only 50 seconds in the large compartment. As the rat was placed in the large compartment at the beginning of the test, part of the 50 seconds must have been a latency measure. The distribution of times between the 2 compartments was therefore in effect so unequal, that any tendency of physostigmine to increase time spent in the small compartment would hardly be measurable.)

(5) Cholinesterase Activity and Human Behaviour.

The effects of changes in ChE activity on both normal and abnormal behaviour have been investigated.

Rowntree, Nevin and Wilson (1950) found that schizophrenics were more resistant to the E.E.G. changes produced by the inhibition of ChE than were

manic depressives or normals. Pope (1952) suggested that this was because of higher ChE levels in schizophrenics. Pope, Caveness and Livingstone (1952) did find evidence suggestive of a higher ChE level in schizophrenic than in non-psychotic patients, but the subjects were of different ages, different sexes, and had received different therapeutic treatments. No significant difference between the brain ChE levels of schizophrenics and other mental patients was reported by Takahashi and Ogushi (1953).

Intraventricular injection of ChE will relieve catatonic symptoms in cats, Sherwood, Ridley and Mc Culloch (1952), and similar injection of ChE will relieve such symptoms in human catatonic schizophrenics, Sherwood (1955). Conversely, inhibition of ChE by intraventricular injection of eserine or DFP will produce catatonic symptoms in cats, Feldberg and Sherwood (1954). These results suggest that schizophrenics may have too <u>low</u> a level of ChE. However, intraventricular injection is a method which circumvents the brain's natural barriers to noxious substances. As ChE, eserine, DFP etc. do not normally occur in the cerebro-spinal fluid (c.s.f.) in large quantities, the effects which they produce when

given intraventricularly may not be related to normal processes in the brain. For example, it is possible that the results are simply an outcome of changes in the osmotic pressure of c.s.f. Some weight is given to this kind of speculation by the fact that drugs differing in structure and pharmacological properties will produce many of the same symptoms, Sherwood (1955).

There have been several reports of poisoning with organophosphorus compounds in normal subjects, Grob (1963).

Intramuscular injection of DFP produced EEG changes which persisted for 1 - 5 weeks after injections were stopped, Grob et al (1947a). These changes were reported to be accompanied by dreaming, insomnia and emotional lability in Grob et al (1947b), but no normal data were given for comparison. The state of subjects following a single injection of an organophosphorus anti-cholinesterase has been described by Bowers (1962) as a moderate delirium, consisting of subjective tenseness, intellectual impairment and sleep disturbances. No intellectual changes are found unless there are also physical signs or symptoms of illness, Durnham, Wolfe and Quinby (1965).

After acute poisoning with organophosphorus compounds recovery is complete, Barnes (1961), Bidstrup (1961). This is in agreement with the experiment of Platt and Wickens (1957) in Section (3) Instrumental reward conditioning.

Some general comments can be made which concern many of the experiments reported above.

(1) Several studies have not included controls for the physiological location of any effects of the drugs used.

(2) Parallel to the above case, little attention has been paid to the location of effects in the behavioural sense - for example, on motivation, on stimulus reception, on mediating processes, or on response mechanisms. An example of this is the experiment, quoted above, of Pfeiffer & Jenny (1957) in which evidence was presented to show that inhibition of a C.A.R. caused by eserine was a central effect. This does not preclude the existence of a central effect directly upon the response measure (in this case latency) quite independently of any effect upon the animal's ability to connect the C.S. and C.R.

(3) Some tasks are more sensitive to "non-associational" effects, that is, motivation, response mechanisms, etc., than others. Data presented in Parts 4, 5 and 6 on DFP and eserine show that lever pressing in the Skinner box is one task sensitive to these drugs, and latency of response is probably another. It follows from this that not too much reliance should be placed on interpretations arising out of a single type of experiment, until they have been checked in other situations.

To summarize, the data reviewed show:-

(1) That there is an effect of reduced ChE on performance, especially for behaviour which is not highly overtrained.

(2) There is, as yet, little evidence that learning ability is affected in any way by reduced ChE, apart from the study of Stratton & Petrinovich (1963).

PART THREE

REDUCED CHOLINESTERASE ACTIVITY AND MOTIVATION

Since the behavioural experiments reported in Part 5 used hunger schedules and food rewards, it is important for a full interpretation of their results, to know whether or not food motivation itself was affected by inhibition of ChE. The following experiments were therefore performed to determine the effects of reduced ChE upon body weight, food intake and water intake.

Experiment 1. Effects of acute and chronic inhibition of cholinesterase activity upon body weight, food intake and water intake.

Method.

Fifty eight female Wistar rats were used. They were received at the age of sixty days and maintained on a free-feeding diet of rat cubes till the age of approximately 120 days. All were then recaged and adapted to a feeding schedule of two hours per day over a period of 5 days. They were kept on this feeding schedule for a further 15 days. Water was available at all times. In this and in

later stages of the experiment food was granulated to prevent hoarding.

Acute and chronic reductions of ChE were produced as described in Part 1.

Subjects were weighed to the nearest gram throughout the 20 day adaptation period. By the end of this time body weight had reached a stable value (about 150 gm.). Water and food intakes to the nearest gram were measured for the last ten days of the adaptation period. All food weights were corrected for spillage but this correction could not be made for water weights.

Feeding was at the same time each day. Body weights were taken one hour before feeding, and the water intake was measured immediately after feeding - that is, there was a 24 hour period from the end of one feeding period to the end of the next. At the conclusion of the 20 days' adaptation the subjects were injected, as shown below :-

Groups	(i.m.)		(i.p.)	N (Acute)	N (Chronic)
1	H_2O	+	H_2O	10	-
2	H_2O	+	C434	10	6
3	DFP	+	H_2O	10	6
4	DFP	+	C434	10	6

The control injections were of sterile pyrogen-free water equivalent in volume to the DFP and C434 injections. All injections were made three hours before the first post-injection body weight was taken; that is, four hours before the start of the feeding period, and six hours before the water intake was measured. The 'booster' injections in the three chronic groups were at the same time as the initial injection.

Results of both acute and chronic experiments were analyzed in the same way. The mean body weight, food intake and water intake for the five days immediately preceding the first injection were calculated for each animal. All post injection measures were then calculated for each animal as a percentage of these pre-injection means, summed for each treatment group, and analyzed by *t* test. The absolute values of body weight, food intake and water intake before injection were 146.8, 20.9 and 9.9 grams respectively.

Results.

1. Acute (Figure 1; Table 1).

There were no significant differences between the H_2O + H_2O and H_2O + C434 groups, except for Day 5, Water Intake, which was probably a chance fluctuation. Data is only presented for H_2O + C434 subjects. Three hours after injection neither DFP + H_2O nor DFP + C434 had produced any change in body weight. However, 24 hours after injection both experimental groups were significantly lighter than the controls. This difference was maintained throughout the 14 days by the DFP + H_2O group; whereas the DFP + C434 group did not differ significantly from the H_2O + C434 controls after day 8. The two experimental groups did not differ three hours to three days after injection, but did thereafter.

The food intake of both experimental groups was less than that of the controls up to days 3-5 but not later. There were no differences between the two experimental groups.

Water intake was also reduced on days 2-5 for the DFP + C434 group, and days 2-7 for the DFP + H_2O group. The 6 hour and 24 hour differences were not significant due to the drop in the H_2O + C434 group. This drop was probably artifactual, as it did not occur in the chronic study. There were no differences between the DFP + H_2O and DFP + C434 groups.

FIGURE 1 ACUTE TREATMENT WITH DFP

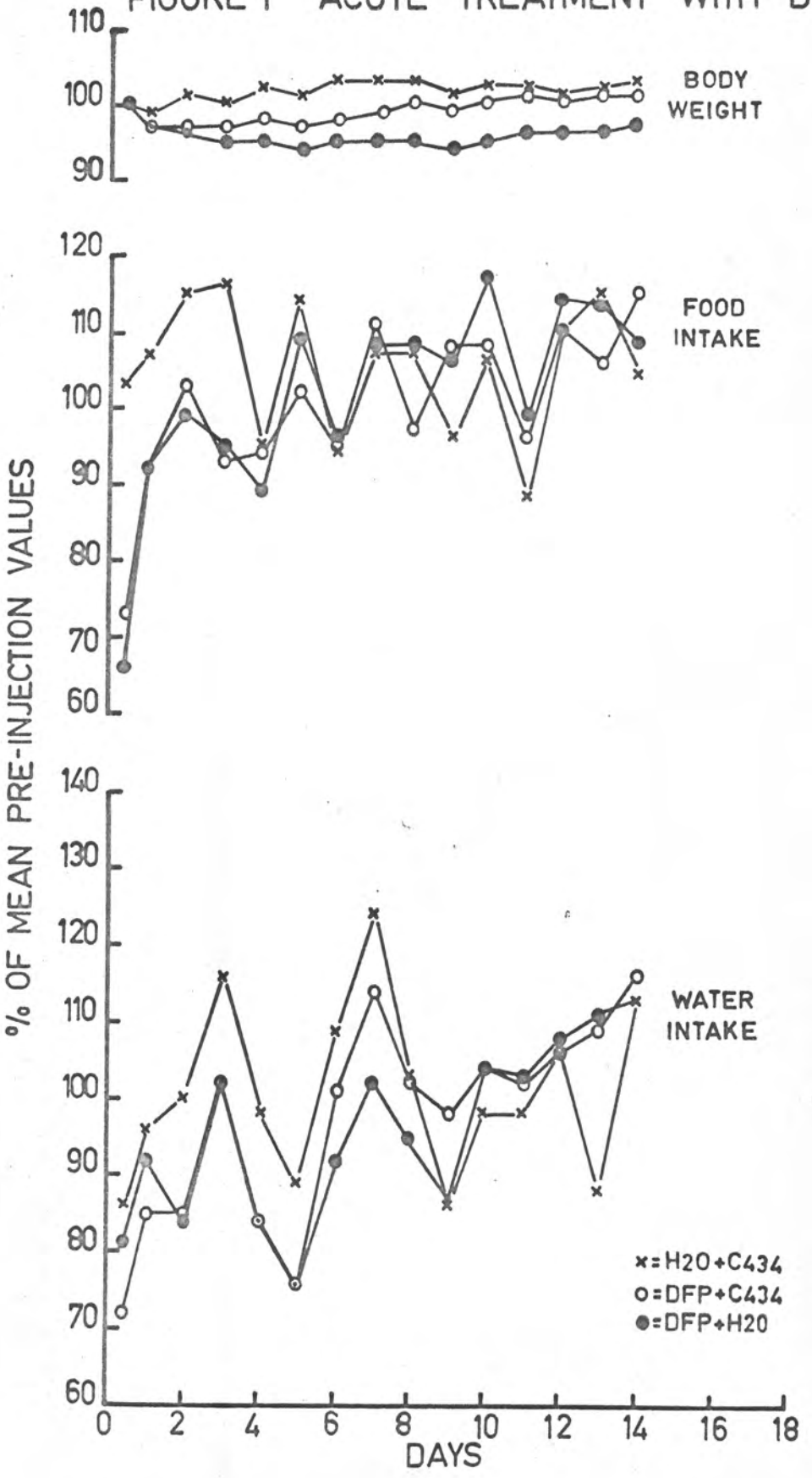


FIGURE 2 CHRONIC TREATMENT WITH DFP

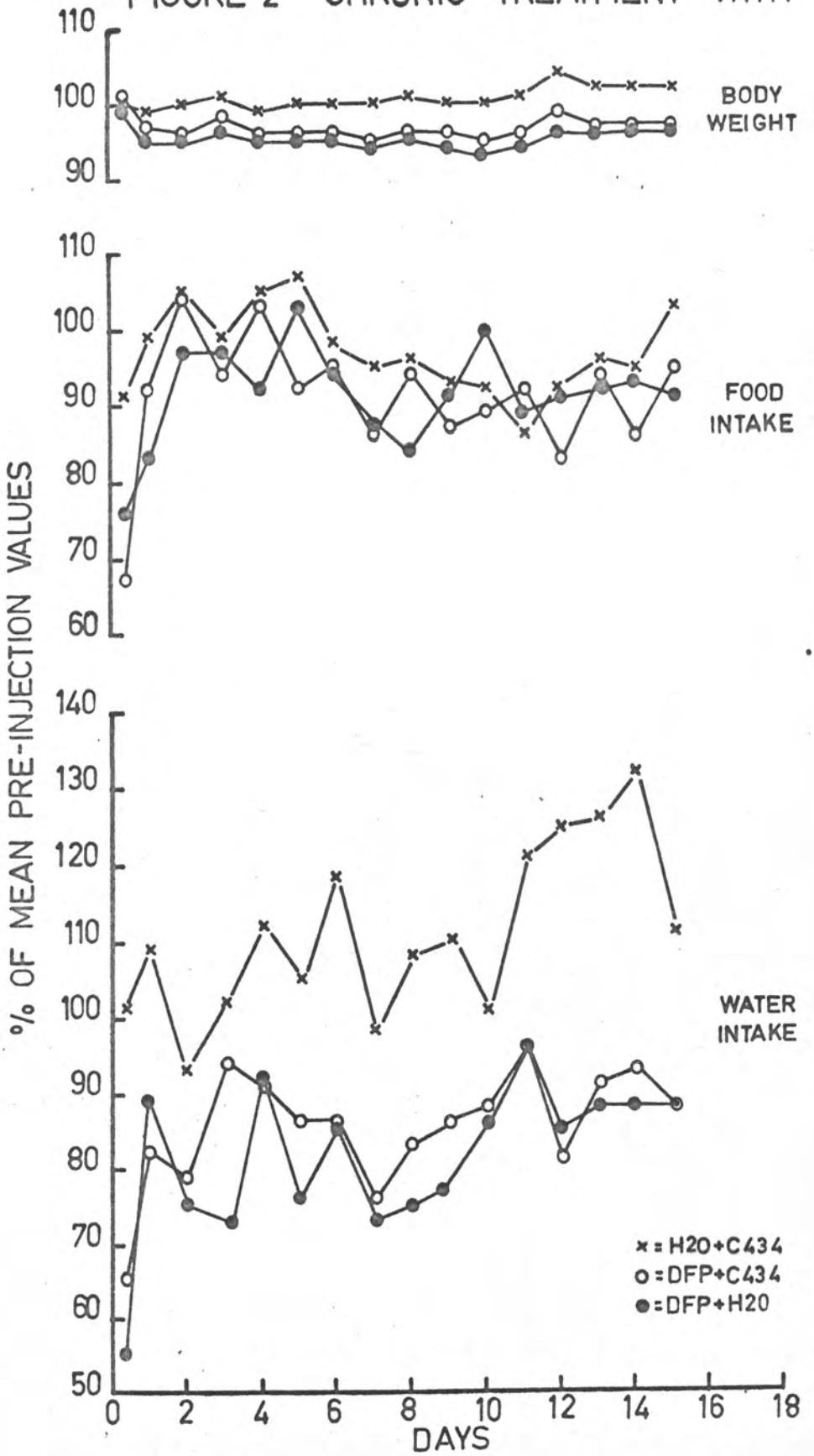


TABLE 1A. Acute Treatment with DFP and Body Weight

Days after initial injection	$H_2O + H_2O$ v. $H_2O + C434$			$H_2O + C434$ v. $DFP + H_2O$			$H_2O + C434$ v. DFP + C434			DFP + C434 v. $DFP + H_2O$		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
+ 3 hrs.	1.13	18	NS	0.63	18	NS	0.09	18	NS	0.78	18	NS
1	0.37	18	NS	3.56	18	1%	2.80	18	2%	0.03	18	NS
2	0.06	18	NS	5.25	18	0.1%	3.62	18	1%	1.98	18	10%
3	0.18	18	NS	4.64	18	0.1%	3.05	18	1%	2.07	18	10%
4	1.00	18	NS	6.47	18	0.1%	3.35	18	1%	2.59	18	2%
5	0.43	18	NS	6.02	18	0.1%	2.41	18	5%	2.41	18	5%
6	0.57	18	NS	5.55	18	0.1%	3.86	18	1%	2.25	18	5%
7	0.33	18	NS	6.31	18	0.1%	3.24	18	1%	3.13	18	1%
8	0.55	18	NS	4.86	18	0.1%	2.35	18	5%	3.17	18	1%
9	0.85	18	NS	4.38	18	0.1%	1.32	18	NS	2.93	18	1%
10	0.20	18	NS	4.84	18	0.1%	1.60	18	NS	3.70	18	1%
11	0.42	18	NS	4.30	18	0.1%	0.89	18	NS	3.08	18	1%
12	0.13	18	NS	3.45	18	1%	0.50	18	NS	2.17	18	5%
13	0.96	18	NS	3.19	18	1%	0.96	18	NS	2.58	18	2%
14	0.14	18	NS	2.57	18	2%	1.13	18	NS	2.21	18	5%

TABLE 18. Acute Treatment with DFP and Food Intake

Days after initial injection	H_2O + H_2O v. H_2O + C434			H_2O + C434 v. DFP + H_2O			H_2O + C434 v. DFP + C434			DFP + C434 v. DFP + H_2O		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
+ 3 hrs.	0.08	18	NS	3.70	18	1%	4.19	18	0.1%	0.80	18	NS
1	1.36	18	NS	3.27	18	1%	2.61	18	2%	0.02	18	NS
2	0.84	18	NS	2.30	18	5%	2.42	18	5%	0.67	18	NS
3	1.32	18	NS	4.25	18	0.1%	4.03	18	0.1%	0.42	18	NS
4	1.49	18	NS	0.75	18	NS	0.12	18	NS	1.19	18	NS
5.	0.67	18	NS	0.63	18	NS	2.21	18	5%	1.14	18	NS
6	0.88	18	NS	0.41	18	NS	0.51	18	NS	0.04	18	NS
7	1.37	18	NS	0.14	18	NS	0.89	18	NS	0.72	18	NS
8	1.01	18	NS	0.00	18	NS	1.28	18	NS	1.44	18	NS
9	1.76	18	NS	0.96	18	NS	1.93	18	10%	0.18	18	NS
10	1.42	18	10%	1.42	18	NS	0.35	18	NS	1.38	18	NS
11	1.28	18	NS	1.84	18	10%	1.34	18	NS	0.39	18	NS
12	0.59	18	NS	0.58	18	NS	0.15	18	NS	0.43	18	NS
13	0.12	18	NS	0.26	18	NS	1.14	18	NS	1.12	18	NS
14	0.83	18	NS	0.54	18	NS	1.60	18	NS	0.70	18	NS

TABLE 1C. Acute Treatment with DFP and Water Intake.

Days after initial injection	H_2O + H_2O v. H_2O + C434			H_2O + C434 v. DFP + H_2O			H_2O + C434 v. DFP + C434			DFP + C434 v. DFP + H_2O		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
+ 3 hrs.	1.04	18	NS	0.37	18	NS	1.28	18	NS	0.34	18	NS
1	0.65	18	NS	0.60	18	NS	1.66	18	NS	1.16	18	NS
2	1.50	18	NS	1.76	18	10%	1.96	18	10%	0.46	18	NS
3	0.64	18	NS	1.42	18	NS	2.15	18	5%	0.22	18	NS
4	1.16	18	NS	1.84	18	10%	1.78	18	10%	0.02	18	NS
5	2.12	18	5%	2.10	18	10%	2.85	18	2%	0.56	18	NS
6	0.03	18	NS	2.64	18	2%	1.48	18	NS	0.94	18	NS
7	1.02	18	NS	2.63	18	2%	1.10	18	NS	0.91	18	NS
8	0.20	18	NS	1.45	18	NS	0.43	18	NS	1.08	18	NS
9	1.21	18	NS	0.38	18	NS	0.31	18	NS	0.95	18	NS
10	0.97	18	NS	0.11	18	NS	1.12	18	NS	0.00	18	NS
11	0.88	18	NS	0.24	18	NS	0.14	18	NS	0.22	18	NS
12	0.77	18	NS	0.06	18	NS	0.27	18	NS	0.35	18	NS
13	1.72	18	NS	0.94	18	NS	0.83	18	NS	0.20	18	NS
14	1.99	18	10%	0.17	18	NS	0.22	18	NS	0.21	18	NS

TABLE 2A. Chronic Treatment with DFP and Body Weight.

Days after initial injection	H_2O + C434 v. DFP + H_2O			H_2O + C434 v. DFP + C434			DFP + C434 v. DFP + H_2O		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
+ 3 hrs.	1.9278	10	10%	0.3771	10	NS	1.9920	10	10%
1	4.0772	10	1%	4.5704	10	1%	1.5278	10	NS
2	6.0817	10	0.1%	5.6647	10	0.1%	1.6792	10	NS
3	5.8308	10	0.1%	3.8921	10	1%	1.5428	10	NS
4	2.7746	10	2%	3.6778	10	1%	0.6869	10	NS
5	4.1392	10	1%	4.9074	10	0.1%	0.9545	10	NS
6	3.1475	10	2%	4.3619	10	1%	0.5384	10	NS
7	3.2742	10	1%	4.2846	10	1%	0.6178	10	NS
8	3.1901	10	1%	2.9056	10	2%	0.7399	10	NS
9	3.0572	10	2%	2.7391	10	5%	1.3587	10	NS
10	2.8141	10	2%	3.0323	10	2%	0.8275	10	NS
11	3.1771	10	1%	2.6696	10	5%	1.3544	10	NS
12	3.0042	10	2%	2.0582	10	10%	2.0148	10	10%
13	2.7565	10	5%	2.2459	10	5%	0.9961	10	NS
14	2.2812	10	5%	1.8787	10	10%	1.1578	10	NS
15	2.4099	10	5%	2.1272	10	10%	0.9154	10	NS

TABLE 2B. Chronic Treatment with DFP and Food Intake.

Days after initial injection	H_2O + C434 v. DFP + H_2O			H_2O + C434 v. DFP + C434			DFP + C434 v. DFP + H_2O		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
+ 3 hrs.	1.4654	10	NS	3.3512	10	1%	0.7769	10	NS
1	2.7984	10	2%	1.1630	10	NS	1.5753	10	NS
2	1.3069	10	NS	0.0756	10	NS	0.9838	10	NS
3	0.2798	10	NS	0.6629	10	NS	0.3413	10	NS
4	2.2955	10	5%	0.3632	10	NS	1.9063	10	10%
5	0.5478	10	NS	1.7159	10	NS	1.5284	10	NS
6	0.4698	10	NS	0.3687	10	NS	0.1559	10	NS
7	0.8843	10	NS	0.9558	10	NS	0.2407	10	NS
8	1.2558	10	NS	0.4121	10	NS	1.0555	10	NS
9	0.4343	10	NS	0.7207	10	NS	0.4057	10	NS
10	1.8876	10	10%	0.3885	10	NS	1.8732	10	10%
11	0.4093	10	NS	0.8101	10	NS	0.5363	10	NS
12	0.2559	10	NS	1.9753	10	10%	1.6306	10	NS
13	1.0119	10	NS	0.2328	10	NS	1.3827	10	NS
14	0.9110	10	NS	1.8479	10	10%	1.9307	10	10%
15	0.1923	10	NS	1.0139	10	NS	0.9327	10	NS

TABLE 2C. Chronic Treatment with DFP and Water Intake.

Days after initial injection	H_2O + C434 v. DFP + H_2O			H_2O + C434 v. DFP + C434			DFP + C434 v. DFP + H_2O		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
+ 3 hrs.	6.8160	10	0.1%	4.1409	10	1%	1.0834	10	NS
1	3.1964	10	1%	3.4456	10	1%	0.6724	10	NS
2	2.9994	10	2%	2.2178	10	10%	1.1315	10	NS
3	3.7856	10	1%	1.0981	10	NS	2.3865	10	5%
4	2.5559	10	5%	3.1525	10	2%	0.1533	10	NS
5	3.8559	10	1%	2.6752	10	5%	1.1634	10	NS
6	4.3534	10	1%	2.8199	10	2%	0.0678	10	NS
7	3.8955	10	1%	3.5186	10	1%	0.3206	10	NS
8	4.8154	10	0.1%	3.2108	10	1%	1.2162	10	NS
9	3.5339	10	1%	2.2054	10	10%	0.7106	10	NS
10	1.9256	10	10%	1.3014	10	NS	0.2196	10	NS
11	2.1457	10	10%	2.3120	10	5%	0.0078	10	NS
12	3.9519	10	1%	3.0099	10	2%	0.3034	10	NS
13	4.8260	10	0.1%	3.2878	10	1%	0.3181	10	NS
14	4.2759	10	1%	2.9093	10	2%	0.4663	10	NS
15	2.5935	10	5%	2.5118	10	5%	0.0908	10	NS

2. Chronic (Figure 2; Table 2.).

Body weight was reduced throughout the experiment in the DFP + H_2O and DFP + C434 groups. There were no differences between these two groups.

Food intake in the DFP + C434 group was only significantly reduced in the very first period after injection. The DFP + H_2O group differed from the controls on days 1 and 4, but no other differences were significant. There were no differences between the two DFP groups.

Water intake was reduced throughout the experiment in both DFP + C434 and DFP + H_2O groups. Once again there were no differences between the two DFP groups, except on day 3, which was probably a chance effect due to opposite fluctuations occurring simultaneously.

Discussion.

1. Acute.

Since disturbances of eating lasted up to five days, and disturbances of drinking up to seven

days, any behavioural change occurring in this period could have a motivational cause. With respect to this, two points should be noted. Firstly, the direction of any change which can be ascribed to motivation is fixed, because the animals both ate and drank less. Secondly, the changes shown in Figures 1 and 2 are perhaps of less motivational importance than they imply. This is because both eating and drinking are related to body weight; therefore if a drug has an independent effect on body weight, then food and water intake will also be affected, simply as a result of the changed body weight.

The lack of any significant differences between the two DFP groups in eating and drinking makes it unlikely that any differential effect upon behaviour of the two treatments was the result of a differential effect upon the animals' motivation. However, since body weights differ for the two groups it is possible that there were motivational differences which did not show up as changes in the amounts eaten and drunk.

2. Chronic.

When both peripheral and central ChE are chronically reduced, the effect on body weight is no greater than for an acute reduction. However, the chronic continuation of the DFP + C434 treatment kept body weight low, whereas after a single injection the body weight recovered. This indicates that C434 has less effect in the chronic situation.

The change in food intake was similar to that found in the acute experiment (65% - 75% of normal) and lasted no longer. As in the acute experiment it is possible to interpret the initial decrease in food intake as an effect of reduced body weight. This data suggests that food-motivational changes in animals with chronically inhibited ChE would only be important in the first few days after injection.

Water intake was the only measure which was very different to the acute experiment. In this case, reduced ChE clearly produced a lowered water intake throughout the experiment.

(The fact that the effect of the first injection of DFP + H_2O was much greater in the chronic than in the acute experiment was probably due solely to sampling differences.) Wide variation among animals in their reaction to reduced ChE has been found in both the present experiments and in several operant lever pressing situations. Since eating and drinking are positively correlated in the rat, it could be argued that any change in water intake would affect food motivated behaviour. From the present chronic study there is little evidence for this; food intake was close to normal while water intake remained depressed. However, in the extinction experiments referred to later, such a correlation would in any case be irrelevant since the animals never actually have to eat. It is in performance type situations, where the subject has actually to consume a food reward, that the relation of eating to drinking is potentially important. As far as extinction experiments are concerned, motivational changes can only possibly be of importance in the first five days, where effects are found on food intake.

In general then, the behavioural importance of changes in eating and drinking will depend on the type of behaviour being considered. There may also be other subtler motivational effects of the DFP, besides the effects on eating and drinking. For example the animal may suffer from a non-specific sickness or weakness. Such a motivational effect could also interact with the behavioural task; it might have no effect on response to a positive stimulus, that is, a stimulus with desirable consequences, and yet combine with a negative stimulus to decrease responsiveness.

Finally, there is the problem of how to account for the results physiologically. There are two main possibilities. Firstly, all the changes may be due to changes in the functioning of neuromuscular junctions, and the peripheral autonomic nervous system. Secondly, they may be due to interference with certain regulating systems in the central nervous system (CNS). The fact that in the acute study no antidotal effect of the C434 was

found upon eating or drinking argues against the peripheral view. However, since it is not known to what extent C434 protects peripheral ChE in tissues other than muscle under the conditions of the acute experiment, not too much reliance should be placed on this negative finding. In both the DFP + H_2O and DFP + C434 groups, faeces were initially much wetter than in the control group, indicating that in both there was some peripheral effect of the DFP.

The central effects of DFP have been studied in dogs by Duke,Pickford and Watt (1950) who found that DFP injected directly into the supra-optic nuclei produced a short initial inhibition of urine flow followed by a prolonged increase of both urine flow, and water intake. They interpret the inhibition as being due to stimulation by acetyl-choline producing an increase in anti-diuretic hormone. The later increase they interpret as being due to excess ACh exerting a blocking effect on the pro-duction of anti-diuretic hormone.

Grossman (1962a) has shown that direct cholinergic stimulation of the hypothalamus by intro-duction of crystalline ACh or Carbachol induced

drinking in water satiated rats. Adrenergic stimulation by nor-adrenalin, induced eating in food satiated rats.

The cross-effects - cholinergic stimulation upon eating and adrenergic stimulation upon drinking - were relatively slight. In a further report, Grossman (1962b) it was shown that the induced eating and drinking could be countered by intra-peritoneal injection of adrenergic and cholinergic blocking agents. The injections of cholinergic and adrenergic stimulating agents were made into the same general region of the hypothalamus from which eating and drinking can be induced by electrical stimulation. Stein (1963) has shown that the cholinergic blocking agents, atropine and scopolamine, when injected intraperitoneally, both inhibit drinking in the rat, whereas their quaternary methyl derivatives do not. Since methyl atropine and methyl scopolamine do not pass into the C.N.S. he concluded from this that the reduction in drinking was due to a central effect. All four compounds produced a marked depression in food intake. From this it was inferred that the effect on eating was peripheral. However, the data does

not preclude both central and peripheral effects. Since the centrally active anticholinergics affect drinking, it is highly likely that they will also, by way of this, affect food intake. Central effects on water intake are not restricted to the hypothalamus alone. Cholinergic stimulation of various limbic structures induces drinking in rats, Fisher and Coury (1962), Grossman (1964).

This data showing that cholinomimetics stimulate drinking, whereas anticholinergics inhibit it, is in direct contrast to the present results where DFP reduced water intake. The discrepancy cannot be accounted for, but it could be related to differences in the methods used. In view of the evidence that a cholinergic mechanism controls water intake it is perhaps noteworthy that water intake was the measure most affected in the chronic experiment.

PART FOUR

REDUCED CHOLINESTERASE ACTIVITY AND MOTOR BEHAVIOUR.

The general aim of this section is to examine the effects of reduced ChE on response mechanisms, as a control for the learning experiments in Part 5.

In certain learning situations, such as operant extinction where the measure of behavioural adaptation is actually rate of response, such control data is essential to distinguish effects directly on response mechanisms from those on mechanisms mediating changes in response. The same is true of situations in which latency of response is the measure of adaptation. In situations where the proportion of correct choice is the measure taken (e.g. in discrimination tasks) interference with the response mechanism is of little direct importance. However, there is still the possibility of an indirect effect on the course of adaptation - for example, by altering the effort involved in making a response, which is often the only factor inhibiting error making in tasks such as discrimination learning.

In the experiments reported below, observations were made of the effects of low ChE on

various aspects of a simple lever pressing response.

Experiment 1. Effects of reduced ChE activity on fixed ratio lever pressing performance.

General Procedures.

The subjects were 120 day old female rats, weighing approximately 150 grams, and maintained on 2 hours feeding per day.

The apparatus was a sound-proofed and ventilated Skinner box. The internal dimensions of the box were 9 ins. x 9 ins. x 9 ins., and the lever was set centrally in one wall, immediately above the food cup and 4 ins. above the floor. The lever was 2 ins. wide, and projected ½ in. from the wall. A force of approximately 30 gms. was necessary to work it. The food pellet reinforcer weighed 75 mgm; its presentation was always accompanied by light and buzzer secondary reinforcers.

The rats were trained to press the lever over a period of 24 days. In the first 7 days, they were habituated to the box, adapted to the

secondary reinforcers, magazine trained and trained to press the lever. They then worked at F.R. 1 for 7 days, before being progressively switched to their allotted final fixed ratio schedule. The change of ratio occupied 3 days, and a further 7 days' training was given on the final ratio. Twenty reinforcements were given each day throughout the 24 day training period, by the end of which, the time taken to obtain them had become stable.

1. Response Form.

Several experiments reported in Part 2 showed that the form of a motor response could be altered by asymmetrical reductions of brain ChE. Since the injection procedures described in Part 1 should produce symmetrical reductions in ChE, such effects on response form would be unlikely to occur. The following observations on paw preference in a lever pressing task were made to check this expectation.

Methods and Results.

Five animals were trained at F.R.1. The paw with which they pressed the bar was observed for a series of 10 reinforcements before, and 24 hours after, injection of 1 mgm/kgm DFP + H_2O.

All 5 animals obtained their 10 pre-injection reinforcements with the same paw each time. After injection their performance was exactly the same.

The general form of a simple motor response is therefore unaffected by reductions of brain ChE activity to 35% of normal.

2. Response Rate.

The normal cage behaviour of rats injected with 1 mgm/kgm of DFP is obviously changed 30 minutes after injection. The animals lie down, fasciculate, salivate, and only move when touched. However, 24 hours after treatment, it is only rarely possible to distinguish them from uninjected controls. The

following experiments on rates of lever pressing were undertaken to see whether effects of DFP could still be detected 24 hours after injection, and if they could, to determine their magnitude and duration.

Methods.

One hundred and twenty animals were trained at F.R. 1, 8 or 16. At the end of training they were injected as shown below.

Drug	Ratio		
	F.R.1 N	F.R.8 N	F.R.16 N
H_2O + C434	10	15	15
DFP + C434	10	15	15
DFP + H_2O	10	15	15

All injections were made according to the standardized procedures described in Part 1 for an acute reduction of ChE. Twenty four hours after injection, and on the following 2 days, all subjects were retested.

Twenty of the DFP + H_2O animals were trained for another 7 days, that is, for a total of 9 days after injection. Five of these were F.R.1, five F.R.8, and ten F.R.16.

Sixty of the animals were re-injected after the third day's testing and maintained on the chronic injection schedule (see Part 1) for a further 7 days. All injections were made approximately 24 hours before the subsequent testing session. The distribution of these 60 animals between the various subgroups is shown below.

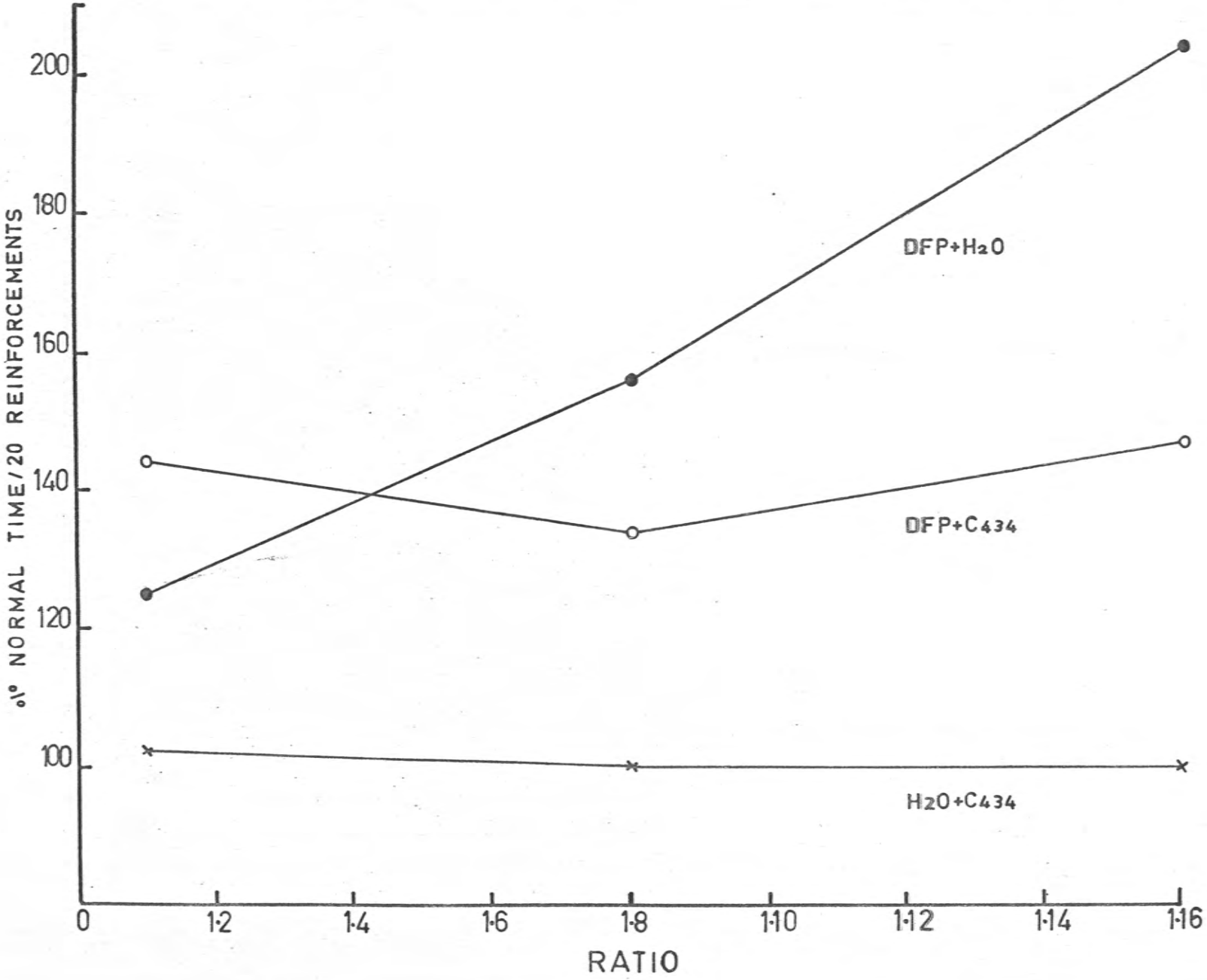
Drug	Ratio F.R.1 N	F.R. 8 N	F.R. 16 N
H_2O + C434	5	10	5
DFP + C434	5	10	5
DFP + H_2O	5	10	5

Results.

The time required to obtain 20 reinforcements varied little over 5 successive days immediately preceding the injection. The averages of the five days were 161.3, 390.1 and 760.0 seconds for ratios 1, 8, and 16, respectively.

The average of the 5 pre-injection days was found for each animal and all post-injection scores calculated as a percentage of it. The percentage scores of the 3 treatment groups were then compared by *t* test.

FIGURE 1. PERFORMANCE ON 3 RATIOS 24 HOURS AFTER INJECTION OF DFP



The interaction of ratio and reduced ChE is shown in Figure 1 and Table 1.

TABLE 1. Operant Lever Pressing Performance Twenty four Hours after Acute Reduction of Cholinesterase Activity.

	H_2O + C434 v. DFP + C434			H_2O + C434 v. DFP + H_2O			DFP + C434 v. DFP + H_2O		
Ratio	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
F.R.1	2.085	18	10%	1.726	18	NS	0.813	18	NS
F.R.8	2.906	28	1%	2.708	28	2%	0.967	28	NS
F.R.16	1.904	28	10%	4.880	28	0.1%	1.755	28	10%

Twenty four hours after injection, H_2O + C434 had no effect. At all three ratios, the performance of this group was 100% of the normal pre-injection mean.

The DFP + C434 group was slower after treatment; their scores being approximately 140% of normal at each ratio. The differences between this group and the H_2O + C434 animals reached acceptable levels of statistical confidence at only ratio 8. However, the differences at ratios 1 and 16 did reach the 10% level, and would therefore have been acceptable using

a one-tailed test. Animals vary widely in their reaction to treatment with DFP, and the increased variability among the DFP-treated animals was probably the major reason for the low level of statistical significance. Since the performance of animals treated with H_2O + C434 was so stable the increases in the DFP and C434 group at ratios 8 and 16 have been treated as real, not sampling, differences.

Those animals which received no reactivator were slower than normal, but especially so at ratios 8 and 16. At ratio 1 the difference did not quite reach the two-tailed value for the 10% level, but the argument that variations of the same size are not expected from control animals applies here too, and the difference should probably be regarded as a real one.

There were no significant differences between the two DFP groups, although at ratio 16 the t value reached the 10% level.

Figure 2 and Table 2 show grouped data for the same 120 animals on the three days following injection, and for the 20 DFP + H_2O animals trained

up to day 9. Data from the 20 chronically injected H_2O + C434 animals has been included for days 4 - 9, to provide a comparison, as the 'booster' H_2O + C434 injections had no noticeable effect.

Differences between the two DFP groups and the H_2O + C434 group were significant at days 1 - 3, but none of the comparisons between the DFP groups was significant, Table 2A.

FIGURE 2. ACUTE DFP AND F.R. PERFORMANCE

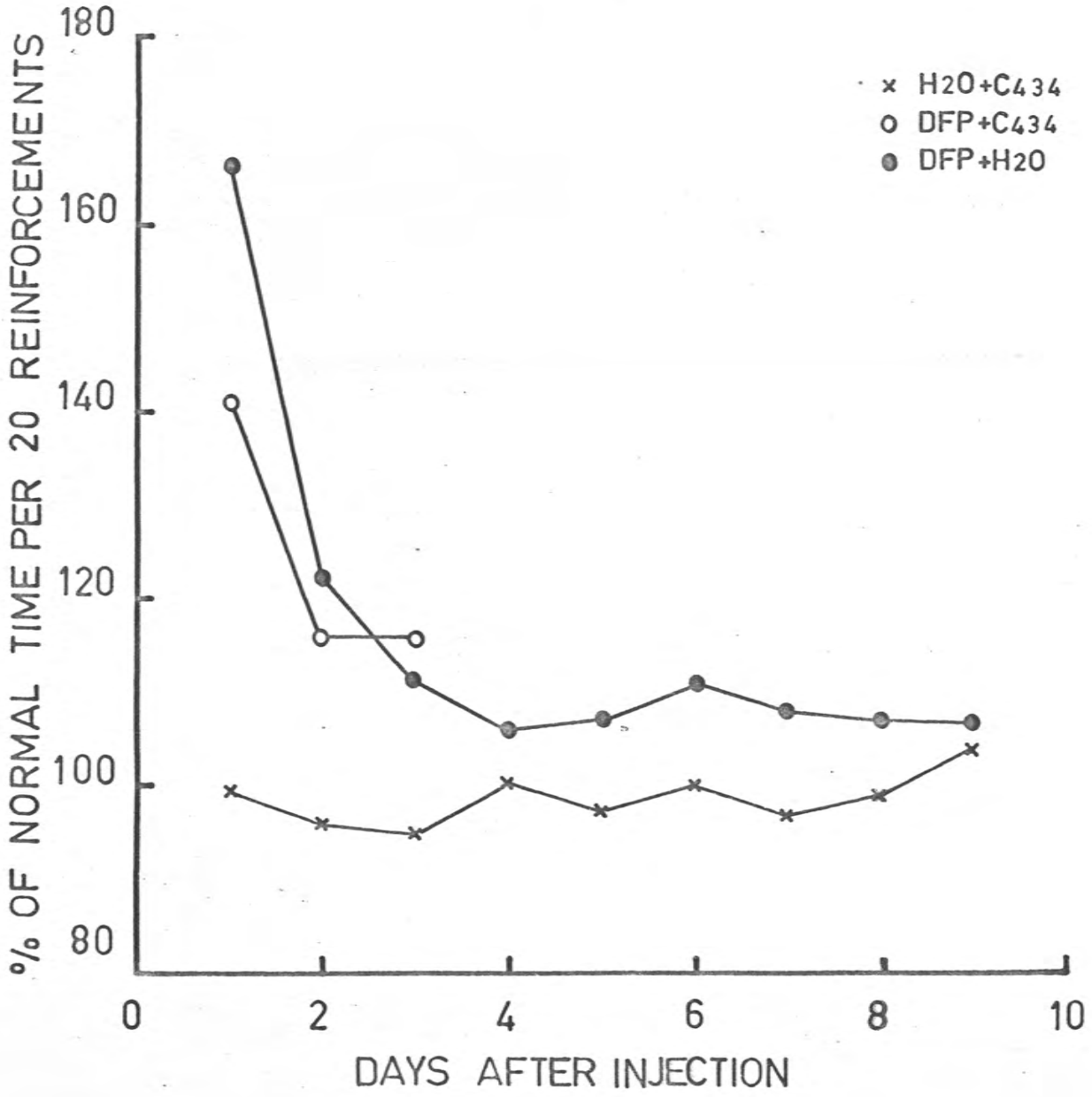


TABLE 2. Operant Lever Pressing Performance After an Acute Reduction of Cholinesterase Activity.

A.

	H_2O + C434 v. DFP + C434			H_2O + C434 v. DFP + H_2O			DFP + C434 v. DFP + H_2O		
Day	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
1	3.695	78	0.1%	5.292	78	0.1%	1.548	78	NS
2	3.159	78	1%	3.938	78	0.1%	0.703	78	NS
3	2.862	78	1%	2.239	78	5%	0.636	78	NS

B.

	H_2O + C434 (Chronic) v. DFP + H_2O (Acute)		
Day	t	df.	Sig.
4	1.110	38	NS
5	1.699	38	10%
6	2.331	38	5%
7	1.737	38	10%
8	0.969	38	NS
9	0.403	38	NS

The 20 DFP + H_2O animals that were trained till day 9 were indistinguishable from the H_2O + C434 controls after day 7, Table 2B. From days 4 - 9 there was only a 6 - 10% difference between the groups.

Grouped data for the 60 animals in which a low level of ChE was chronically maintained are shown in Figure 3 and Table 3. Both the DFP treatments slowed down lever pressing, but the effect was most marked in the DFP + H_2O group. The difference between these two groups was significant on day 1. In the DFP + H_2O group the effects of the chronic injections progressively diminished, whereas the DFP + C434 group remained at approximately the same level throughout.

FIGURE 3 CHRONIC DFP AND F.R. PERFORMANCE

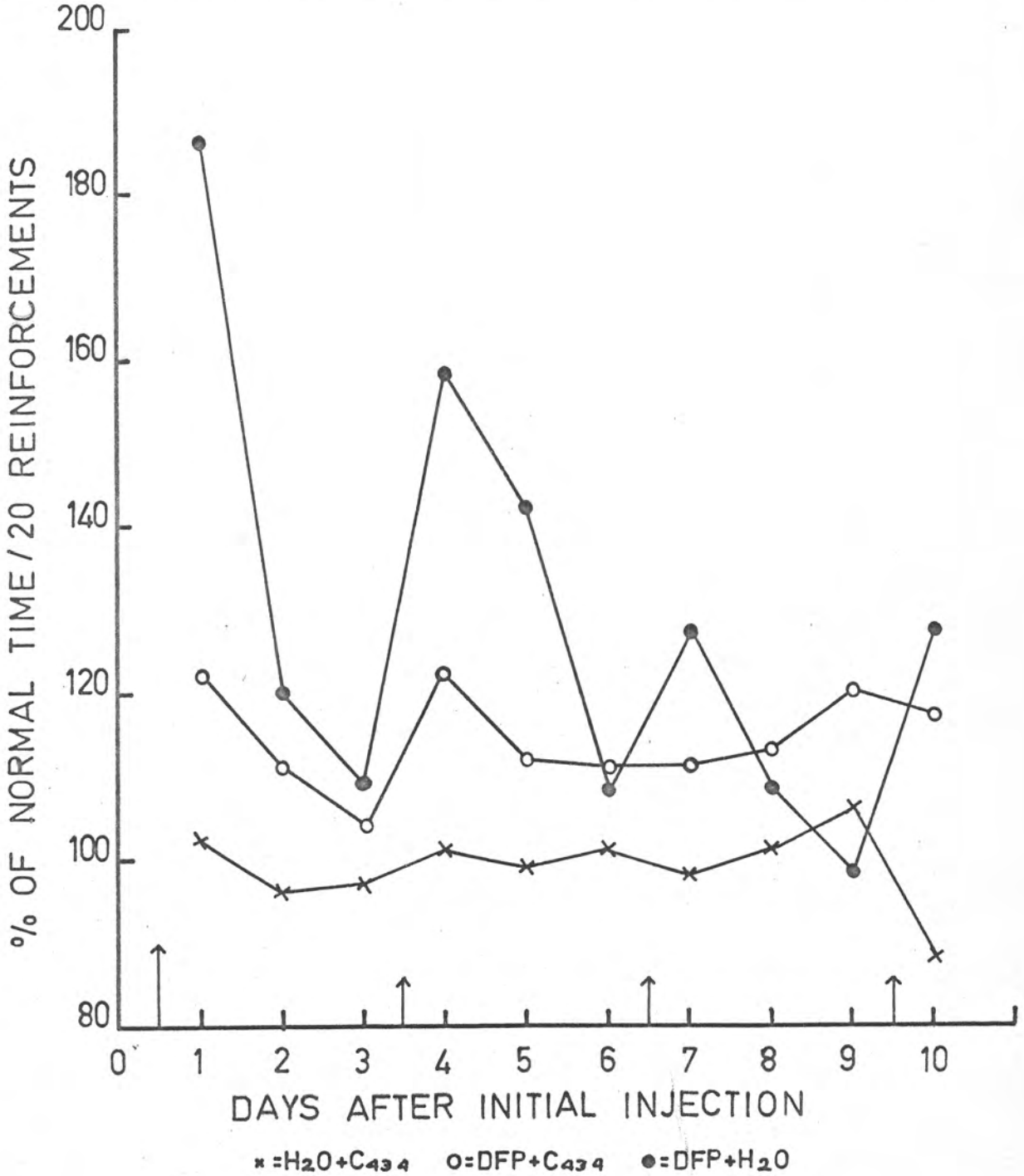


TABLE 3. Operant Lever Pressing Performance During Chronic Reduction of Cholinesterase Activity.

Days After Initial Injection	H_2O + C434 v. DFP + C434			H_2O + C434 v. DFP + H_2O			DFP + C434 v. DFP + H_2O		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
1	1.836	38	10%	4.050	38	0.1%	2.891	38	1%
2	2.018	38	10%	2.440	38	2%	0.761	38	NS
3	1.105	38	NS	1.615	38	NS	0.599	38	NS
4	1.604	38	NS	3.736	38	0.1%	1.863	38	10%
5	1.277	38	NS	2.597	38	2%	1.634	38	NS
6	0.905	38	NS	1.094	38	NS	0.230	38	NS
7	1.107	38	NS	2.362	38	5%	0.941	38	NS
8	1.253	38	NS	0.737	38	NS	0.617	38	NS
9	1.183	38	NS	1.004	38	NS	1.809	38	10%
10	2.061	38	5%	2.021	38	5%	0.441	38	NS

These data show:-

(1) That reduced ChE undoubtedly affects motor behaviour, and that it appears to do so most strongly where the

task involves a greater amount of work.

(2) That the effect of an acute injection rapidly diminishes, and is not apparent in fixed ratio lever pressing situations after 7 days, at which point brain ChE activity would be 55%, and gastrocnemius muscle 80 - 90%, Glow, Rose and Richardson (1966).

(3) That reactivation of part of the peripheral ChE, although it may have some effect, does not always have a marked effect. The major effect of C434 appeared to be to prevent extreme cases of slowing down.

(4) That the effect on lever pressing of successive injections of DFP diminishes, even though ChE is maintained at a low level, Part 1, Figure 1.

(5) There are wide variations in response to DFP among normal animals.

The increasing effect of DFP + H_2O with increasing ratio, and the fact that the largest difference between the DFP + H_2O and DFP + C434 groups was found at ratio 16, points to the 'effort' involved in obtaining reinforcement as a crucial factor. This would, at first sight, suggest that DFP impairs muscular efficiency and produces slower responding -

the more work to be performed the worse does the animal fare. However, observations made during testing showed up certain facts which do not fit this hypothesis. When the DFP-treated rats were actually pressing the lever their behaviour appeared to be quite normal. The increased time taken by such animals was apparently due to a lengthening of the periods in which the animals were not actually working at the lever - that is, the time-out (TO). The next experiment investigates this point more closely.

3. Response Distribution.

Methods.

Nine rats were trained at F.R. 8 until their response rates were highly stable. They were then given 10 days' further training, 10 minutes per day. During the last five days, records were taken of their responding on a Both paper recorder. After the tenth day, five animals were injected with 1 mgm/kgm DFP + 50 mgm/kgm C434, and four with

H_2O + C434. Twenty four hours after injection, training was continued in the same way as before, for a further six days.

Results.

The number of responses emitted in 10 minutes was reduced after injection, but recovered over succeeding days as in 2, Response Rate. Table 4 below shows mean daily responses per 10 minutes for the H_2O + C434 and DFP + C434 animals.

TABLE 4. Mean Responses in 10 Minutes Before and After Injection.

	Pre-Injection Days			Post-Injection Days					
	1	2	3	1	2	3	4	5	6
H_2O + C434	199	211	240	243	213	230	226	232	233
DFP + C434	201	199	213	65	151	191	196	210	188

Table 5 shows data for the DFP + C434 animals, 24 hours before and after injection. The first reinforcement obtained was treated as a 'warm-up' period, and was not used in the calculations. Time out (TO)

was the time from any one reinforcement to the next lever press. Response Time (RT) was the time taken to make a block of 8 lever presses for reinforcement. These values were obtained for the first ten reinforcements on the day before and the day after injection. (Some animals did not obtain 10 reinforcements after injection and the means are then on reduced numbers.) The TO/RT ratios were calculated by dividing each RT measure by its preceding TO measure.

TABLE 5. Response Time and Time Out Before and After Treatment with DFP + C434.

Animal	Response Time (1)		Time Out (2)		Ratio TO/RT	
	Before	After	Before	After	Before	After
1	6.55 (3)	7.31	22.05	50.25	3.57	6.90
2	10.20	8.15	17.35	30.70	1.78	4.07
3	6.80	16.00	20.30	57.50	3.16	4.03
4	7.15	8.50	19.90	53.00	2.97	6.71
5	4.56	5.40	9.82	28.10	2.69	5.37
$\bar{X}$	7.012	9.012	17.884	43.910	2.834	5.416

(1) Response Time = $\bar{X}$ time taken to make 8 presses.

(2) Time out = $\bar{X}$ time between reinforcement and next lever press.

(3) All data is in seconds.

Although in four animals out of five the RT was increased by treatment with DFP, by far the largest effect was on TO. Mean RT increased by 28.5% after injection whereas mean TO increased by 145.5%. This is borne out by the analysis of individual TO/RT ratios. For each of the five animals the ratio increased, indicating relatively greater increase in TO.

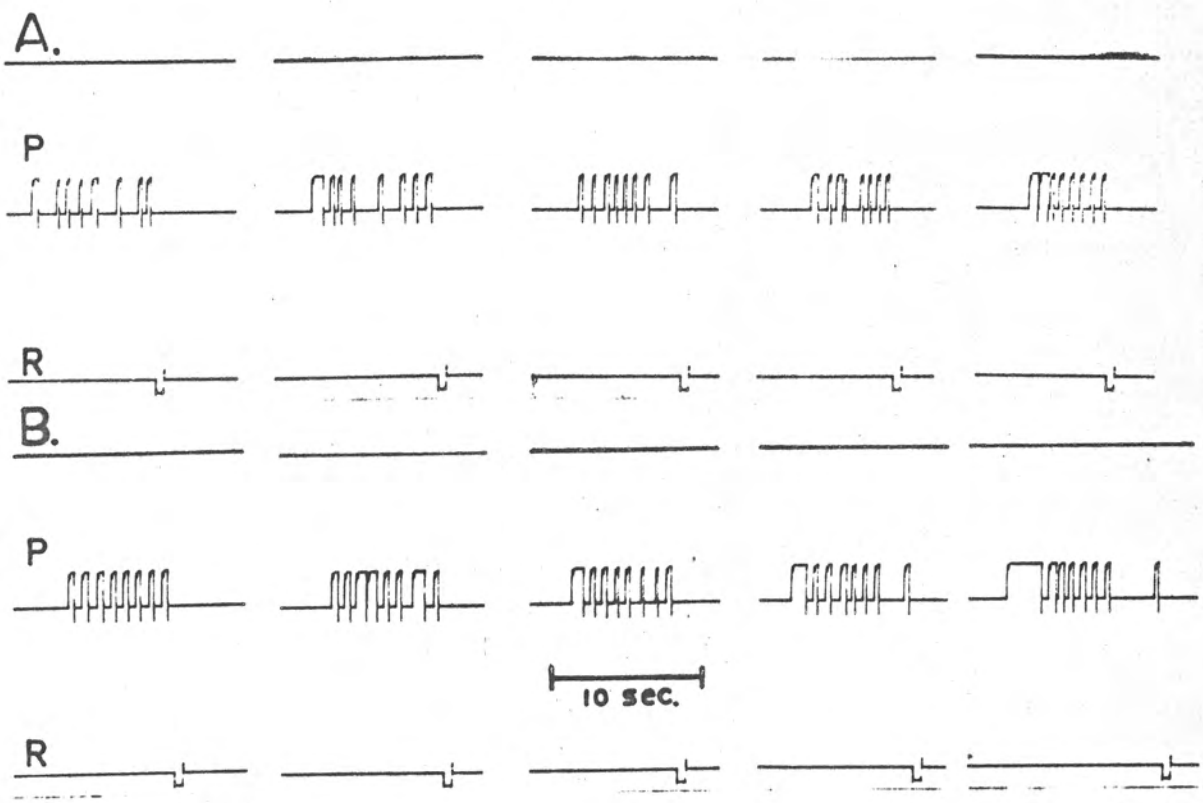
Pre- and post-injection traces of a single animal for reinforcements 2-6 are shown in Figure 4. There were no obvious differences between the traces; time for each individual response was equivalent, RT for each block of eight responses was approximately equal, and there was no disruption of the grouping of responses. Scores for this animal were 245, 255 and 273 responses for 10 mins. on the three days before injection, and 72, 179, 223, 255, 206 and 207 for the six days after injection. The relatively greater effect of low ChE on TO than on RT shows that the slower overall response rate was not simply a matter of muscular inefficiency. Neither is it likely that the effect was due to fatigue lasting

longer in the low ChE animals and preventing resumption of lever pressing, since similarly treated animals will respond more than eight times in a row in extinction situations.

As the measure of TO did not differentiate time used in eating the pellet, from time spent in other activities, it is possible that the increase in TO could have been produced by a longer time spent in eating. However there was no readily observable indication of this happening. It appeared that the usual routine of 8 presses - take pellet - eat pellet near lever - explore box - return to lever, was unchanged except for the 'explore box' segment.

This leads on to the final possibility to be considered: that the effects on lever pressing were not motor but motivational. Lever pressing for food can be considered an aversive task for the rat since it is rapidly stopped when food is withheld. Any decrease in food motivation (or general sickness, not necessarily related to food motivation) leads to the prediction that the aversive properties of the lever would be ignored less often. However, since in highly trained F.R. performance, the probability is high, that any run of responses commenced will be continued until the terminal reinforcement is obtained, the response time measure should be relatively unaffected, Mechner (1958).

FIGURE 4. LEVER PRESSING AT RATIO EIGHT



A = 24 HOURS BEFORE INJECTION

B = 24 HOURS AFTER INJECTION OF 1 MGM/KGM DFP + 50 MGM/KGM C434

P = LEVER PRESS

R = REINFORCEMENT

This possibility, as was mentioned in the introductory section, is of critical importance for any learning task in which the measure of an animal's progress depends on the aversiveness of certain responses that could be made in the situation. Most of the tasks used in Part 5 do in fact fit into this category.

PART FIVE.

REDUCED CHOLINESTERASE ACTIVITY AND DISCRIMINATIVE BEHAVIOUR.

1. INTRODUCTION.
2. STIMULUS ANALYSIS.
3. ASSOCIATIVE LEARNING.
4. RESPONSE CONTROL.

1. INTRODUCTION.

The theoretical approach adopted towards discrimination learning will be briefly discussed since it has influenced not only the kind of experiments performed but also their analysis.

Discrimination learning has been assumed to involve three separable kinds of behaviour - stimulus analysis, associative learning, and response control. Stimulus analysis is the isolation of stimulus differences in the environment. Whether this is an "innate" ability, or involves "perceptual learning" of the kind discussed by Hebb (1949) is not a crucial question in this context. The point to be made is simply that this kind of behaviour can be both theoretically and empirically distinguished from the use of a particular stimulus as a signal. Associative learning, as it implies, is simply the correlation of a stimulus signal with some particular consequence. The simplest cases of this are found in discrimination and avoidance learning situations where there is an invariant relationship between a single stimulus value and a single response. By varying the complexity of the relationship between the stimulus and the response to be learned, the task can

be made more difficult. Response control is concerned with performance of an already established habit. This class of behaviour may be thought to be redundant, since the presence or absence of response control is usually taken to indicate the presence or absence of learning. However, some of the later experimental results appear to demand such a category.

2. STIMULUS ANALYSIS.

Experiment 1. The effects of reduced ChE activity upon stimulus analysis.

Introduction.

Following the previous theoretical discussion of discrimination learning, this experiment was designed to investigate the effect of reduced cholinesterase activity upon the ability to isolate stimulus differences. Three discrimination learning tasks, known to require different amounts of practice for solution, were used. In each task the structure of the habit to be learned was the same, the only difference being in the stimuli to be discriminated.

Therefore, if reduced cholinesterase affects stimulus analysis, its effects should be greater upon the more difficult discriminations. That is, there should be a significant interaction between ChE activity and task difficulty. On the other hand, if there is no relationship between ChE activity and stimulus analysis, then no such interaction should be found.

Methods.

A two choice discrimination box was used. This was a single unit of the 4 unit apparatus described by Bruner, Matter, and Papanek (1955). The box was basically a straight alley, leading from a goal box to 2 top-hinged gates. Before choosing one of the gates the animals had to cross a 3 in. air-gap. The gates were locked by a bar ½ in. behind their bottom edge, and behind them the alley led into a goal box containing a pot of granulated dry food. The discriminative stimuli were black versus white (B v. W.), horizontal versus vertical stripes (H v. V), and circle versus triangle (C v T.). In the B v. W discrimination one gate was completely black and the other white; in the H v. V one gate had horizontal, and

the other vertical, alternating black and white stripes; and in the C v. T one gate had a 1.2 inch diameter black circle on a white ground, and the other a 1.5 inch side black equilateral triangle, also on a white ground.

Twenty rats were trained on each discrimination problem. Ten were normal controls and ten were experimental animals with reduced ChE. Half of each group were trained with one of the two stimuli positive and half with the other.

The animals were all females, 120 days old and approximately 150 grams at the beginning of the experiment. They were adapted to a routine of two hours' feeding each day before preliminary training in the apparatus, as in Part 3. Feeding was at the same time each day, and training was always approximately six hours before the feeding period.

The pretraining covered 5 days, as follows:- Day 1, 10 minutes free exploration of the box with gates removed. Day 2, trained to run from start box to goal box with gates removed. Day 3, trained to open grey gates. Day 4, trained to go to opposite gate when chosen gate locked.

Day 5, 10 trials with gates locked in a balanced random sequence (5 left, 5 right).

On the day before discrimination training began the animals were injected in two groups. (1) DFP + C434, (2) H_2O + C434. These injections were repeated (at half the original dosage) on every third day. All injections were approximately 17 hours before the next training session. Details of this procedure have already been set out in Part 1.

Discrimination training consisted of 10 self correction trials each day. The positive stimulus was on the right-hand side 5 times and on the left 5 times in a balanced random order, Gellerman (1933). An error was scored when the negative gate was pushed back far enough to contact the locking bar. Fifteen seconds feeding in the goal box was allowed after each trial, and there was another 15 second interval between trials. Control and experimental animals were run alternately on any given day, and the order of running was reversed on successive days. Training was continued to a criterion of 18 correct responses out of 20 on two consecutive days.

(Note:- Some animals died, or were killed because of middle ear disease, in this and subsequent experiments. These cases are not specifically mentioned in the text. Only in Experiment 3 of Part 6 was death obviously due to the drug treatment.)

Results.

Table 1 shows that chronically reduced ChE produced increased trials and errors to criterion measures for the learning of both horizontal v. vertical stripes and circle v. triangle discriminations. However, the black v. white discrimination was unaffected, although the means were in the same relation to each other as for the other two groups.

TABLE 1. Discrimination Learning: Statistical Analysis of Measures to Criterion.

		H_2O + C434			DFP + C434			Statistical Comparison		
		$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df	Sig.
Black v. White	Total Trials	71.0	24.3	10	79.0	21.7	10	0.738	18	NS
	Error Trials	23.1	10.8	10	28.8	11.8	10	1.069	18	NS
Horizontal v. Vertical	Total Trials	100.0	28.6	10	176.0	41.3	10	4.538	18	0.1%
	Error Trials	35.4	13.2	10	62.9	17.6	10	3.746	18	1%
Circle v. Triangle	Total Trials	422.1	231.7	9	639.9	200.3	10	2.078	17	10%
	Error Trials	171.3	103.7	9	270.9	85.7	10	2.165	17	5%

A regression analysis was made of errors to criterion against level of difficulty. The relative difficulty of the three problems was determined on the basis of the control groups' error scores to criterion, taking the mean for the black-white discrimination as = 1. A straight line model was fitted to the data. The fit was significant for both groups. (Experimental:- $F = 122.483$, 1,28 df; $P < 0.001$; Control:- $F = 25.259$; 1,27 df; $P < 0.001$).

The slopes of the two lines differed significantly ($F = 10.036$; 1,55 df; $P < 0.01$), as did their adjusted means, ($F = 10.495$; 1,55 df; $P < 0.01$).

Discussion.

The results show that reduced ChE activity interacts with discriminative difficulty. This therefore suggests that the ability to isolate stimulus differences is in some way impaired by low ChE. However, the experimental data does not allow any more specific interpretation to be made. The cause of the impairment in stimulus analysis could

be anywhere in the visual pathway, since DFP has been shown to affect ChE in the retina, the colliculi and the visual cortex, Glow, Rose and Richardson (1966).

In addition, the interpretation of the differences found as being strictly of a visual nature, is only true if low ChE does not affect some other ability which itself interacts with visual discrimination. From observation of the animals' behaviour it appeared that low ChE subjects tended to make more errors in the later stages of training, even when they had quite obviously learned the discrimination, than did controls. The usual pattern of behaviour was for the animals to adopt position habits early in training, and gradually learn to return from the negative gate, when it was on the preferred side, without actually pushing it. In most animals the position habit was quite evident even when performance was 100% correct, since they would cross the air gap to the same side of the apparatus on every trial.

This was the general pattern for both the control and the experimental subjects, but the latter would more often make an error before

recrossing the air gap, as though they could not inhibit response to a negative stimulus as well as did the controls.

Such an instability in response control, if it was a genuine effect, could explain the interaction found, since it seems likely that efficiency of response control would be a function of the discriminability of the stimuli towards which response was made. These questions are investigated more fully in the experiments that follow.

Experiment 2. Acute reduction of cholinesterase activity and post-criterion discrimination performance.

Introduction.

Experiment 1 showed that chronically reduced ChE activity slowed down the learning of visual pattern discriminations. However, the data presented did not allow the reasons for the slowing down to be determined. This experiment is designed to check the possibility that low ChE causes defective vision - i.e., some degree of blindness.

If this was the case in Experiment 1, then the performance of previously learned discriminations should also be impaired by reduction of ChE activity.

Method.

The animals used were control H_2O + C434 subjects from the Horizontal v. Vertical and Circle v. Triangle discriminations of Experiment 1, (N = 10,8) together with 5 other H_2O + C434 rats trained on a Black v. White discrimination.

Once the animals had reached criterion on the original task, they were given a further two days' training - 10 trials per day. On the following day they were injected with 1 mgm/kgm DFP (i-m) + 50 mgm/kgm C434 (i-p), and tested two hours after injection. The final testing session was on the day after injection.

Results.

Figure 1 shows that for all discriminations there was a drop in the accuracy of

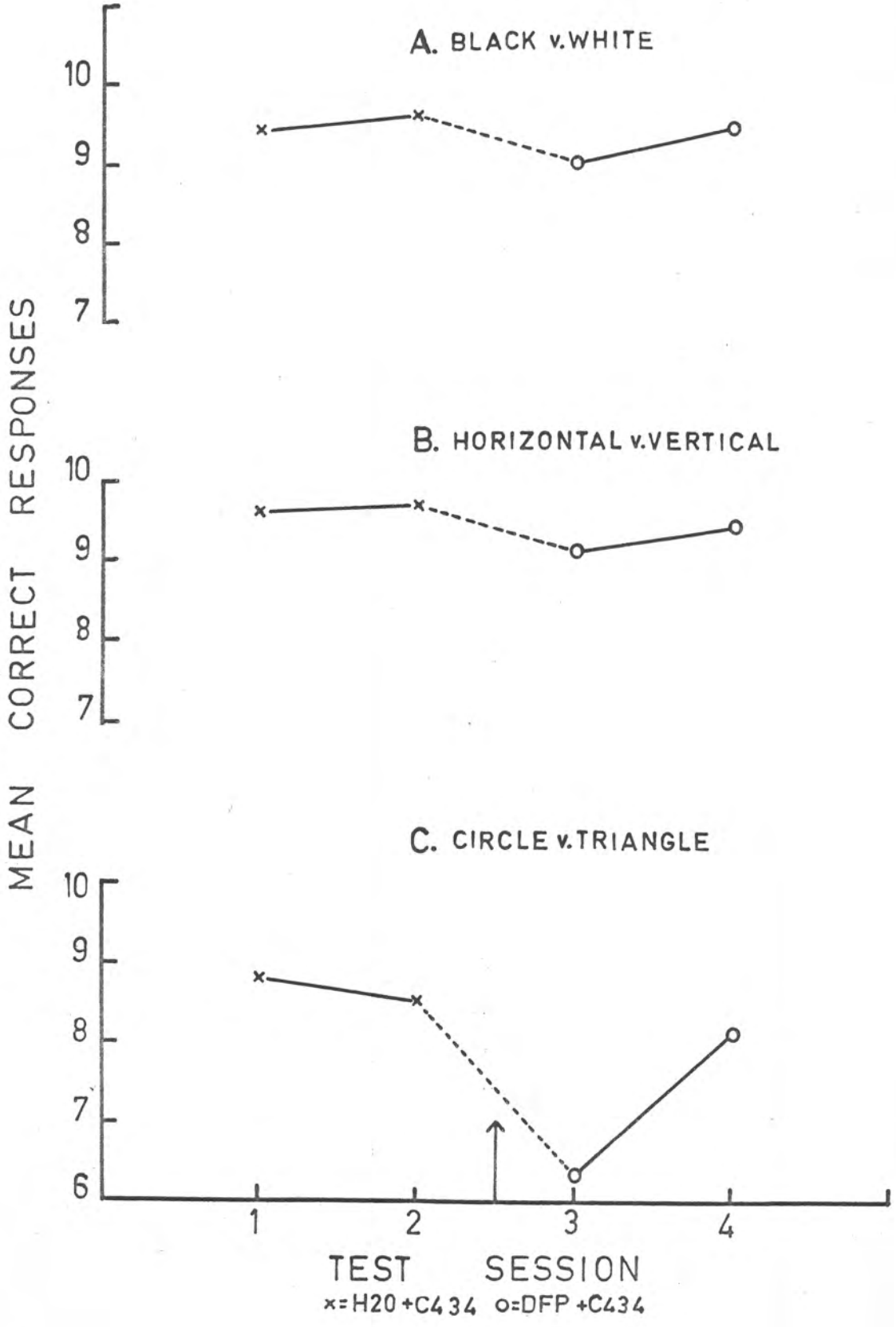
performance 2 hours after injection, but recovery to normal values in the second testing session, 24 hours later. The results of *t* tests between trials correct on the session before injection, and the 2 post-injection sessions, are shown in Table 2. The only comparison to reach significance was for the first test session in the circle v. triangle group.

TABLE 2. Acute Reduction of ChE Activity and Performance of Three Previously Learned Discriminations.

Discrimination	Session 2 v. 3*			Session 3 v.4		
	t	df	Sig.	*t*	df	Sig.
Black v. White	0.885	8	NS	0.426	8	NS
Horizontal v.Vertical	1.138	18	NS	0.976	18	NS
Circle v. Triangle	2.826	14	2%	0.728	14	NS

* Session 2 = 24 hours pre-injection
Session 3 = 2 hours post-injection
Session 4 = 24 hours post-injection

FIGURE 1 DISCRIMINATION PERFORMANCE



Discussion.

The lack of any effect 24 hours after injection, suggests that if visual defect is produced by low ChE it must be of a very minor kind, and is unlikely to account for the results of Experiment 1. The possibility of some visual defect occurring is not completely ruled out, since the present task is probably less sensitive to such defects than are the learning situations of Experiment 1. (It is easier to detect a difference already known to exist, than to discover an unknown one.)

The significant change found in the circle v. triangle group 2 hours after injection was very likely a motivational (or motor) effect, since the animals still showed gross effects of the drug - (see Parts 3 and 4) and moved very slowly in the apparatus. The effort involved in returning from the negative gate to the positive gate (see Experiment 1 : Discussion) would be more than usually aversive under such conditions, and thus increase the tendency to make an error.

Finally, not all the animals in the 2 hour circle-triangle group were affected by the injection, and those that were still made some observing responses, indicating that the ability to discriminate was still present.

Experiment 3. Effects of chronically reduced ChE activity upon learning a Horizontal/Vertical Stripes Discrimination with shock for errors.

Introduction.

In Experiment 1 it was suggested that one possible reason for the poorer discrimination learning of low ChE animals might be an instability in their performance. Such instability could produce inflated trials or errors to criterion measures that did not really indicate a slower rate of discrimination learning. In terms of the discussion in 1, Introduction, the effects of reduced ChE might be on response control rather than stimulus analysis.

This hypothesis can be tested experimentally by increasing the 'cost' of making an error.

In the normal hunger motivated discrimination situation an error involves nothing except a short detour and a slight delay in obtaining food. The present experiment was designed to increase this low degree of aversiveness by punishing the animal for errors with an electric shock. If the hypothesis outlined above were true then increased error 'cost' should either attenuate or abolish the effects of low ChE on discrimination learning.

Methods.

The discrimination box used in Experiment 1 was modified by replacing the solid floor on each side of the air gap with independently electrifiable grids. The grid between the start box and the air gap will be referred to as 'Grid 1', and that in front of the gates as 'Grid 2'. The voltage applied across the grids could be varied from 0-50.v. The voltage used was always just below that which produced squealing - usually 25 v. The start box was fitted with a movable false wall to force the animals into the apparatus.

Maintenance and pretraining of animals (N=20) was basically the same as in Experiment 1, but

an extra 2 days' training with grey gates was given to all animals to ensure that they were well adapted to the shock. By the end of pretraining, the crouching, defecating, and urinating found initially had stopped completely.

The animals were divided into two groups matched on the basis of position habit strength on the last 2 days of pretraining. One group was started on the chronic H_2O + C434 treatment, and the other on DFP + C434.

Discrimination training was also similar to Experiment 1, with the following modifications. The animal was forced from the start box onto Grid 1. If no choice response was made within 5 seconds then the grid was electrified with brief shocks at a frequency of 1/sec. until the animal crossed the air gap onto Grid 2. If an error (defined as in Experiment 1) were made, then Grid 2 was electrified in the same way until the animal crossed back to Grid 1. If no response were made within a further 5 seconds then the whole procedure was repeated until the correct choice had been made. (Originally it had been intended to give shock <u>only</u> for errors. However, Grid 1 had to be electrified because the pilot animals refused to approach either

half of Grid 2 in the early stages of training). Fifteen seconds eating was allowed and there was a 15 second intertrial interval as in Experiment 1.

Results.

The results are shown in Table 3 below.

TABLE 3. Effects of chronically reduced ChE on learning a Horizontal versus Vertical Stripes Discrimination, with Shock for Errors.

	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df	Sig.
Total Trials	62.0	20.9	10	68.0	26.4	10	0.535	18	NS
Error Trials	20.2	9.5	10	23.2	11.2	10	0.733	18	NS

The difference between the 2 drug treatments is extremely slight and nowhere near accepted levels of statistical significance.

Discussion.

Observation of the animals' behaviour showed that the 'cost' of errors was in fact increased. Whereas in the food motivated experiments, animals would often cross the air gap and move close to the locked gate before returning to make a correct response, in the present experiment the discrimination was usually made before the air gap was crossed. (The animals would run up and down in front of the gates before making a response.) The negative results therefore strongly support the hypothesis that the results of Experiment 1 were due to an effect upon response control rather than directly upon stimulus analysis.

In addition, the fact that the discrimination was learned far more quickly with shock than with food reward alone gives added weight to this conclusion, since more of the learning would have occurred in the period when adaptation to the effects of low ChE was least, and the effects on motivation and motor behaviour greatest, Parts 3 and 4.

Experiment 4. Horizontal/Vertical Stripes Discrimination in a Water Maze.

Introduction.

The purpose of this experiment was similar to that of Experiment 3. Firstly, it was thought that escape from water would be a stronger motivator than food reward and increase the 'cost' of errors. Secondly, since reduced ChE has been shown to affect food and water intake (Experiment 1, Part 3) this experimental situation should enable food motivational effects to be separated from effects directly upon stimulus analysing mechanisms.

Methods.

The apparatus was basically the normal discrimination box built as a water-filled tank. The gates were hinged at the sides instead of at the top.

Maintenance and pretraining were the same as in Experiment 1, but the animals were allowed

free-feeding throughout the experiment.

Discrimination training was also as in Experiment 1 but in this case an error was defined simply as touching the negative gate.

Results.

The results are shown in Table 4 below.

TABLE 4. Effects of Chronically Reduced ChE on Learning a Horizontal versus Vertical Stripes Discrimination in a Water Maze.

	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df	Sig.
Total Trials	96.0	46.9	10	87.0	22.4	10	0.519	18	NS
Error Trials	33.6	17.2	10	30.1	8.2	10	0.550	18	NS

It can be seen that as in the shock experiment the difference between the groups was slight, but that in this case the DFP + C434 group was actually the better of the two. The difference did not approach statistical significance.

Discussion.

The results of this experiment support the conclusions reached in Experiment 3 that the effects of low ChE on discrimination learning found in Experiment 1 were not due to a direct interference with stimulus analysis. In view of this it appears that the major effect of low ChE is simply to reduce the stability of performance.

3. ASSOCIATIVE LEARNING.

The three experiments that follow examine the effect of reduced ChE activity upon the ability to associate a stimulus with a particular response. If this ability is affected by reduced ChE activity then it can be argued, as in the stimulus analysis case, that complex associations of stimulus and response would be expected to show the effect more clearly than simpler ones.

Experiment 5. The effects of reduced Cholinesterase activity upon discrimination and reversal learning.

Introduction.

A series of three learning tasks was used:- (1) A simple position habit, (2) A simultaneous brightness discrimination, (3) A successive brightness discrimination which involved making a position response contingent upon a particular brightness value. Since the stimulus conditions were very simple - and the same for the two most difficult discriminations - it was assumed that any effect on stimulus analysis should not produce an interaction with task difficulty. (The original intention was to use black v. white stimuli for all discriminations, the series being (1) black v. white simultaneous (2) black v. white successive (3) black v. white conditional, in which either black or white was the positive gate depending on the nature of a third stimulus. However, the conditional discrimination proved too difficult and was discarded.) On the

other hand, if ChE affects associative learning ability, then there should be a significant interaction of ChE level and task difficulty.

Methods.

Maintenance and pretraining of the animals was the same as in Experiment 1.

The 20 animals trained on the position discrimination were matched for strength of position habit before training began. They were all run to the non-preferred side. The gates were the mid-grey ones used in the pretraining. Upon reaching criterion (18/20 on 2 consecutive days) training was continued on the reverse discrimination until the same criterion was reached. The habit was then reversed a second time and training continued to criterion. Twenty animals were also trained on a black v. white simultaneous discrimination/reversal task. The discrimination performance of these animals has already been reported in Experiment 1. Nineteen animals learned the successive brightness discrimination task, in which two white gates signified left-side gate open and two black gates right-side gate open. The relationship

between brightness and position was reversed once the criterion level was met, as in the other two tasks.

Results.

It can be seen from Table 5 that there was no evidence whatever of low ChE affecting either the learning or the reversal of a position habit. In the case of the simple brightness discrimination, the low ChE animals took significantly more trials to reach criterion on the first reversal than the controls, but the error trials to criterion measures differed at only the 10% level of confidence. There was no difference between the groups on the second reversal. The successive brightness discrimination likewise showed no difference between the drug groups, but the reversals were learned <u>faster</u> by the low ChE subjects, although the difference was significant only for the second reversal.

When scores for the discriminations and 2 reversals were combined there were no significant differences between the treatment groups on any task, although the comparison for the successive discrimination did reach the 10% level of confidence.

TABLE 5. Chronic Reduction of ChE Activity and Associative Discrimination Learning.

Task	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df	Sig.
Right v. Left Discrimination									
Trials	41.0	17.0	10	45.0	19.6	10	0.462	18	NS
Errors	9.6	6.8	10	8.6	5.0	10	0.355	18	NS
Reversal 1									
Trials	46.0	11.1	10	45.0	10.2	10	0.198	18	NS
Errors	14.3	5.1	10	15.6	5.3	10	0.527	18	NS
Reversal 2									
Trials	37.0	7.8	10	40.0	8.9	10	0.758	18	NS
Errors	10.6	4.9	10	10.7	4.9	10	0.043	18	NS
Combined									
Trials	124.0	29.7	10	130.0	25.7	10	0.458	18	NS
Errors	34.5	11.8	10	34.9	7.9	10	0.085	18	NS
Black v. White Discrimination									
Trials	71.0	24.3	10	79.0	21.7	10	0.738	18	NS
Errors	23.1	10.8	10	28.8	11.8	10	1.069	18	NS
Reversal 1									
Trials	124.0	40.8	10	187.0	66.2	10	2.431	18	5%
Errors	52.7	19.4	10	78.3	31.8	10	2.061	18	10%
Reversal 2									
Trials	183.0	33.2	10	190.0	50.6	9	0.341	17	NS
Errors	72.3	14.1	10	74.3	14.5	9	0.293	17	NS
Combined									
Trials	385.0	65.6	10	424.4	109.3	9	0.912	17	NS
Errors	148.6	33.6	10	174.3	41.3	9	1.414	17	NS
Black/R v. White/L Discrimination									
Trials	336.7	147.4	9	359.0	162.0	10	0.296	17	NS
Errors	145.4	64.1	9	140.9	63.3	10	0.147	17	NS
Reversal 1									
Trials	483.3	239.5	9	379.0	229.7	10	0.916	17	NS
Errors	218.6	107.4	9	166.2	114.4	10	0.970	17	NS
Reversal 2									
Trials	360.0	134.9	8	190.0	62.4	9	3.188	15	1%
Errors	150.8	60.0	8	77.0	28.3	9	3.097	15	1%
Combined									
Trials	1076.3	355.0	8	817.8	162.2	9	1.846	15	10%
Errors	475.0	163.8	8	333.9	85.3	9	2.124	15	10%

A regression analysis, similar to that in Experiment 1, was made of error scores against level of difficulty for the three original discriminations. Relative difficulty was determined as in the previous experiment, using the mean errors to criterion of the left versus right group as equal to 1. A straight line model was fitted to the data. The fit was significant: (Experimental; $F = 67.644$; 1,28 df; $P < 0.001$; Control; $F = 67.646$; 1,27 df; $P < 0.001$.) However, there were no significant differences between either the slopes, or the adjusted means, of the lines of the two groups. (Slopes : $F = 0.064$; 1,54 df; $P > 0.05$. Adjusted means: $F = 0.002$; 1,54 df; $P > 0.05$.)

Discussion.

The lack of any interaction between drug treatment and task difficulty indicates that the ability to associate a stimulus with a particular response is not affected by reduced ChE activity. This conclusion cannot be extrapolated beyond the range of task difficulty studied, but it is noteworthy that the most complex discrimination task used

(black/white v. white/left) needed more trials to learn than the horizontal versus vertical discrimination of Experiment 1. Since the horizontal v. vertical task was learned far more quickly by the controls than the experimentals, it cannot be said of the present experiment that the range of difficulty was too small to show a difference. The lack of any effect of reduced Ch E upon the black/right v. white/left discrimination suggests that the positive results found in Experiment 1 were due to the nature of the stimuli, and not to any interference with associative learning ability.

The reversals of the three tasks gave inconsistent results and are consequently difficult to interpret. No significant differences were found for either of the position habit reversals. The first reversal of the simultaneous black/white discrimination was learned more quickly by the controls whereas there were no differences on the second reversal. In contrast, both the reversals of the successive black/white v. white/left discrimination were learned more quickly by the experimental animals, although the difference was only significant for the second reversal.

There was some suggestion that the experimental animals performed poorly on the first reversal

of the simultaneous brightness discrimination because they showed the same defect in response control as the animals in Experiment 1. This possibility is checked in Experiment 7.

The contrary results found for reversals of the successive discrimination habit are open to various explanations. It could be said that the effect of low ChE varies with type of task. However, this is a completely arbitrary assumption lacking any theoretical justification. There is a possibility that the long period animals were maintained with low ChE in this task was important, since adaptation to the effects of DFP occurs (See Part 4). However, simple adaptation cannot account for an absolute improvement in performance. In view of the general trend of the results obtained in this and in earlier experiments (that is, either no effect or impairment of the low ChE animals) the improvement could be a chance sampling error. In any case, this result shows conclusively that low ChE does not necessarily impair learning ability.

The following Experiment investigates the effects of low ChE on the retention and performance of these 3 discrimination - reversal tasks.

Experiment 6. Reduced ChE and Post - Criterion Discrimination Reversal Performance.

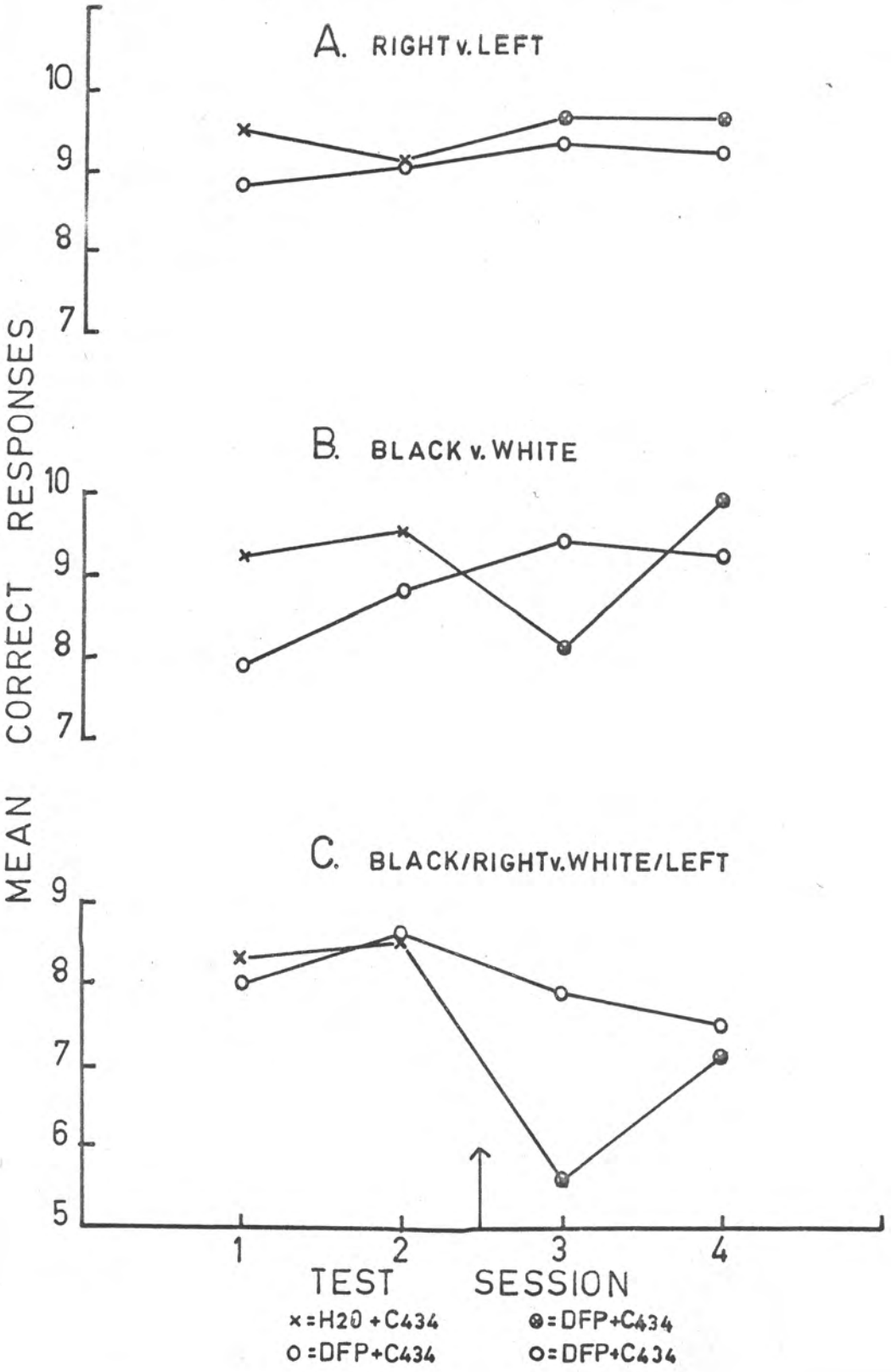
Methods.

The subjects were those trained on the 3 tasks of Experiment 5. Upon reaching criterion on the second reversal all animals were left in their cages for 5 days, after which they received 2 days' further training - 10 trials on each day. On the following day the control H_2O + C434 animals were injected with 1 mgm/kgm DFP + 50 mgm/kgm C434, and the experimental DFP + C434 animals with the usual 0.5 mgm/kgm DFP + 25 mgm/kgm C434. They were then given 10 test trials 2 hours and 24 hours after the injection.

Results.

The results are shown graphically in Figure 2. There was some evidence that retention of the response over 5 days was less for the low ChE animals. Figure 2 shows that the mean for the DFP + C434 animals

FIGURE 2 REVERSAL PERFORMANCE



was less than that of the H_2O + C434 animals in the first test session of all 3 discriminations. The differences reached the 10% and 5% levels of confidence for the Left v Right and Black v White discriminations respectively, Table 6 A. This point will be returned to in Experiment 9.

The effects upon performance of the acute and chronic injections were analysed by comparing the second pre-injection test session with each of the 2 post-injection sessions, Table 6B.

TABLE 6. Discrimination - Reversal Retention and Performance.

Discrimination	Session 1* H_2O + C434 v. DFP + C434			Session 2 v.3 H_2O + C434			Session 2 v.3. DFP + C434		
	t	df	Sig.	t	df	Sig.	t	df	Sig.
Right v. Left	2.044	17	10%	1.140	18	NS	0.632	16	NS
Black v. White	2.286	17	5%	3.126	18	1%	1.272	16	NS
Black/R v. White/L	0.424	14	NS	3.407	14	1%	1.047	14	NS

* Session 1 = First retraining session after 5 day gap.
Session 2 = 24 hours pre-injection
Session 3 = 2 hours post-injection

In no task did the chronic injection of DFP + C434 have any clear-cut effect upon performance. (The decrease found 24 hours after injection in the Black/Right v. White/Left experiment was probably an artifact due to the unstable performance on this task. The lack of any effect 2 hours after injection supports this.)

The acute injection of DFP + C434 to the control group had no effect upon the Right v. Left task, but produced a significant decrease in performance 2 hours after injection in the other two discriminations. This effect was not present 24 hours later.

Discussion.

The effects of acute DFP upon performance 2 hours after injection were probably due to general sickness making the effort involved in retracing from the negative gate more aversive than under normal conditions. (See Experiment 2, Discussion).

Experiment 7. Black versus White reversal learning with shock for errors.

Introduction.

In Experiment 5 there was some evidence that low ChE slowed down the reversal of a simple brightness discrimination, but had no effect upon initial learning of the habit. This Experiment is designed to determine whether any deficit in associative learning ability need be postulated to account for the result. An electric shock was given, as in Experiment 3, to increase 'error cost'. In addition the animals were only put on the low ChE treatment after learning the discrimination. This allowed the control and experimental groups to be matched for learning ability, and made the reversal learning trials occur within the period where adaptation to the drug was least.

Methods.

Maintenance and training of the animals was the same as for Experiment 3 with the exception that

the stimuli used were black v. white. 20 animals were trained to the criterion of 18/20 and divided into two matched groups on the basis of errors to criterion.

They were then given 2 days' overtraining to ensure that all animals were still at criterion when reversal training commenced. On the first day all animals received the usual 10 trials. On the second day a further 10 trials were given, followed by a second block of 10 trials for those animals which had still not reached criterion.

This was sufficient to bring all subjects to the level of 18/20 on 2 successive blocks of 10 trials.

After the second day's overlearning one group of 10 animals was started on the chronic H_2O + C434 injection schedule and the other group on DFP + C434.

Reversal training began on the following day and was carried to a criterion of 18/20.

Results

Table 7 shows that chronically low ChE had no effect whatever on the subjects' ability to reverse

the brightness discrimination. Means for both total trials and error trials to criterion are almost identical for the two groups.

TABLE 7. Black v. White Reversal Learning with Shock for Errors.

	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df	Sig.
Discrimination (Pre-injection)									
Total Trials	41.0	9.4	10	42.0	10.8	10	0.210	18	NS
Error Trials	9.50	4.2	10	10.40	4.6	10	0.434	18	NS
Reversal (Post-Injection)									
Total Trials	57.0	10.0	10	58.0	16.0	10	0.159	18	NS
Error Trials	22.8	7.1	10	22.4	8.4	10	0.109	18	NS

Discussion.

The results support the hypothesis that the inferior performance of low ChE animals found in the black/white reversal task of Experiment 1 was an effect

upon accuracy of performance rather than upon the ability to utilize information.

The method used in this experiment of matching the groups for learning ability should increase sensitivity to any possible drug effect, and therefore strengthens the negative result obtained.

Finally since approximately six days were needed to learn the reversal, the entire course of learning took place within the period where motivational and motor effects are greatest (see Parts 3 and 4). Assuming that the effects on motor abilities and general motivational state would themselves tend to produce slower learning, the conclusion that low ChE does not decrease associative ability seems unavoidable.

3. RESPONSE CONTROL.

The data reported in sections 2 and 3 pointed to response control as the main class of behaviour affected by low ChE. The experiments described below give further information on this point.

Experiment 8. Chronic Reduction of Cholinesterase Activity and Discrimination Performance.

Introduction.

Experiments 3 and 4 were based on the assumption that the poorer discrimination learning of low ChE animals found in Experiment 1 was due to unstable performance and not to reduced ability to discriminate. The present experiment is an attempt to positively identify this assumed instability.

If response control is poorer in animals with chronically lowered ChE activity, then the post-criterion performance of such animals should be worse than that of controls. Furthermore, this inferiority should be less in a situation in which error 'cost' is increased by punishment with shock.

Methods.

Twenty animals were trained to a criterion of 18/20 on a horizontal/vertical stripes discrimination for food reward, and twenty on the same discrimination

with shock for errors. 10 animals of each group received the chronic DFP + C434 treatment, and 10 H_2O + C434.

The animals were stopped on reaching criterion, but the injection schedule was maintained. This procedure enabled all animals to be at the same stage of the injection schedule during over-training. When the entire group, in each experimental situation, had finished training, they received 100 trials over-learning. Ten trials were given daily, the first day's trials being 24 hours after an injection.

Results.

The results are shown in Table 8 below, and graphically in Figure 3.

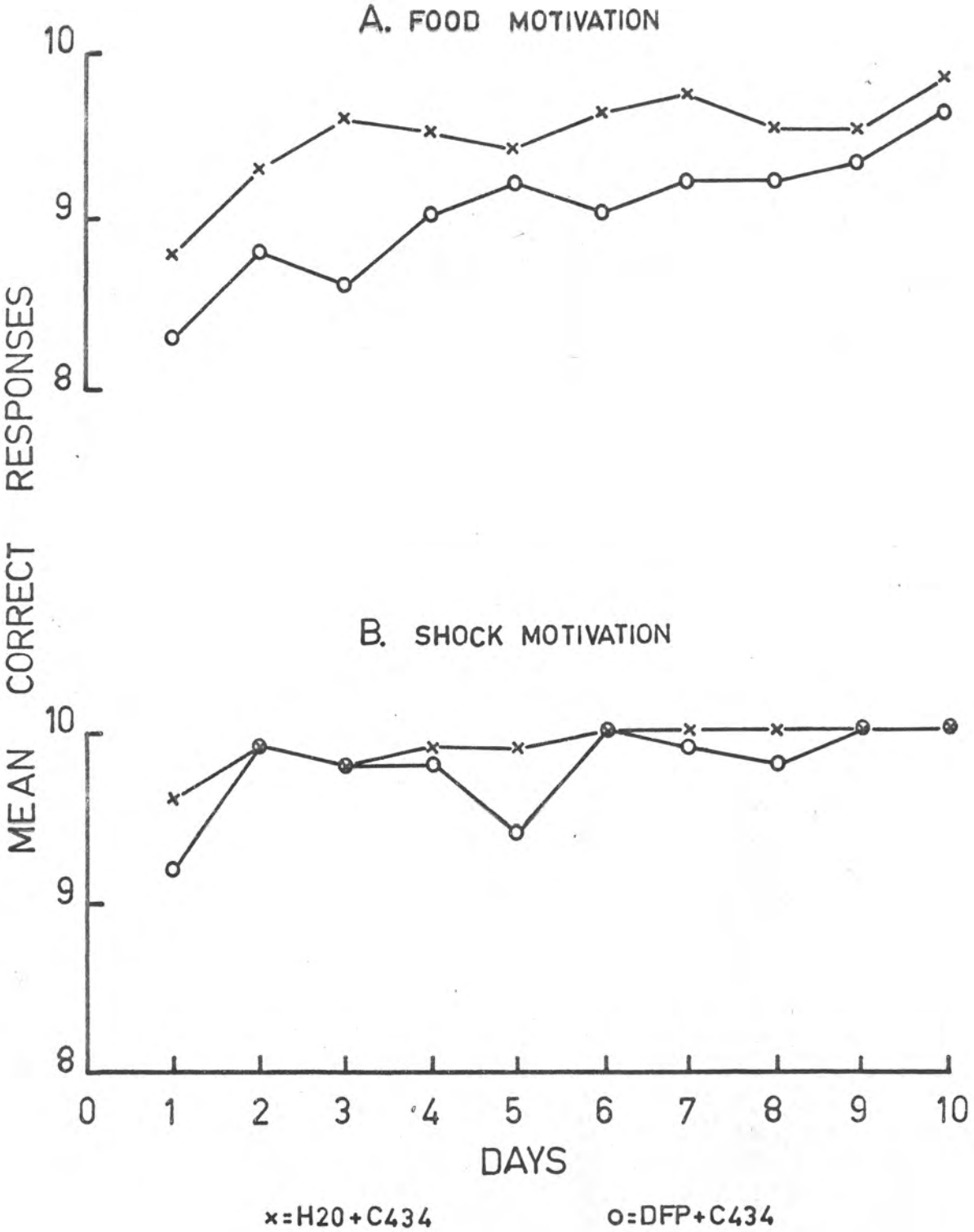
TABLE 8. Post-criterion Horizontal v. Vertical Stripes Discrimination.

	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$(1)	SD	N	$\bar{X}$	SD	N	t	df	Sig.
Food Motivation									
Days (1-5)	3.4	3.0	10	6.1	3.8	9	1.650	17	NS
(6-10)	1.9	1.6	10	3.7	1.9	9	2.031	17	10%
(1-10)	5.3	4.1	10	9.8	5.1	9	1.995	17	10%
Shock Motivation									
Days (1-5)	1.0	0.9	9	1.9	1.2	9	1.650	16	NS
(6-10)	0.0	0.0	9	0.3	0.7	9	1.414	16	NS
(1-10)	1.0	0.9	9	2.2	1.5	9	1.976	16	10%

(1) $\bar{X}$ = Mean total errors for days (1-5) etc.

In both the food and shock motivated conditions the DFP + C434 group tended to make more errors than the H_2O + C434 group. This difference was far larger in the food motivated situation, but diminished as the experiment progressed. The average performance level of both treatment groups in the shock condition was superior to that of their food motivated counterparts, which was expected following the results obtained in Experiment 3.

FIGURE 3. DISCRIMINATION PERFORMANCE



The statistical analysis in Table 8 shows that none of the comparisons of total error scores for days 1 - 5, 6 - 10 and 1 - 10, reached acceptable levels of significance, although most were either at, or close to, the 10% level.

Discussion.

The results obtained do provide some support for the hypothesis proposed in the Introduction, but statistically the evidence is inadequate.

It is possible that continued over-training tends to raise performance to such a level that the effect of the drug treatment is obscured. In this case the present experiment would not be an adequate test of the suggested explanation of post-criterion performance.

Experiment 9. Chronic reduction of cholinesterase activity and visual discrimination learning, performance, and retention.

Introduction.

This experiment was designed to complement Experiment 3 in which error cost was increased by shock. By filling in the air-gap in the discrimination apparatus it was hoped to reduce the cost of making errors. In addition, by testing animals at various periods after training, it was aimed to clarify the suggestion made in Experiment 8 that continued overtraining abolishes the effect of DFP on response control.

Methods.

Twenty rats were trained on a horizontal versus vertical stripes discrimination as in Experiment 1, with the exception that the air-gap was filled in to make a continuous floor. Ten were chronically injected with H_2O + C434, and 10 with DFP + C434. Upon reaching criterion training was stopped, but the injection schedule was maintained, as in Experiment 8. When all animals had finished training they were given 50 trials overlearning, 10 trials each day. The first block of overlearning trials was 24 hours after an

injection.

At the conclusion of these 5 days' training, blocks of 10 trials were given at successive intervals of 3, 6, 12 and 36 days. Each block fell on the second day after an injection day.

Results.

The DFP and C434 animals took more trials and made more errors in reaching criterion than did the H_2O + C434 controls, but neither difference was statistically significant, Table 9A.

DFP + C434 animals also made more errors than controls in the 5 day performance phase, but once again the difference was not significant, Table 9B, Figure 4.

There were no differences between the groups in the retention tests made after intervals of 3 or 6 days, but for the 12 and 36 day intervals the low ChE animals made more errors, Table 9C.

Discussion.

Comparison of the control group's learning results with those obtained in Experiment 1 shows that

the lack of an air-gap had little effect. Comparison of the performance results (Figure 4) with those of the food motivated group in Experiment 8 (Figure 3) supports this conclusion.

The trend of the results in the learning phase was similar to that of Experiment 1, but the fact that the inferiority of the DFP + C434 group was not significant suggests that the original experiment over-estimated the true difference between the 2 populations (H_2O + C434 and DFP + C434). Combining the error scores of the two experiments gave the following results:-

H_2O + C434 : $\bar{x}$ = 36.1; SD = 12.1; N = 20.

DFP + C434 : $\bar{x}$ = 60.4; SD = 26.4; N = 20.

$\underline{t}$ = 3.6346; 38 df.; $p < 0.001$.

The performance results were similar to those of the equivalent group in Experiment 8. Combining the results for days 0 - 5 from these experiments raised the difference between the normal and low ChE animals to the 2% level of confidence:-

(H_2O + C434; $\bar{x}$ = 3.8; SD = 2.6; N = 19.

DFP + C434: $\bar{x}$ = 6.7, SD = 4.1; N = 18.

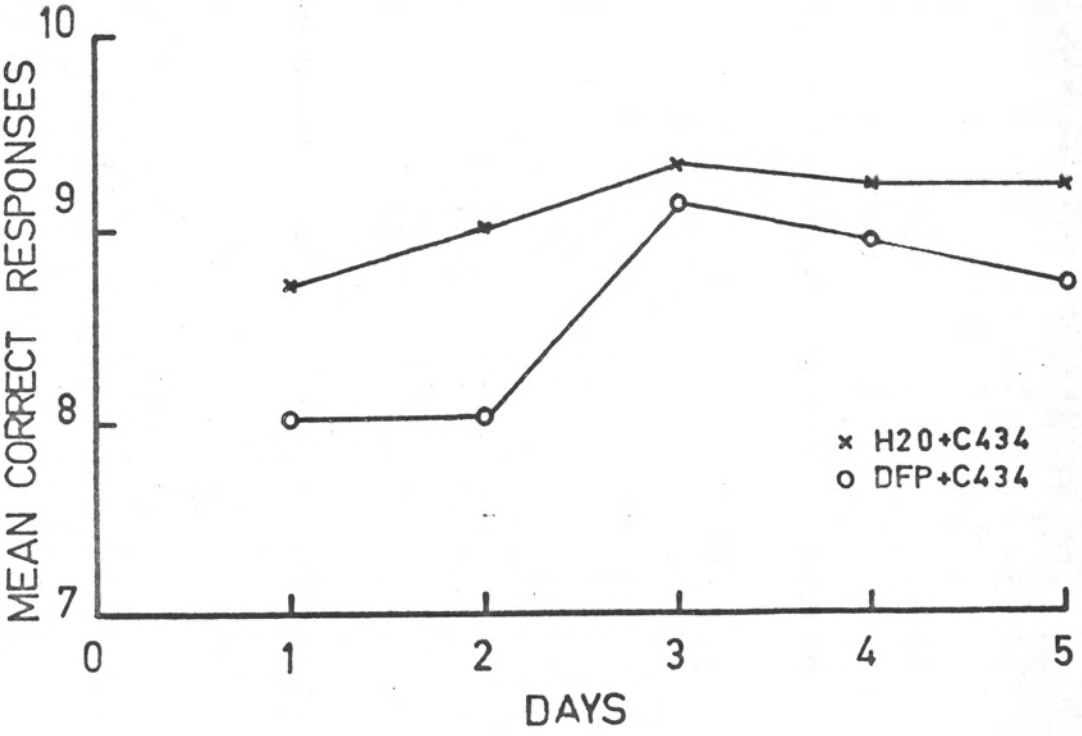
$\underline{t}$ = 2.453; 35 df.; $p < 0.02$.)

TABLE 9. DFP and Horizontal v. Vertical Stripes Discrimination.

	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df	Sig.
A. Learning									
Trials	111.0	23.9	10	145.0	65.2	10	1.470	18	NS
Errors	36.8	10.9	10	57.8	32.8	10	1.825	18	10%
B. Performance									
Total Errors	4.3	1.9	9	7.3	45	9	1.723	16	NS
C. Retention (1)									
3 days	0.667	0.67	9	0.889	0.74	9	0.632	16	NS
6 days	1.111	0.88	9	1.000	0.82	9	0.263	16	NS
12 days	0.556	0.68	9	1.778	1.23	9	2.460	16	5%
36 days	0.444	0.96	9	1.778	1.81	9	1.841	16	10%
Total Errors	2.778	2.70	9	5.444	2.06	9	2.222	16	5%

(1) = Errors in 10 trials.

FIGURE 4 DISCRIMINATION PERFORMANCE



The difference between the H_2O + C434 and DFP + C434 groups decreased with continued training in both Experiments 8 and 9. After 5 days' overtraining, leaving the animals for firstly 3 days, and then a further 6 days had no effect, but intervals of 12 and 36 days between tests increased the difference between the groups. This substantiates the suggestion made in Experiment 8 that overtraining tends to diminish the effect of low ChE on response control.

In the experiments that follow, an attempt was made to clarify the effects of reduced ChE in various operant lever pressing tasks. In all the experiments the basic aim was to train an animal in a situation which maximized the probability of "unnecessary" responses being made.

Experiment 10. Chronically Reduced ChE Activity and Operant Extinction.

An operant extinction situation (for example, lever pressing in the Skinner box) can be thought of as

a successive discrimination task. The lever is firstly associated with reinforcement - that is, it becomes an S+, and later, during extinction, is associated with no reinforcement - that is, it becomes an S-. Initially, the change from the lever being S+ to S- involves some degree of learning in the associative sense used in section 2. However, this learning normally occurs very rapidly; responding declines quickly at first, and then more slowly until it stops. The latter stage, where the response level is low (that is where the lever has already become an S-) is probably mainly a measure of how well an animal can control its responding, rather than of its rate of learning.

Methods.

Eighty-six female rats were used in the experiment. All were on the usual schedule of 2 hours feeding each day. They were trained to press the lever of a Skinner box at fixed ratio 8 as described in Part 4, General Procedures. At the end of the training period the animals were placed in the box, with the feeder and secondary reinforcers switched off, for a 20 minute period on each of the following 16 days.

Six drug treatments were used, as shown in

Table 10 below. The purposes of these were firstly, to compare the DFP + H_2O and DFP + C434 treatments, and secondly, to study the time course of the effects of low ChE.

TABLE 10. Experimental Design.

Drug Group	Time on Drug Before Extinction	N.
1. H_2O + C434	0 Days	14
2. DFP + H_2O	0 "	15
3. DFP + C434	0 "	15
4. DFP + C434	9 "	14
5. DFP + C434	18 "	14
6. DFP + C434	36 "	14

Details of these chronic drug treatments have been set out in Part 1. They were begun at various times before extinction, but the first extinction session was always 24 hours after an injection.

FIGURE 5 CHRONIC TREATMENT WITH DFP AND EXTINCTION

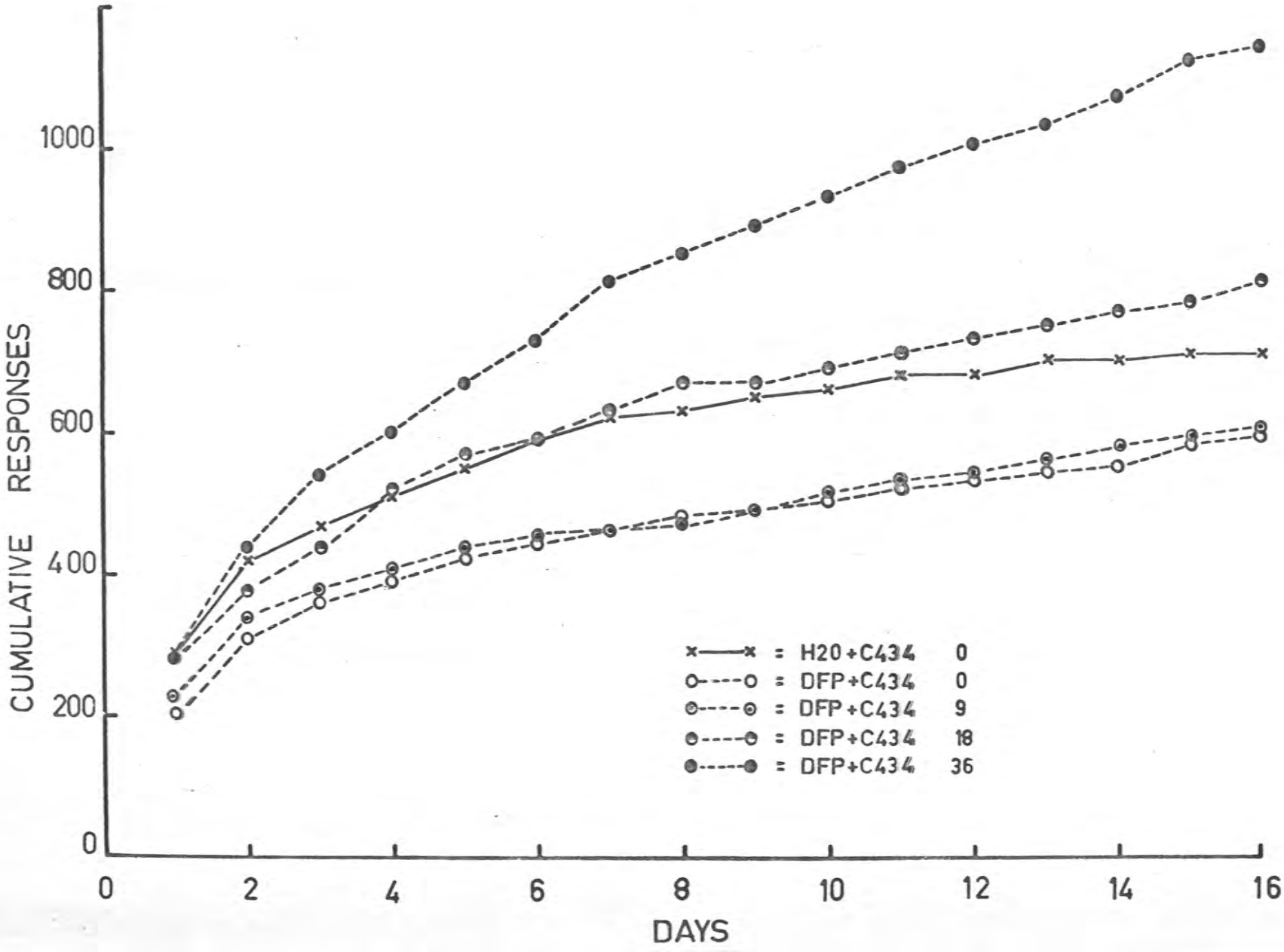


TABLE 11. Chronic Treatment with DFP and Cumulated Extinction Responses.

	H_2O + C434(0)* v. DFP + H_2O(0)			H_2O + C434(0) v. DFP + C434(0)			DFP + C434(0) v. DFP + H_2O(0)			H_2O + C434(0) v. DFP + C434(9)			H_2O + C434(0) v. DFP + C434(18)		
Day	t	df	Sig.	t	df	Sig.	t	df	Sig.	t	df	Sig.	t	df	Sig.
1	3.520	27	1%	2.996	27	1%	0.454	28	NS	1.442	26	NS	0.421	26	NS
2	3.268	27	1%	2.611	27	2%	0.530	28	NS	1.546	26	NS	0.726	26	NS
3	3.483	27	1%	2.617	27	2%	0.928	28	NS	1.444	26	NS	0.490	26	NS
4	3.199	26	1%	2.566	27	2%	0.773	27	NS	1.470	26	NS	0.134	26	NS
5	2.502	26	2%	1.990	27	NS	0.687	27	NS	1.491	26	NS	0.255	26	NS
6	2.708	26	2%	2.126	27	5%	0.715	27	NS	1.712	26	10%	0.027	26	NS
7.	2.441	26	5%	2.011	27	NS	0.507	27	NS	1.813	26	10%	0.183	26	NS
8	2.282	26	5%	1.945	27	NS	0.388	27	NS	1.807	26	10%	0.447	26	NS
9	2.299	26	5%	1.968	27	NS	0.388	27	NS	1.692	26	NS	0.293	26	NS
10	2.304	26	5%	2.053	27	5%	0.284	27	NS	1.621	26	NS	0.243	26	NS
11	2.242	26	5%	1.949	27	NS	0.360	27	NS	1.498	26	NS	0.380	26	NS
12	2.127	26	5%	1.835	27	NS	0.395	27	NS	1.342	26	NS	0.475	26	NS
13	2.006	26	NS	1.752	26	NS	0.258	26	NS	1.212	26	NS	0.552	26	NS
14	1.876	26	NS	1.666	26	NS	0.207	26	NS	1.063	26	NS	0.648	26	NS
15	1.689	26	NS	1.388	26	NS	0.284	26	NS	0.978	26	NS	0.708	26	NS
16	1.564	26	NS	1.256	26	NS	0.287	26	NS	0.897	26	NS	0.911	26	NS

* Figure in bracket ( ) = Number of days animals were kept on drug schedule prior to extinction.

TABLE 11 (Contd.)

	H_2O + C434(0) v. DFP + C434(36)			DFP + C434(0) v. DFP + C434(9)			DFP + C434(0) v. DFP + C434(18)			DFP + C434(0) v. DFP + C434(36)			DFP + C434(18) v. DFP + C434(36)		
Day	t	df	Sig.	t	df	Sig.	t	df	Sig.	t	df	Sig.	t	df	Sig.
1	0.217	26	NS	0.933	27	NS	2.383	27	5%	2.286	27	5%	0.214	26	NS
2	0.307	26	NS	0.643	27	NS	1.765	27	10%	2.808	27	1%	1.391	26	NS
3	1.058	26	NS	0.466	27	NS	1.919	27	10%	3.598	27	1%	2.006	26	10%
4	1.438	26	NS	0.360	27	NS	2.599	27	1%	3.964	27	0.1%	1.658	26	NS
5	1.653	26	NS	0.345	27	NS	2.823	27	1%	4.367	27	0.1%	1.690	26	NS
6	1.840	26	10%	0.157	27	NS	2.571	27	2%	4.339	27	0.1%	2.032	26	10%
7	1.924	26	10%	0.006	27	NS	2.597	27	2%	4.026	27	0.1%	1.920	26	10%
8	2.085	26	5%	0.058	27	NS	2.690	27	2%	4.022	27	0.1%	1.796	26	10%
9	2.146	26	5%	0.000	27	NS	2.562	27	2%	4.090	27	0.1%	2.027	26	10%
10	2.251	26	5%	0.113	27	NS	2.552	27	2%	4.171	27	0.1%	2.158	26	5%
11	2.374	26	5%	0.122	27	NS	2.586	27	2%	4.206	27	0.1%	2.166	26	5%
12	2.457	26	5%	0.133	27	NS	2.563	27	2%	4.169	27	0.1%	2.165	26	5%
13	2.516	26	2%	0.231	26	NS	2.551	26	2%	4.093	26	0.1%	2.161	26	5%
14	2.658	26	2%	0.287	26	NS	2.526	26	2%	4.113	26	0.1%	2.215	26	5%
15	2.786	26	1%	0.127	26	NS	2.269	26	5%	3.996	26	0.1%	2.294	26	5%
16	2.850	26	1%	0.100	26	NS	2.274	26	5%	3.927	26	0.1%	2.147	26	5%

Results.

The number of responses in each 20 minute extinction period was recorded for all animals. Cumulative curves derived from these scores are shown in Figure 4 and their statistical analysis in Table 11.

Both the DFP + H_2O and DFP + C434 groups in which injections started 24 hours before extinction had lower cumulative curves than the controls. There were no significant differences between these 2 groups. (The DFP + H_2O curve is not shown on Figure 4 to avoid confusion, since it is almost a duplicate of the DFP + C434 curve).

With increasing time on drug before extinction there was a corresponding increase in the extinction curve until at 36 days it was greater than that of the control group.

Finally, although the H_2O + C434 group was asymptotic from days 13 - 16, all the low ChE groups' curves were still rising. Over the last 8 days of extinction the response rate was almost constant for all six groups. The average response rates for this period are shown in Table 12 below.

TABLE 12. Average Response Rates for the last 8 days of Extinction.

Group	Responses per day
1. H_2O + C434 (0)	10.00
2. DFP + H_2O (0)	13.63
3. DFP + C434 (0)	14.25
4. DFP + C434 (9)	16.75
5. DFP + C434 (18)	18.13
6. DFP + C434 (36)	32.00

The difference between Group 6 and Group 1 was significant at the 5% level by t test. ($t = 2.3567$; 26 df; $p < 0.05$). There was no significant difference between Groups 5 and 1. ($t = 1.5546$; 26 df; $p > 0.05$).

Discussion.

The lack of any difference between DFP + H_2O and DFP + C434 (Day 0) animals is consistent with the results on F.R. performance presented in Part 4, where it was found that the reactivator had little,

if any, effect upon ongoing F.R. responding of animals injected with DFP. The decreased cumulative curves of the DFP + C434 animals in groups (0) and (9) is probably a consequence of the effects shown in Part 4. That is, lever pressing is simply more aversive to animals with lowChE.

The large increase in the 36 day group cannot be explained in this way, and it is necessary to postulate some additional mechanism.

Since the cumulative curve for the 18 day DFP group was close to the control one, lever pressing itself can be assumed to no longer have aversive properties. The <u>increase</u> in the 36 day group can be regarded as a reduced power of the S- provided by the lever, to effectively control the animals' responding.

Experiment 11. Effects of chronically reduced ChE on successive acquisition and extinction.

Introduction.

This experiment is basically similar to Experiment 10. Its main purpose was to see whether an effect of DFP on response control would be more

apparent if habit strength during extinction was maintained at a higher level by repeated re-acquisition periods.

It has been shown previously that rats trained on a program of alternating reinforcement-extinction will gradually decrease the number of responses which they make in the extinction periods, Wickens & Miles (1954). This phenomenon has been treated as an instance of a reinforcement based discrimination by Bullock & Smith (1953). The situation has been shown to have the characteristics of normal extinction in that the effect appears less readily after partial than after continuous reinforcement, Perkins & Caccioppo (1950).

Methods.

Twenty female rats were trained, as in Experiment 10, to press the lever of a Skinner box at F.R. 4. At the end of the training period 10 animals were injected with H_2O + C434, and 10 with DFP + C434, as described in Part 1. On the following day the animals were allowed to obtain 20 reinforcements at F.R. 4., the feeder and secondary reinforcers were then switched off, and the animals were all given 20

minutes extinction pressing. This procedure was repeated daily for 14 days.

Results.

Comparison of the H_2O + C434 group with data from a normal extinction experiment in which there was no periodic reacquisition (Glow & Rose, 1966) showed that response strength was increased by the daily 20 pellets, Table 13 Groups A and B.

However, there were no significant differences between the two acquisition - extinction groups. When responses were summed over all 14 days for the four 5 minute segments of each extinction period, and for the entire 20 minutes, there were likewise no differences, Table 14.

TABLE 13. Normal Extinction Compared to Successive Acquisition and Extinction.

	Group (1)			Statistical Comparison			
	A	B	C	A versus B		B versus C	
Days	$\bar{X}$(2)	$\bar{X}$	$\bar{X}$	t	Sig.	t	Sig.
.1	240.5	190.2	169.0	1.742	10%	0.817	NS
2	98.3	83.2	83.4	0.732	NS	0.009	NS
3	53.1	56.6	64.1	0.300	NS	0.572	NS
4	28.8	60.2	61.9	2.772	2%	0.097	NS
5	14.8	54.6	50.9	2.544	5%	0.154	NS
6	15.3	29.7	46.7	1.858	10%	1.394	NS
7	14.2	34.8	39.8	2.761	2%	0.433	NS
8	12.9	28.8	30.1	2.255	5%	0.135	NS
9	9.1	28.4	42.0	2.717	2%	1.092	NS
10	5.3	23.0	34.8	1.900	10%	0.867	NS
11	9.5	19.2	26.6	1.453	NS	0.504	NS
12	3.0	16.7	26.8	3.445	1%	1.203	NS
13	0.5	18.3	24.5	2.844	2%	0.534	NS
14	9.6	19.4	22.9	0.697	NS	0.358	NS
	N=10	N=10	N=10				

(1) Group A = Normal Extinction (Taken from Glow and Rose (in press)). B = Acquisition-Extinction, H_2O + C434. C = Acquisition-Extinction, DFP + C434.

(2) $\bar{X}$ = Mean Daily Responses per 20 min. Extinction Period.

TABLE 14. Cumulated extinction responses for days 1 - 14.

Extinction Period	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df	Sig.
0-5 mins.	378	79	10	341	164	10	0.619	18	NS
5-10 mins.	128	53	10	145	93	10	0.476	18	NS
10-15 mins.	94	50	10	113	67	10	0.708	18	NS
15-20 mins.	52	33	10	87	85	10	1.148	18	NS
0-20 mins.	652	184	10	686	374	10	0.245	18	NS

The time taken to obtain 20 reinforcements was longer in the DFP + C434 group (See Table 15), as was expected from the results of Part 4.

TABLE 15. Times per Twenty Reinforcements.

Day	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$(1)	SD	N	$\bar{X}$	SD	N	t	df	Sig.
0 (2)	235	52	10	256	46	10	0.908	18	NS
1	224	55	10	281	91	10	1.601	18	NS
2	263	83	10	365	181	10	1.540	18	NS
3	339	97	10	443	221	10	1.227	18	NS
4	281	159	10	566	334	10	2.090	18	10%
5	288	88	10	383	246	10	1.097	18	NS
6	272	64	10	361	189	10	1.326	18	NS
7	279	41	10	391	207	10	1.517	18	NS
8	274	58	10	338	97	10	1.681	18	NS
9	290	68	10	359	175	10	1.052	18	NS
10	350	194	10	500	229	10	1.501	18	NS
11	324	96	10	444	236	10	1.411	18	NS
12	331	129	10	401	131	10	1.099	18	NS
13	296	63	10	571	228	10	3.483	18	1%
14	342	86	10	518	228	10	2.166	18	5%

(1) Mean time in seconds.

(2) Mean time for the 5 days before extinction.

Discussion.

The expectation that an increase in habit strength should increase the difference between normal and low ChE animals was not fulfilled. The slowing in the reinforced press-rate could plausibly be held responsible for this; and it is possible that if the extinction rate could be calculated relative to the reinforced rate, then it would in fact be higher in the low ChE group.

The next experiment in this series was a simple form of single stimulus visual discrimination. The reasons for choosing this situation were firstly, that it provided a simple measure of response control in the S- periods, and secondly, it had the advantage of giving a concurrent measure of response strength to the S+, measured in the same way as response to S-.

Experiment 12. Chronic reduction of ChE activity and operant discrimination learning.

Methods.

Forty female rats, maintained on 2 hours feeding each day were used. The apparatus was a Skinner box, in which the lever produced food reinforcement, together with visual and auditory secondary reinforcers, at F.R. 8 in the S+ periods but not in the S-. In the S+ period a small light (2w.) was on below the lever; in S- periods the light was off. S+ and S- were equal in length and were presented in alternation. The length of the stimulus periods varied from day to day. Animals worked on this routine for 10 minutes each day, after being trained to lever press over 5 days for continuous reinforcement. On days 1 to 4 the lengths of the stimulus periods were 30, 30, 20 and 20 secs. respectively. The periods were then 30, 15, 20; 30, 15, 20; and so on. Training continued for 25 days.

Two groups of 20 were run. One group received chronic treatment with H_2O + C434 and one with DFP + C434. An additional control group of 5 H_2O + C434

animals was run with the stimulus light on in both S+ and S- periods.

Results.

The group that had no differential stimulus to signal reinforcement or no reinforcement showed no sign of learning the problem, Table 16. This shows that although the stimulus periods were not randomized, the animals did respond to the light, not to the presence of the reinforcer or to the temporal sequence of reinforced and un-reinforced periods.

TABLE 16. Ratio of responses in reinforced periods to total responses, for animals which had no differential stimulation.

Day	Average Ratio
1-5	0.442
6-10	0.446
11-15	0.426
16-20	0.422
21-25	0.426

In these 5 animals, responding in the unreinforced periods was consistently slightly greater than in the reinforced, which brought the ratio of reinforced to total responses below 0.5.

Curves for responding in S+ and S- and the ratio of daily S+ to (S+ + S-) responses, are shown in Figure 5 and Table 17.

The number of responses made in S+ periods was less in the DFP + C434 animals than in the controls, but the positions were reversed in the case of responses in S-. When each animal's S- score was expressed as a proportion of the total number of responses in ten minutes, the DFP + C434 group was consistently and significantly less than the H_2O + C434 animals.

Discussion.

The results indicate that although free responding for food is decreased (which is consistent with the results reported in Part 4, and for the extinction groups DFP + C434, Days 0 and 9, in the previous experiment) the ability to withhold response to an unrewarded stimulus is also reduced. No new mechanism

is necessary to account for these results. The hypothesis used to explain the increased responding of Group 6 in Experiment 10 - that the 'power' of S-over behaviour is in some way weakened by low ChE - is adequate in this case too.

FIGURE 6. OPERANT DISCRIMINATION LEARNING

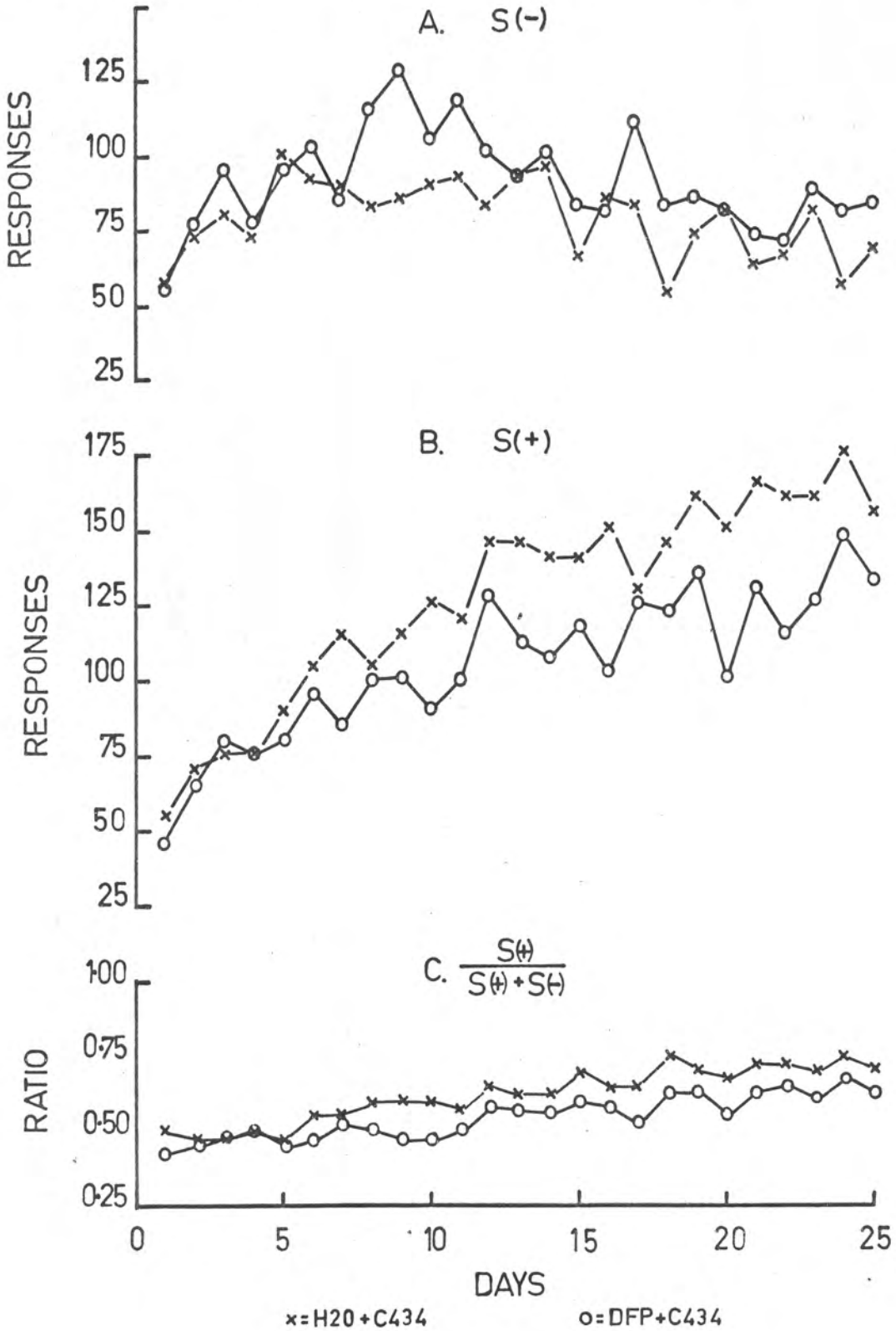


TABLE 17. Operant Discrimination Learning: Statistical Comparison of H_2O + C434 and DFP + C434 Treatments.

	A. S-			B. S+			C $\frac{S+}{S+ + S-}$		
Day	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
1*	0.042	38	NS	1.314	38	NS	2.018	38	10%
2	0.323	38	NS	0.511	38	NS	1.144	38	NS
3	0.967	38	NS	0.278	38	NS	0.448	38	NS
4	0.307	38	NS	0.244	38	NS	0.288	38	NS
5	0.233	38	NS	1.008	38	NS	0.637	38	NS
6	0.688	38	NS	0.888	38	NS	2.633	38	2%
7	0.314	38	NS	2.530	38	2%	1.013	38	NS
8	1.665	38	NS	0.601	38	NS	3.104	38	1%
9	1.959	38	10%	0.863	38	NS	3.859	38	0.1%
10	0.908	38	NS	2.481	38	2%	3.509	38	1%
11	1.305	38	NS	1.402	38	NS	2.071	38	5%
12	1.153	38	NS	1.204	38	NS	1.938	38	10%
13	0.046	38	NS	2.150	38	5%	1.780	38	10%
14	0.285	38	NS	2.008	38	10%	2.051	38	5%
15	1.341	38	NS	1.201	38	NS	2.619	38	2%
16	0.337	38	NS	2.804	38	1%	1.790	38	10%
17	1.926	38	10%	0.359	38	NS	2.647	38	2%
18	1.956	38	10%	1.317	38	NS	2.447	38	2%
19	0.793	38	NS	1.445	38	NS	1.563	38	NS
20	0.135	38	NS	2.779	38	1%	1.832	38	10%
21	0.557	38	NS	2.221	38	5%	2.168	38	5%
22	0.120	38	NS	2.934	38	1%	1.474	38	NS
23	0.298	38	NS	1.883	38	10%	1.734	38	10%
24	1.299	38	NS	1.375	38	NS	1.098	38	NS
25	0.820	38	NS	1.228	38	NS	1.420	38	NS

* - shows point of injection.

Experiment 13. Effects of chronically reduced ChE on double-lever alternation performance.

Introduction.

The basic plan of this experiment was to provide a task consisting of two separate segments, in which one segment had to be completed before the final segment would produce reinforcement. Any effects of low ChE on response control were expected to show up as an attempt to perform the final segment before completing the first.

Methods.

Sixteen animals were trained to F.R. 8, in a single lever Skinner box, as described in Experiment 1, Part 4. They were then transferred to a 2 lever box and trained to press the two levers in alternation. The dimensions of the box were 11 ins. x 9 ins. x 9 ins. The 2 levers were set in the 11 in. wall and were 6 in. apart, centre to centre.

The food pot was between the 2 levers.

The right-hand side (R.H.S.) lever was the one which produced reinforcement. No reinforcement could be obtained before the specified number of responses had been made on the left-hand side (L.H.S.) lever.

The training procedure in the 2 lever box is shown below.

Days	Responses on L.H.S. Lever.	Responses on R.H.S. Lever
1 - 3	1	1
4 - 5	4	1
6 - 8	8	1
9 - 14	8	1
15 - 19	8	1

On days 1 - 8 a small 2 w. light was on beneath the bar to be pressed. (When reinforcement could be obtained it was on under the R.H.S. lever, and under the L.H.S. lever when the specified number of responses had not yet been made).

From day 9 onwards there was no signal light, and a 400 cycles per second square wave was introduced to mask machine noise.

From days 1 - 14 the animals worked for 20

pellets daily, and from days 15 - 19 for 10 pellets. Delivery of the pellet was always accompanied by light and buzzer secondary reinforcers.

After training on day 19, 8 animals were started on the chronic H_2O + C434 schedule, and 8 on DFP + C434. Training was continued in the same way for the next 5 days. Responses made on the R.H.S. lever before completing the required number on the L.H.S. lever were counted for all animals. These responses will henceforth be referred to as R.H.S. Error Responses.

Results.

For the 5 days before and after injection, the total number of R.H.S. error responses was calculated for each animal. The group means are shown in Table 18.

For each animal the post-injection total was subtracted from the pre-injection one. The resultant difference scores of the 2 groups and also the pre and post injection raw scores, were then compared by t tests. The increase after injection was significantly greater in the DFP + C434 group than in the H_2O + C434 controls.

TABLE 18. R.H.S. Lever Error Responses before and after DFP.

	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{x}$	SD	N	$\bar{x}$	SD	N	t	df	Sig.
Pre-injection	42.2	13.9	8	46.1	25.6	8	0.3536	14	NS
Post-injection	51.7	21.5	8	82.3	30.3	8	2.1748	14	5%
Difference	9.5	15.4	8	36.2	16.2	8	3.1560	14	1%

Discussion

The results show that reduced ChE increased R.H.S. lever error responses, as was predicted. This is in agreement with the interpretation of the extinction and operant discrimination experiments, that the ability to inhibit a response is lessened by reduced ChE.

The fact that there was a change in the first five days after injection suggests that either the alternation situation is more sensitive to the effects of low ChE on response control than are simple extinction and operant discrimination, or else that

other factors besides loss of response control helped to determine the increased R.H.S. responses. It is possible that the results could be accounted for by the hypothesis proposed in Part 4, of an increase in the aversiveness of lever pressing after DFP. Such increased aversiveness could make the animal try to avoid that part of the task requiring the greatest amount of work. The animal could be thought to attempt a "short-circuit" of the normal procedure. Against this hypothesis is the fact that the total amount of work performed increased after DFP, but such an objection is not crucial unless it is assumed that the subjects' behaviour must necessarily be 'rational', that is, such as to keep the amount of effort involved in obtaining reinforcement to a minimum.

Information on the necessity for another explanatory hypothesis, additional to that of reduced ability to inhibit response, could be obtained by using single lever situations, analogous to the extinction and discrimination ones, but in which greater stress is put on the animal to control response. Two such situations would be fixed interval and drl responding. In both these there should be increased responding after DFP, if the alternation results were

in fact due to loss of response control.

To summarize:- the bulk of the evidence indicates that reduced ChE has some effect on the accuracy of performance - that is response control - but not on discrimination and reversal learning ability. The effect on response control is attenuated by continued overtraining and also by giving shock for errors.

PART SIX.

PHYSIOLOGICAL EXPLANATIONS OF THE EFFECTS OF REDUCED CHOLINESTERASE ACTIVITY.

The principal fact to be explained by any physiological account of the relationship between the ACh - S and behaviour is the lack of any marked effect upon learning ability or retention after reduction of ChE. To check that the results on which this statement is based were in fact due to low ChE, and not specifically to the use of DFP, some of the observations made in Parts 4 & 5 were repeated, using a different inhibitor of ChE.

Experiment 1. The effects of eserine sulphate on fixed ratio lever pressing, and post-criterion discrimination performance.

Methods.

Eight animals were trained at F.R.8, as in Part 4, until their response rates were stable. They were given 5 minutes testing each day, and the number of responses made in this time was counted. Eserine sulphate and equivalent volumes of water were injected intraperitoneally on alternate days. (Eserine sulphate is a reversible inhibitor of ChE, Goodman and

Gilman (1955)). Three doses were used, 0.25, 0.50 and 1.00 mgm/kgm - and for each dose a test was made 10 mins., 30 mins and 60 mins. after injection. The 10 minute determinations were made first, followed by those at 30 and 60 minutes. The order of the doses was 0.25, 0.50 and 1.00 at each time.

Ten animals which had previously learned a black versus white discrimination as described in Part 5, Experiment 1, were overtrained for 5 days, 10 trials per day. They were then injected in the same ways as the lever pressing subjects, and tested at the same intervals and in the same order.

Results.

Eserine reduced the number of responses made per 5 minutes. The effect increased with increasing dose and decreased over time, Table 1A. (Since no animal responded 10 minutes after 0.50 mgm/kgm, no test was made at 1.00 mgm/kgm.) Paired *t* tests were made of each eserine session against the preceding water session. These were significant for all except the 60 minutes after 0.25 mgm/kgm comparison, Table 1B.

TABLE 1A. Mean responses after eserine.

DOSE (mgm/kgm)	TIME AFTER INJECTION					
	10 min.		30 min.		60 min.	
	W	E	W	E	W	E
0.25	136.3	35.6	141.1	109.6	140.3	133.8
0.50	146.1	0.0	147.4	94.6	135.8	113.6
1.00	-	-	150.1	33.2	141.4	79.4

W = Water Day E = Eserine Day

TABLE 1B. Statistical Comparison of W. and E. Days.

DOSE. (mgm/kgm)	10 min.			30 min.			60 min.		
	t	df.	Sig.	t	df.	Sig.	t	df.	Sig.
0.25	4.421	7	1%	4.007	7	1%	1.154	7	NS
0.50	14.622	7	0.1%	3.823	7	1%	3.840	7	1%
1.00	-	-	-	4.459	7	1%	8.223	7	0.1%

In contrast the discrimination results show no effect of eserine at the same doses and testing times, Table 2. (There was some slight suggestion that at 0,50 mgm/kgm + 10 minutes, performance was worse. Three animals made errors - they scored 8, 9 and 9 respectively, whereas no errors were made on the preceding water day. Since the equivalent F.R. animals did not press at all this slight decrease could be due to the generally poor condition of the animals.) Ten and 30 minutes after 1.00 mgm/kgm the animals were too sick to run.

TABLE 2. Mean correct trials after eserine.

DOSE (mgm/kgm)	TIME AFTER INJECTION					
	10 min.		30 min.		60 min.	
	W	E	W	E	W	E
0.25	10.0	9.9	10.0	9.9	9.8	10.0
0.50	10.0	9.5	10.0	10.0	9.9	10.0
1.00	10.0	-	10.0	-	10.0	10.0

W = Water Day E = Eserine day.

Discussion.

The general slowing effect of eserine on F.R. lever pressing was similar to that of DFP. Paper recordings of response distribution (as in Part 4) showed that the usual clustering of responses was maintained but that time out (T O) was much longer. This effect was also similar to that of DFP.

The lack of change in black versus white discrimination performance is the same as the results obtained from this task 2 hours after DFP; see Part 5 Experiment 2.

The eserine results therefore parallel those of DFP on both lever pressing and discrimination, and it can be assumed that this is due to the drugs' common ability to inhibit ChE.

In the remainder of this part various hypotheses which could account for the data will be discussed.

(1) The simplest is that the ACh - S is not directly involved in the learning process. There is good evidence from the studies of Aprison (1958) and White (1956) on the production of forced circling by anticholinesterases that the ACh - S is involved in the motor pathway, especially in the caudate nucleus. It is possible that the effects on response control could be due to interference with the functions of the caudate since similar kinds of behaviour have been reported after caudate lesion by Denny-Brown (1962) and Whittier & Orr (1962). However, ChE is present in most areas of the brain, even those with no motor function, and to assume that it is present without function runs counter to the idea of the general adaptiveness of bodily mechanisms. Even if this idea is rejected as teleological, there remains considerable physiological evidence that the ACh - S is in some way related to central neural activity, (see Part 1). ACh sensitive neurones have been found in the visual cortex, Salmoiraghi & Bloom (1964) and DFP influences evoked potentials in the visual cortex, Marrazzi (1953), which suggests that the ACh - S would

play some part in visual discrimination. The hypothesis of non-involvement in learning will therefore be provisionally rejected, although on the behavioural evidence alone it is certainly plausible.

(2) The second hypothesis is that the nervous system can function adequately in various states. For example, if all the ChE of the brain were simultaneously reduced, there would presumably be altered firing in all cholinergic neurones, but the overall 'balance' of the system would be retained. The production of forced circling by reduced ChE which was discussed in Part 2 provides an example of this. Reduction of ChE on one side of the brain resulted in circling, whereas equivalent reductions on both sides had no effect. A general change of state in this case, allowed the system to function normally.

The ACh content of rat brain varies with the animal's state of behavioural excitation, being higher in anaesthesia and sleep, and lower during electric - shock, and convulsions, Richter & Crossland (1949). This is presumably due to changes in the release, and consequent inactivation of ACh.

Transient variations in released ACh (and hence in firing rate of central neurones) are therefore probably a normal part of an animal's experience, and any manipulation which tended to mimic such variations would be predicted to have relatively little effect.

Overton (1964) has reported a phenomenon which is in some respects related to the ideas discussed above. He found that rats could learn a T maze problem whether or not they were treated with 25 mgm/kgm of sodium pentobarbital. However, learning in the drugged state was not apparent in the undrugged state, nor was learning in the undrugged state apparent in the drugged state. That is, although function was possible in either state, there was a dissociation between them. The Experiments in Part 5 show that this does not occur for the case of learning under normal ChE, with testing under reduced ChE. The experiment reported below examined the converse case of learning under reduced ChE, with retention under normal ChE.

Experiment 2. Learning under chronically reduced ChE activity and retention after recovery.

Methods.

A group of 20 rats was trained to choose the black gate in a black/white discrimination problem. Five rats were chronically treated with H_2O + C434, and 15 with DFP + C434. Maintenance and training conditions were the same as for Experiment 1, Part 5.

After reaching criterion each rat was left in its home cage for 30 days. During this time the 5 H_2O + C434 rats and 5 of the DFP + C434 group continued on the same injection schedule, while the other 10 DFP animals were injected with H_2O + C434.

At the end of 30 days (± one day to make the final day fall on an injection day) 5 of the DFP - recovery animals were reinjected with 1mgm/kgm DFP + 50 mgm/kgm C434.

On the following day retraining was begun on the original discrimination, and 10 trials given on successive days to a criterion of 18/20.

Results.

The statistical analysis of the retention scores is shown in Tables 3A and 3B. (C refers to H_2O + C434, and D refers to DFP + C434. C-C-C means H_2O + C434 in the learning, delay and retention periods, etc.)

The only noticeable variation among the 4 groups was the inferior retention of the D-D-D animals, which differed from each of the other 3 groups at the 10% level. This effect is similar to that found in Experiment 9, Part 5. No other comparison approached significance.

TABLE 3A. Learning and Relearning Scores.

Group (1)	Learning			Relearning		
	$\bar{X}$(2)	SD	N	$\bar{X}$	SD	N
C-C-C	26.8	9.1	5	2.0	2.1	5
D-D-D	33.4	3.9	5	11.6	9.0	5
D-C-C	37.0	21.1	5	2.4	3.8	5
D-C-D	29.4	8.1	5	3.0	1.8	5

TABLE 3B. Statistical Analysis.

Groups	Comparison of Retention Scores		
	t	df.	Sig.
C-C-C v. D-D-D	2.082	8	10%
C-C-C v. D-C-D	0.725	8	NS
C-C-C v. D-C-C	0.183	8	NS
D-D-D v. D-C-D	1.878	8	10%
D-D-D v. D-C-C	1.885	8	10%
D-C-D v. D-C-C	0.284	8	NS

(1) C = Control; D = Drug

(2) $\bar{X}$ = Mean errors to criterion.

Discussion.

The results indicate that learning during low ChE is retained when ChE has recovered to near normal values (See Part 1 - Fig. 1). "Dissociation", of the kind found by Overton (1964) therefore does not occur in the case of DFP.

(3) Another possible explanation is that the neurone rapidly regulates the amount of released ACh by either a redistribution of the remaining enzyme, or by some feedback mechanism that inhibits the production and/or release of ACh. Michaelis et al (1954) found that 'free' ACh in the cerebral cortex of rabbits rose sharply after injection of DFP but returned to normal within 24 hours. At this time the ChE activity was still only 20% of normal (determined from the data of Mazur and Bodansky (1946)). The fact that ChE activity was still low does not mean that it could not be responsible for the fall in ACh. McIsaac and Koelle (1959) found that only very small amounts of ChE were necessary to prevent facilitation of

ganglionic potentials by DFP, and suggested that only 'external' ChE is functional, while reserve ChE within the cell plays no immediate part in transmission. Fleisher et al (1960a) found evidence of a similar kind using an isolated nerve-muscle preparation. Neuromuscular block produced by an anticholinesterase was reversed by C434 before there was any detectable increase in ChE activity.

If this regulation were the case, and if ACh is in fact involved in learning, then problems learned soon after the injection of an anticholinesterase should be more affected than those learned later. Experiment 3 gives data relevant to this hypothesis.

Experiment 3. Daily treatment with DFP and reversal learning.

Methods.

The subjects used were those from Experiment 7, Part 5. Ten animals had been treated with DFP + C434 and ten with H_2O + C434, for 12 days. All had learned a black/white discrimination and the

reversal of it.

The subjects were retrained to criterion on the first reversal, in exactly the same way as in Experiment 7, on the two days before the present experiment began.

On the second day of overtraining all subjects received their final injection, on the normal chronic injection schedule.

On the following day, the experimental animals received 0.25 mgm/kgm DFP + 12.5 mgm/kgm C434, while the controls were given equivalent volumes of H_2O + C434. Ten training trials were given on the reversal of the previous task 2 hours after the injection. The injections and training sessions 2 hours after injection, were continued until a criterion of 18/20 was achieved.

When the experiment was finished 5 experimental animals were killed 2 hours after injection, and their brains assayed for ChE.

Results.

Table 4 shows that the injection procedure did not significantly affect reversal learning. The average whole brain ChE value for the 5 experimental animals was 3.7% of normal, SD = 2.8.

TABLE 4. Daily treatment with DFP, and reversal learning.

	H_2O + C434			DFP + C434			Statistical Comparison		
	$\bar{X}$	SD	N	$\bar{X}$	SD	N	t	df.	Sig.
Total Trials	71.0	15.8	10	77.5	20.5	8	0.717	16	NS
Error Trials	26.2	7.6	10	30.6	11.4	8	0.931	16	NS

Discussion.

The daily injections produced gross motor disabilities in the experimental animals. Some would move through the apparatus in an abnormal hopping manner, while others had complete paralysis of the hind limbs and could only drag themselves along. Two of the DFP subjects died during the experiment.

Since these symptoms were evident during training, either the ACh - S is not crucial to learning, or, if it is crucial, then the cholinergic structures necessary for learning must adapt more quickly than the structures responsible for the motor symptoms.

In addition, this adaptation must occur within 2 hours after injection, in the presence of an extremely low level of ChE.

An attempt was made to repeat this kind of experiment under different conditions. The aim was to train animals to criterion in a single block of trials at various times after the first injection of DFP. However, this was unsuccessful for two reasons. Firstly massing of trials increased the variability among animals and greatly extended the learning period; the control H_2O + C 434 animals (Table 5) made over twice as many errors as did comparable control animals that were given 10 trials each day (Part 5, Experiment 7, Table 7). Secondly, the animals would not run until 24 hours after injection. This last observation is interesting since it shows that the effect of DFP on general condition of an animal interacts with the amount of practice it has had at the task required of it. No animal was incapable of running 2 hours after injection in the acute post-criterion experiments of Part 5.

The data which was obtained is summarized in Table 5 below. It gives no indication that DFP has any greater effect than in the reversal task.

TABLE 5. Black versus white discrimination learning of animals trained to criterion in a single block of trials, 24 hours after injection of 1 mgm/kgm DFP.

	H_2O + C434		DFP + C434	
	$\bar{X}$	N	$\bar{X}$	N
Error Trials	24.5	4	20.8	4

The results do not support the hypothesis that ACh is directly involved in learning, but so rapidly regulated by the neurone that changes in ChE have little effect. However, behavioural evidence alone is insufficient to completely exclude this possibility. The crucial information needed is the effect of the reduced ChE levels used in the above experiments on brain ACh levels. Without this basic data any hypothetical regulatory mechanisms are speculations about a speculation.

(4) The final possibility to be considered is that if the synapse could be regarded as a form of chemical

switch that can take only two positions, 'on' or 'off', then any manipulation which simply made the switch close more strongly should have little effect on behaviour. Reduction of ChE can be thought of as such a manipulation since it should increase transmitter action (up to the point at which transmission is blocked by excess ACh). However, any manipulation that prevented the switch from closing would be predicted to have a greater effect. The hypothesis makes the assumption that it is the connection of various on/off elements that mediates learning and memory, and that frequency of firing in the elements is responsible for only the intensive aspects of these processes. Information on this prediction was obtained by giving an anticholinergic drug to animals trained on various visual discriminations.

Experiment 4. The effects of atropine and atropine methyl nitrate upon discrimination performance.

Methods.

The animals used had been controls

(H_2O + C434) in various discrimination experiments. They were all given 100 trials of overlearning (10 trials per day) before being tested in the present experiment. Atropine sulphate (AS) and Atropine Methyl Nitrate (AMN) were injected at various concentrations 30 minutes before the animals were tested. (Atropine is thought to prevent ACh from combining with post-synaptic receptor sites, Goodman & Gilman (1955).

Atropine Methyl Nitrate has similar effects to AS, but since it passes the blood brain barrier only in small amounts, these are mainly peripheral, Paul-David et al (1960). The drugs were made up in distilled pyrogen free water immediately before injection, at concentrations which resulted in 1 ml. of solution being given per kgm. body weight to obtain the required dose.

The conditions and results of the various experiments are set out in Table 6.

Results.

For groups 2, 4, 5, 6, 7 & 8, the error scores for each animal after AS. were compared to the preceding day's scores after injection of water.

TABLE 6. Performance of learned discriminations after atropine and atropine methyl nitrate.

Group		Task	Days									
			1	2	3	4	5	6	7	8	9	10
1.	A	H. v. V.	W	AS	W	AMN	W	AS	W	AS	W	AS
	B	Shock		5		5		10		50		100
	C	N = 4	10	10	10	10	10	10	10	10	10	10
2	A	H. v. V.	W	AS	W	AMN	W					
	B	Shock		100		100						
	C	N = 4	10	8.8	10	10	10					
3	A	H. v. V.	W	AMN								
	B	Shock		100								
	C	N = 5	10	9.8								
4	A	H. v. V.	W	AS	W	AS	W	AS	W	AS	W	
	B	Food		100		100		100		100		
	C	N = 5	10	7.6	9.8	6.2	9.8	9.4	10	9.5	10	
5	A	C. v. T.	W	AS	W	AMN	W	AS				
	B	Shock		100		100		100				
	C	N = 1	9	5	9	10	9	9				
6	A	B. v. W.	W	AS	W	AMN	W					
	B	Shock		100		100						
	C	N = 4	10	10	10	10	10					
7	A	H. v. V.	W	AS	W	AS	W	AS	W	AS	W	
	B	Food		2		2		2		2		
	C	N = 5	9.2	8.0	9.0	8.0	9.4	7.0	9.8	7.8	9.6	
8	A	H. v. V.	W	W	AS							
	B	Shock			2							
	C	N = 5	10	10	9.8							

H. v. V. = Horizontal versus Vertical Stripes

B. v. W. = Black versus White.

C. v. T. = Circle versus Triangle.

Row A = Drug injected (W - Water, AS - Atropine Sulphate, AMN - Atropine Methyl Nitrate)

Row B = Dose in mgm/kgm.

Row C = Mean Correct Trials.

Fifty-five such paired observations were made, and of these, 29 showed an increase of errors after AS., 2 a decrease, and 24 no change. The mean difference between post-water and post-AS error scores was 1.426. A *t* test on the difference scores showed that this mean was significantly greater than zero ($t = 5.2063$, 54 df., $P < 0.001$.)

The numbers in the 8 experimental groups were insufficient for separate statistical analysis, but the means do give some idea of the amount of change produced by AS, and AMN, especially as performance levels after water were both high and stable. Group 1. No animal made an error under any of the treatment conditions. However, since adaptation to atropine is known to occur (Goodman & Gilman 1955) it is possible that the higher doses would have some effect if given to previously untreated animals. Group 2. Shows that this suggestion is true. Three out of 4 animals made more errors after 100 mgm/kgm of AS. than after water. A subsequent injection of AMN. had no effect. After AS two animals scored 8/10, one 9/10 and one 10/10. The magnitude of the effect of AS was therefore quite small, and was possibly an artefact due to the general sickness of

the animals. (Some in fact could barely move through the apparatus).

Group 3. This group was run to determine whether the results obtained with AMN in group 2 were due to adaptation produced by the earlier dose of AS. There is little evidence that this was the case, since 4 animals scored 10/10 and one 9/10.

Group 4. The absence of shock for errors increased the effect of AS. After the first injection of AS, scores were 5/10, 5/10, 8/10, 10/10, 10/10; and after the second, 10/10, 6/10, 8/10, 3/10, 4/10. However, the effect of the following two injections of AS was considerably less and no animal scored worse than 8/10. With respect to this situation which depends solely on food motivation, the parasympathetic effect of atropine on salivation is probably important. The animals ate only with great difficulty and retained large lumps of semi-masticated food in their mouths.

Group 5. The single animal of this group performed similarly to the H. v. V. - shock animals of Group 2. The magnitude of the decrease after AS was greater, but this could be either individual variation, or an interaction of atropine effect and task. Like the

animals in Groups 1 and 4 adaptation to the atropine occurred.

Group 6. There was no effect of either AS or AMN upon the black & white discrimination. This does provide some evidence of an interaction with task difficulty since similar injections affected both H. v. V. and C. v. T. discrimination (Groups 2 & 5).

Group 7. Lowering the dose to 2 mgm/kgm did not abolish the effect of AS in a food-motivated discrimination, and adaptation did not occur.

Group 8. A dose of 2 mgm/kgm had little or no effect upon a shock motivated version of the above experiment. This shows again that the effect of AS is greater when response cost is less, and also that dosage is of some importance, since an effect of AS 100 mgm/kgm was apparent in Group 2.

Discussion

It is clear that AS can affect discrimination performance, but any finer analysis of the probable causes of atropine action is suspect due to the small numbers in the 8 experimental groups.

The argument that the effect is due solely

to the animals' general condition after AS, is weakened by the results of the AMN group. There was little evidence of change in this group although the peripheral symptoms were as acute as in the AS animals.

The slightness of the effect of AS on highly over-learned behaviour agrees with the report of Bradley (1964).

As in the experiments with DFP a difference was found between food and shock motivated conditions. This suggests that response control is again the facet of performance affected. However, in Groups 4 & 7 several animals made repetitive errors. One animal made a run of 10 consecutive repetitive errors, and one runs of 5 and 8. This normally never occurs in such well-trained animals, and must indicate some effect of the atropine; but it is difficult to attribute it to general sickness. It never occurred after injection of DFP.

What looks like the same kind of behaviour has been reported before in operant lever pressing situations. Atropine increased responding on an irrelevant lever in a Sidman avoidance situation, Carlton (1962). Scopolamine, which is related to atropine, increased perseveration on the wrong lever

in a 2 lever discrimination situation, and when given part-way through extinction increased response rate and the tendency for bursts to occur on one lever, Hearst (1959).

Carlton (1963) has attempted to integrate these and other results by assuming that the effects of non-reinforcement are mediated by a cholinergic inhibitory system, and that blockage of the system by atropine allows previously suppressed behaviour to reappear. In the terminology of Part 5, atropine is claimed to have its effect upon response control.

Whitehouse (1964) and Whitehouse et al (1965) found that 2 mgm/kgm of atropine before each daily practice session, slowed down the learning of a successive brightness discrimination. It was claimed that the effect was upon the acquisition of information rather than on performance, since there was no sudden improvement in animals given atropine when the injections were stopped. Atropine was also shown to impair post-criterion performance, which is consistent with the theory of Carlton (1963) described above.

Since there is much evidence that atropine will affect performance, and little which distinguishes performance and learning, the claim of Whitehouse (1964) to have shown an effect on learning should

probably be treated with reserve until it has been repeated.

It is notable that Whitehouse found reliable effects with a relatively small dose of atropine (2 mgm/kgm). In the present experiment 2 mgm/kgm had approximately as great an effect as 100 mgm/kgm (groups 7 & 4). Several investigators have reported that atropine and similar compounds have a bimodal effect - facilitating lever pressing at low doses and inhibiting it at higher ones, Boren & Navarro (1959), Herrnstein (1958). There is therefore some doubt as to whether the effects at low doses arose from interference with the same behavioural functions as do those at high doses.

Taking the results of this experiment together with those reported in the literature there is some support for the suggestion made in the Introduction that atropine should have a greater effect than low ChE. To check this suggestion rigorously, animals would have to be trained on the same task with the two treatments arranged to give 'extraneous' effects (e.g., on food intake, motor behaviour, etc.) of equal magnitude. However, it is obvious from the results of group 2 that high level performance <u>is</u> still possible even after massive,

near lethal, doses of atropine.

The data presented above, in Part 5, and in the review by Carlton (1963) of work on anti-cholinergics, all point to response control as the kind of behaviour affected by manipulation of the ACh - S. There is little evidence that learning ability is altered by such treatment.

In section (1) the caudate nucleus was suggested as a possible site of this effect on response control. McLennan (1964) has shown that there is a resting release of ACh from the caudate which is increased by stimulation of the thalamic ventral anterior nucleus. The caudate contains high amounts of ChE and is one of the areas most affected by DFP, (See Part 1). As was noted in section (1) caudate lesion produces effects on motor control, Whittier and Orr (1962); and Ruch (1960) has argued that the function of the caudate is to modulate cortically initiated movement. The involvement of this area could perhaps be tested by a combination of lesion and direct injection techniques.

Another mechanism that could possibly mediate the behavioural effects is the non-specific arousal

system. The reticular activating system has been claimed to be a cholinergic structure by Himwich and Rinaldi (1955) and White (1962). Cholinergic stimulation of the reticular formation retards both learning and performance of an avoidance habit, Grossman et al (1965). However, the fact that section of the brain stem above the reticular formation does not prevent the E.E.G. arousal effect of cholinergic drugs, shows that their effect is not confined to the reticular formation alone, Killam (1962). Whether all, or part of the arousal mechanism is itself cholinergic there is much evidence that brain arousal and ACh are in some ways related. Some of this evidence was mentioned in Part 1. One of the most striking demonstrations is that of Pepeu and Mantegazzini (1964) who showed that hemisection at the collicular level produced an assymmetrical E.E.G. pattern, one hemisphere showing a 'sleep' pattern while the other showed a 'waking' pattern. The sleeping hemisphere had higher cortical ACh than the waking hemisphere but there were no differences in the caudate nucleus. When the E.E.G. difference was eliminated by an arousing drug there were only small differences in ACh. It is interesting that atropine produces a synchronized E.E.G., but reduced

total brain ACh, Giarman & Pepeu (1962), (1963), and behavioural activation. The behavioural and biochemical observations are here consistent, but do not agree with the E.E.G. records. (The lack of correlation between E.E.G. pattern and behaviour after atropine is well established, Wikler (1952) Bradley (1958)).

These results can be rationalized in line with the theory of Carlton (1963) in the following way:- Atropine could produce cortical deafferentation which produces a synchronized E.E.G. If the inhibitory system proposed by Carlton is a cortical one, then reducing the effectiveness of cortical function could result in a loss of response control through a "runaway excitation" of other brain areas. This "excitation" produces the behavioural effects and also the reduced total brain ACh levels. If this were the case then ACh levels in the cortex of atropinized animals should be higher than normal, in keeping with the observation of Pepeu and Mantegazzini (1964) described above, that synchronized E.E.G. patterns are correlated with higher cortical ACh levels.

The fact that DFP produces some of the same kind of behavioural results as atropine but is thought

to have an opposite physiological effect is not necessarily as contradictory as it first appears. Anticholinesterases have frequently been found to have bimodal effects, stimulating at low concentrations and inhibiting at high ones. Any comparison to the effect of other drugs must therefore take this into account.

This comment in fact applies generally to all the studies reported. For valid comparisons to be made it is necessary to know how the behavioural function studied varies with dose and time, and how dose is related to changes in the relevant biochemical and physiological variables.

PART SEVEN.

CONCLUSIONS.

In Part 5 it was concluded that reduced ChE activity impaired response control, but not associative learning ability. Experiment 3 of Part 6, in which ChE was reduced to less than 5% of normal without significant impairment of learning ability, supports this conclusion.

It is still possible of course that other tasks may be more sensitive to the effects of low ChE than discrimination-reversal learning. Lashley (1929) found that brightness discrimination learning was unaffected by lesions that produced poorer maze learning than controls, even though the number of trials needed to learn the discrimination was more than for the maze. He suggested that this was because of the greater complexity of the habit learned in the maze - "It seems not unlikely that the actual associations required for the latch boxes and discrimination habit are simpler than those for the mazes and that the escape of these habits from the influence of brain lesions is the result of their greater simplicity." (P. 140). Any serial task, such as maze learning, would be expected to put a greater stress on memory than a single stimulus-response association, of the kind learned in discriminations.

Transfer tasks, in which old information must be interpreted in a new manner, are in humans thought to be a more complex task than simple association, and could also provide a sensitive test of any intellectual impairment resulting from reduced ChE. (Reversal learning is logically a transfer task, but it appears to be so difficult for the rat that each reversal is in fact treated as a completely separate problem.) Incidental learning might similarly be a sensitive test, on the assumption that while two animals may learn the same task equally quickly, the more "intelligent" of the two should gain more total information about the task than the other.

However, up to the limits of complexity used, the conclusion that learning ability is unaffected by low ChE holds good.

The actual nature of the effect of low ChE on response control remains to be elucidated. There are 2 main possibilities:-

(1) Hyperactivity. Russell et al (1961) suggested that the "oscillation" of rats with low ChE in an

avoidance-extinction situation, "may represent a form of behavioural hyperactivity associated with decreased brain ChE activity level." Simple motor hyperactivity could possibly account for some of the results described above. In the discrimination box experiments, the fact that position habits persist throughout training (Part 5, Experiment 1, Discussion) means that the animal is more likely to make 'false error' than 'false correct' responses. An animal with a 100% preference for one side would meet the positive gate 5 times and the negative 5 times, in each block of 10 trials. A hyperactive animal, on being confronted with the negative gate, would be more likely to make an error than a normal control. Since the actual frequency of meeting the positive gate would be unaffected, the frequency of correct responses should also be unaffected by hyperactivity.

Hyperactivity could also contribute to the increased extinction responding found in Part 5, Experiment 10, but it does not appear to be the complete explanation. Increased responding was found in Experiment 13 in the first 5 days after reduction of ChE, during which period lever pressing activity is reduced, Experiment 1, Part 4. A relative increase

in S- responding, while free responding for food remained depressed, was also found in Experiment 12.

The question of the influence of chronically reduced ChE upon motor activity levels could be experimentally tested by activity wheel or open field methods.

(2) Inability to withold response. In several of the experiments reported in Part 5, an inability to inhibit response, that is, to form negative conditioned responses, was suggested as the explanation of the effects of low ChE. This proposal is not completely distinct from that of hyperactivity, since the latter could be an effect of inability to withold response; especially if this inability is considered to extend to the inhibition of approach responses to neutral stimuli.

If low ChE affects only response to negative stimuli and not to neutral ones, then no increase in activity would be predicted in an open field situation. An effect on response to neutral stimuli could be distinguished from a simple increase in motor activity by performing tests in the presence and absence of exteroceptive stimulation. Simple motor hyperactivity would be predicted to persist

when stimulation was reduced, whereas hyperactivity due to response to neutral stimuli should be attenuated.

In addition to the nature of the deficiency in response control produced by low ChE, the time course of the deficiency also remains to be investigated. It has already been mentioned that the initial effect of ChE reduction is to decrease motor activity such as lever pressing. In some situations it is possible that this initial motor effect could mask a change in response control which would otherwise be observable. This was apparently the case in Experiment 10 of Part 5, whereas Experiments 12 and 13 showed effects on response control sooner after reduction of ChE. To chart the time-course of the DFP effect on response control it would be necessary to separate it from any changes in the interfering effects. One approach might be by using a task such as the drl where only a minimal amount of motor effort is required.

Two principal hypotheses were advanced in Part 6 to account for the results of manipulations of the ACh - S on response control.

(1) That such effects were due to interference with the caudate nucleus.

(2) That they were due to an action on the cortex that freed lower centres from its control.

These hypotheses are not mutually exclusive; the caudate could be one of the subcortical structures freed from cortical control; but more importantly, it is perhaps mistaken to attempt to see the effects of anticholinesterases such as DFP, and anticholinergics such as atropine, as all being due to interference with the same structures. One aspect of performance after atropine, the repetetive error making found in some animals (Part 6, Experiment 4), was unique to that drug. The same kind of repetitive behaviour follows lesions of the cortex, and one of the assumptions underlying hypothesis (2) was that atropine reduced the efficiency of cortical function (see Part 6). In fact the description given by Lashley (1929) of behaviour after cortical lesions could apply as readily to the behaviour after injection

of atropine :- "The normal animal almost never re-enters a cul-de-sac without intervening exploration of other parts of the maze. An animal with severe lesions may repeat a single error as many as two hundred times before passing to other parts of the maze." (P.138).

A case could therefore be made out for the repetetive behaviour after atropine having a cortical origin; but whether or not this also applies to behaviour after DFP is uncertain. The systemic injection methods employed in Parts 5 and 6 are really inadequate to answer such questions. More relevant data on the localization of effects could be obtained by direct injection techniques, e.g. White (1956), Grossman (1962a), or by combining either direct or indirect injections of drug with lesions in the areas assumed to be affected.

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PART EIGHT.

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