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Anticholinesterase Action and Electroconvulsive Shock-Induced Disruption of Taste-Illness Association

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ANTICHOLINESTERASE ACTION AND ELECTROCONVULSIVE SHOCK-INDUCED DISRUPTION OF TASTE-ILLNESS ASSOCIATION

A Thesis Submitted to the Graduate Division in Partial Fulfillment of the Requirements for the Degree of Master of Science

By

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ABSTRACT

Electroconvulsive shock (ECS) interpolated temporally between the pairing of a novel taste and an induced gustatory illness prevents the taste from being associated with the illness (Kral, 1971). Physostigmine has been shown to protect against retrograde amnesic effects of ECS on learning of a passive avoidance task (Davis, Thomas, and Adams, 1971). Physostigmine protection of ECS induced disruption of a conditioned taste aversion was investigated using a 2 (physostigmine vs. saline) x 2 (ECS vs. sham shock) x 2 (conditioned vs. nonconditioned) x 2 (conditioning day vs. test day) factorial design with repeated measures over the last factor. Results indicated physostigmine pretreatment to be ineffective in protecting against ECS disruption of the taste-illness association. However, physostigmine pretreatment alone, interfered with the formation of the conditioned taste aversion. The results implicated the involvement of the cholinergic system in association formation but did not indicate physostigmine as being effective in the amelioration of ECS disruption of learning.

INTRODUCTION

Human and animal experiments have shown that electroconvulsive shock (ECS) interferes with memory (Barbizet, 1970, Grossman, 1967). Clinical observations suggest that following ECS treatment, patients often experience a state of initial confusion where they can not recall their name or where they are. The patient's memory slowly returns and recovery is usually complete except for a short period surrounding treatment (Barbizet, 1970). Clinical studies suggest that ECS can induce retrograde amnesia in which the most familiar situations are recalled first, followed by less familiar situations (Williams, 1950; Rocheford and Williams, 1962).

Animal experiments have shown that learning can be impaired when the learning task is followed within a short time period by administration of ECS (Duncan, 1949; Chorover and Schiller, 1965). The learning impairment, i.e. retrograde amnesia, is most generally thought to be related to a rapid, massive increase in neuroelectrical firing within the brain (Grossman, 1967). Hebb (1949) maintained that newly acquired memories are apparently maintained in a relatively delicate, unstable state for a short period of time as the memories become consolidated into a more permanent, stable state. The massive increase in electrical activity induced by ECS is thought to disrupt the consolidation process by means of an as yet undetermined mechanism, thereby preventing new memories from becoming fixed in long-term memory storage (McGaugh, 1966, Deutch, 1973a).

ECS-Induced Disruption of Association

Animal experiments designed to elucidate the nature of ECS learning and memory impairments have used traditional experimental learning tasks which require close temporal contiguity between the conditioned and non-conditioned stimulus. Because of the necessity of close temporal contiguity in these traditional experimental learning tasks, the ECS had to be administered either prior to or following the stimulus pairing and the results were, therefore, usually discussed in terms of prograde or retrograde amnesia. Garcia and Ervin (1968), however, demonstrated that a temporal interval between stimuli of up to several hours was effective in producing an aversion to a novel taste. The relatively long temporal interval of the conditioned taste aversion allowed ECS to be interpolated between the conditioned and unconditioned stimuli, which in turn permitted an alternate interpretation of ECS disruption.

Kral (1970) interpolated ECS within the taste-illness interval which interfered with conditioning of the taste aversion. Kral theorized that the interpolated ECS interference could disrupt conditioning either by means of retrograde amnesia which would obscure memory of the taste, by proactive interference which would negate the illness, or by preventing the association of the taste with the illness.

Kral (1971) made use of two paradigms to determine if retrograde amnesia or protective interference could account for the interference of the interpolated ECS.

To test whether ECS induced retrograde amnesia for the novel taste, Kral made use of the finding that a deprived animal will habituate to a novel tasting, though moderately unpalatable food over repeated exposures (Barnett, 1963). Water deprived animals were exposed to a sour-tasting

solution of very dilute hydrochloric acid for a period of 10 minutes. ECS was administered within 30 seconds following the drinking period. Animals were allowed a 10-minute period to drink tap water on each of the next two days. The third day after the initial exposure to sour water, animals were again presented sour water during the 10-minute drinking period. Habituation to sour water occurred, indicating that memory of the first sour water exposure was recalled, and not affected by ECS administration. Kral concluded that ECS did not induce retrograde amnesia for the taste.

In the second paradigm, the possibility of proactive ECS interference with the illness was examined. Proactive ECS effects on performance have been shown to decrease with time (Kopp, Bohandanecky and Jarvick, 1968). Therefore, Kral reasoned, proactive interference with the gustatory illness should be time dependent. Animals experienced ECS interpolated either zero, two or fours after the drinking period within a four hour taste-illness interval. Re-exposure to sweet water indicated that the conditioned taste aversion was not time dependent over a four-hour time span. Kral concluded that ECS interference could not be accounted for by proactive interference with the illness.

Kral and Beggarly (1973) presented evidence which suggested ECS interferes with conditioned taste aversion by disrupting the association of the taste with the illness. ECS was effective at disrupting the taste aversion only when it was interpolated between the taste and the illness. ECS delayed shortly after the injection of the gustatory toxicant was ineffective at inducing any disruption of learning. Therefore, they concluded that interpolated ECS disrupts the mechanism by which the conditioned stimulus is associated with the unconditioned stimulus, and not by retrograde amnesia or proactive interference.

Cholinergic System and ECS Induced Learning Deficits

The cholinergic system, a diffuse network of neurons in the brain, appears to be involved with learning and learning deficits (Deutsch, 1973b, Dauron and McGaugh, 1973). Evidence is beginning to appear which implicates cholinergic involvement in learning impairments induced by electroconvulsive shock (Deutsch, 1973a). Neurons in the cholinergic system are sensitive to the neurotransmitter, aceytlycholine (ACH). Stimulation of a neuron generates an electrochemical impulse which travels the length of the neuron's axon. When the impulse reaches the end of the neuron, neurotransmitters are released from vesicles located at the tip of the axon. Neurotransmitters cross the synapse, a small space between neurons, and act to stimulate the next neuron. Following the stimulation of the post-synaptic neuron, a specific neurotransmitter, ACH, is then rendered inactive by the presence of the enzyme aceytlycholinesterase (ACHE). The neutralization of ACH by ACHE appears to be necessary to prevent the continued restimulation of the post-synaptic neurons.

Deutsch (1973b) cited several recent research findings which suggest the electric current which passes through the brain in ECS treatment may result in a neurochemical imbalance in the cholinergic system. It appears that in the process of recovering the neurochemical balance, the cholinergic system influences the retrievability of memory.

Adams, Hoblit and Suther (1969) examined the effect of ECS on whole brain ACHE activity. Rats were decapitated either 10 seconds, 4 hours, 24 hours, or 96 hours after a series of four ECS treatments. Animals in a control group were decapitated within 10 seconds following sham shock (earclips were attached, but no shock was given).

The results showed that immediately following ECS treatments there is a twenty-one percent increase in whole-brain ACHE activity. The ACHE activity level gradually returned to the pre-ECS control level somewhere between 24 and 96 hours. Adams et al. suggested that ECS treatment may interfere with retention by the elevation of the ACHE activity level.

Adams et al., in a second experiment, examined the hypothesis that if ECS-induced retention deficits are caused by the increase in ACHE activity levels, then administration of anticholinergic or anticholinesterose agents should facilitate or eliminate the retention deficit. Rats were trained in a one-way active avoidance task. Training was followed immediately by either ECS or sham shock. Animals were returned to the apparatus four hours later and tested for retention. Half an hour prior to testing, the animals received an injection of either physostigmine (an anticholinesterose which lowers ACHE activity), scopolamine (an anticholinergic drug which blocks ACH post-synaptic stimulation), or saline. Scopolamine appeared to partially eliminate the ECS retention deficit whereas physostigmine appeared to increase the deficit. Physostigmine alone, without ECS treatment, impaired the test performance for retention. Adams et al. concluded that ECS influenced the retention of learning through a reversible mechanism of neurochemical changes in the cholinergic system.

Davis, Thomas and Adams (1971) examined the interactive effects of scopolamine and physostigmine on ECS-induced disruption of one-trial passive avoidance learning. Animals were administered ECS or sham

shock immediately following the learning trial. Testing for retention occurred four hours after shock treatment. Animals received intraperitoneal injections of physostigmine, scopolamine or saline either 30 minutes prior to the learning trial or prior to the test trial. Physostigmine, when injected prior to the learning trial, and scopolamine, when injected prior to the test trial, resulted in increased response latencies. The increase in response latency is an indication that retention of learning occurred. It was concluded that pre-ECS administration of physostigmine and post-ECS administration of scopolamine protects learning from ECS-induced disruption.

The conclusion of Adam et al. and Davis et al. strongly implicates the cholinergic system as being capable of altering the disruptive influence of ECS on learning, at least in paradigms which rely to a large extent on footshock, locomotor activity and close temporal CS-US intervals. Especially exciting is the finding that pre-administration of physostigmine may negate ECS disruption, possibly by maintaining the necessary neurochemical balance which appears vital for retention and possibly (if Kral's hypothesis about the mechanism of ECS disruption is correct) for the formation of association.

The purpose of the present experiment was to determine if pretreatment with physostigmine will protect the association of a taste with an illness against the interference of ECS. The main hypothesis was that a physostigmine pretreated group, which experienced ECS interpolated midway in the taste-illness interval, would drink more sweet water on test day than a saline pretreated group which was

otherwise subjected to similar conditions.

Several sub-hypotheses were formulated to show the effect of conditioning, ECS and physostigmine on sweet water consumption. The sub-hypotheses:

1. A group which experienced gustatory illness following the novel taste of sweet water on conditioning day would drink less sweet water on test day than a group which did not experience gustatory illness following the novel taste.
2. A group which received ECS midway between the novel taste of sweet water and gustatory illness on conditioning day would drink more sweet water on test day than a group which received sham shock midway between the novel taste and gustatory illness.
3. A group which received physostigmine prior to experiencing the novel taste and gustatory illness would drink a statistically equivalent amount of sweet water on test day as a group which received saline prior to experiencing the novel taste and gustatory illness.
4. Groups which did not experience a gustatory illness following the novel taste of sweet water on conditioning day would drink statistically equivalent amounts of sweet water on test day regardless of whether the groups received physostigmine or saline pretreatment, ECS or sham shock, or any combination of drug pretreatment with shock treatment.

METHOD

Subjects. Sixty-four male Holtzman albino rats weighing between 220-250 grams and approximately eight weeks of age at the start of the experiment, were housed in individual cages under a 16-hour light, 8-hour dark cycle. The animals were fed Purina Rat Chow lib. throughout the experiment.

Apparatus. A solution of sweet water (0.1% W/W sodium saccharin) was the conditioned stimulus (CS). The unconditioned stimulus (US) was gustatory illness induced by intraperitoneal injection of 0.4 M LiCl (10 ml/kg of body weight). The ECS was produced by an electroconvulsive shock generator similar to the one designed by Woodbury and Davenport (1952) except that a plate supply transformer with a 1000 Vac secondary was used. The circuit diagram of the shock apparatus is presented in Appendix A. A 60 milliamp current was delivered for a duration of 0.5 seconds across the animals' ear by means of alligator clip electrodes padded with saline-soaked cotton. The shock intensity was limited by a resistor in series with the animal. Water consumption was measured by using 50 ml. graduated cylinders fitted with water drinking tubes.

Design. The present experiment incorporated a 2x2x2x2 factorial design with repeated measures on one factor and equal cell frequency. The four independent variables were physostigmine vs. saline, ECS vs. sham shock, LiCl vs. saline, and conditioning day vs. test day. The dependent measure was the amount of sweet water consumption within a 15-minute drinking period.

Procedure. Animals, upon arrival, were assigned at random to individual cages where they remained except when they were being weighed, injected or shocked. Weighing and injection occurred in the colony room. Animals were given water ad lib for the first 48 hours to offset water deprivation which occurred during shipping. Water bottles were removed after the 48-hour period. Twenty-four hours later, the animals were placed on a 15-minute per day water drinking schedule for the remainder of the experiment. Animals were weighed 15 minutes prior to their drinking period and water consumption was measured and recorded each day of the schedule.

Animals were weighed and given regular tap water for the first four days of the schedule to acustom them to the 15-minute water drinking period. On days 5 through 10, animals were weighed and then injected intraperitoneally with either physostigmine sulfate (1.0 mg/kg of body weight) or saline (1.0 ml/kg body weight) 15 minutes prior to presentation of tap water. Previous pilot studies had shown that administration of physostigmine initially lowered water intake during the drinking period and that the animals need several days to habituate to the drug effect on water consumption.

Conditioning of the taste aversion occurred on day 11 of the schedule. Animals were weighed and injected with either physostigmine or saline 10 minutes prior to the drinking period just as they had been on days 5 through 10. However, instead of tap water, the animals were presented sweet water during the drinking period. Following the drinking period by 15 minutes, animals were placed in a plastic carrying pan and received ECS or sham shock in a room other than the colony room

and then immediately returned to rest in their home cages. Fifteen minutes after shock treatment, animals were injected with either LiCl (10 ml/kg body weight) or saline (10 ml/kg body weight).

During the next two days, days 12 and 13, the animals were weighed and then given tap water during the drinking period. On the thirteenth day, animals were tested for aversion to sweet water. The animals were weighed and then presented sweet water during the drinking period. Day 14 concluded the schedule.

Animal running times were organized in a staggered sequential pattern to minimize the daily total running time, as shown in Appendix B. Randomized cage placement determined which condition an animal was assigned to. All animals in one condition were housed and run in a sequential order to minimize the possibility of handling errors such as the injection of physostigmine to an animal which should receive saline. Animals were run in two squads of thirty-two animals each because of limited housing facilities. All conditions were present in each squad.

RESULTS

The group mean water consumption over days is presented graphically in Figure 1. It can be seen that administration of physostigmine on day 5, decreased tap water intake. Water consumption for the physostigmine group increased and began stabilizing over the following five days which indicated the successful habituation to the drug suppression of water consumption.

The group mean water intake for conditioning day and test day are graphically depicted in Figure 2. A summary table of the multifactor analysis of variance with repeated measures on one factor which was employed to determine significance between and within group differences is presented in Table 1. Summary tables for multifactor analysis of between group differences on condition day and on test day are presented in Tables 2 and 3 respectively.

Physostigmine pretreated groups differed significantly from saline groups (p 0.001, Table 1) and physostigmine pretreatment interacted significantly with days (p 0.001, Table 1). A significant difference was found for physostigmine on conditioning day (p 0.001, Table 2) but not on test day (Table 3) which properly reflects the discontinuation of physostigmine after conditioning day.

Electroconvulsive shock groups differed significantly from sham shock groups (p 0.001, Table 1) and the difference was found to be significant on test day (p 0.001, Table 3) but not on conditioning day (Table 2). The results coincide with expectations since shock treatment occurred after water intake was measured on conditioning day.

Fig. 1

GROUP MEAN WATER CONSUMPTION OVER DAYS

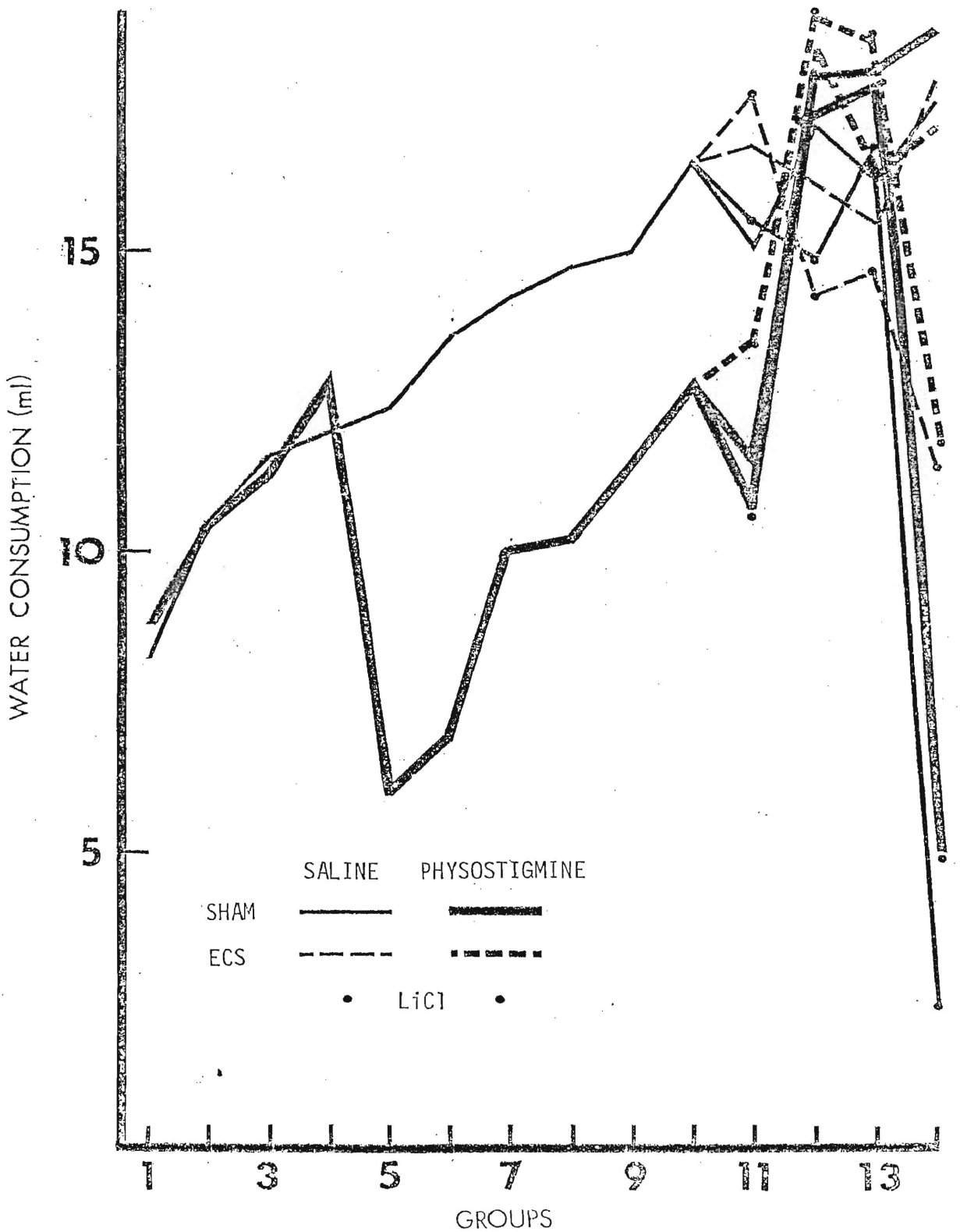

Figure 2

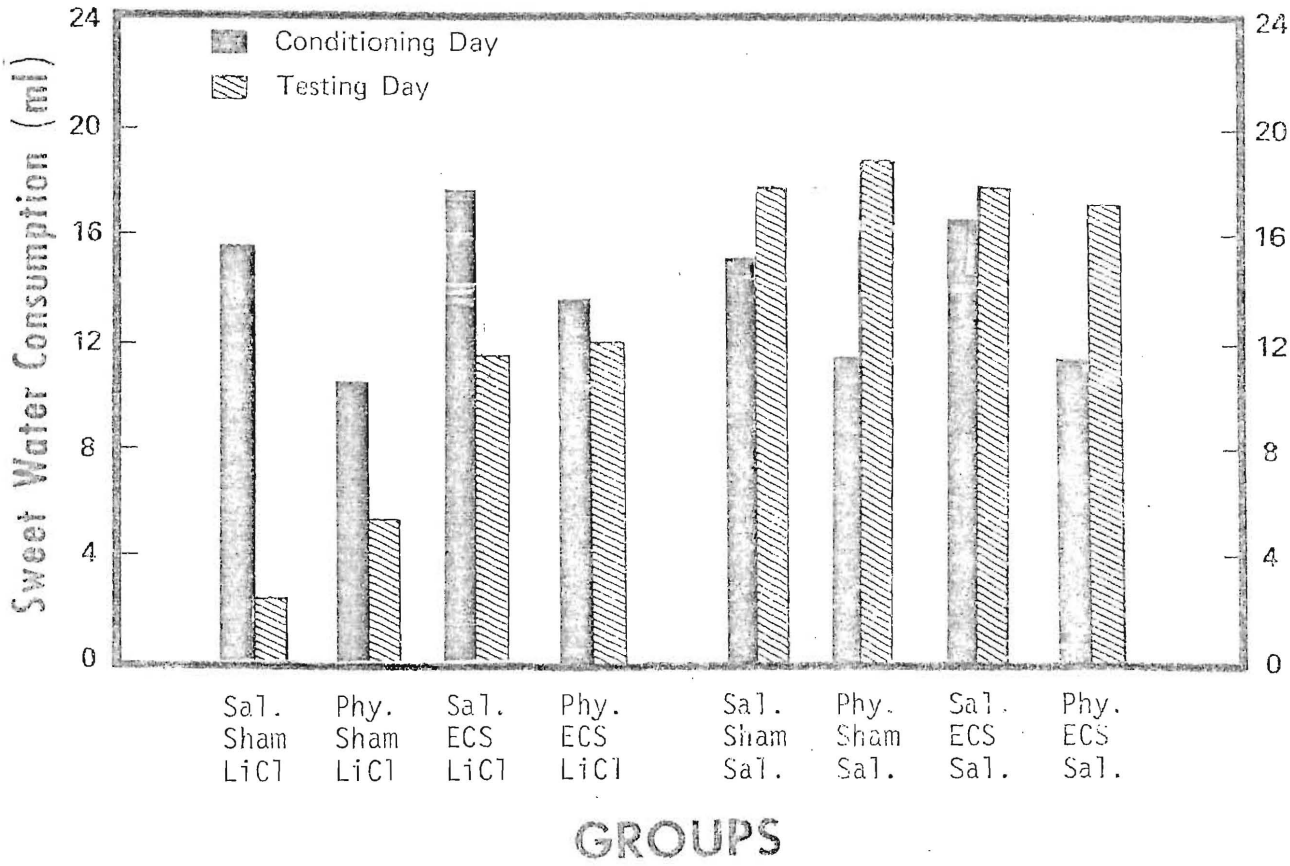

Fig. 2 Mean sweet water consumption for all groups on conditioning and test day. Physostigmine pretreatment did not ameliarate the disruptive effect of ECS on learning, however, physostigmine alone mildly interfered with the taste-illness association.

TABLE I

ANALYSIS OF VARIANCE SUMMARY TABLE FOR SWEET WATER CONSUMPTION DATA ON CONDITIONING AND TEST DAYS

SOURCE OF VAR.	SS	df	MS	F
BETWEEN S		63		
A physo		1	98.00	10.31*
B ECS		1	205.03	21.57*
C cond. vs		1	731.53	76.96*
D replic.		1	2.00	---
AB		1	13.78	1.45
AC		1	3.78	---
AD		1	10.12	1.06
BC		1	210.12	22.11*
BD		1	2.53	---
CD		1	26.28	2.76
ABC		1	1.12	---
ABD		1	11.28	1.19
ACD		1	2.53	---
BCD		1	32.00	3.37
ABCD		1	4.50	---
SUBJ. W. GROUPS	456.25	48	9.50	
WITHIN S		64		
E days		1	47.53	6.78*

TABLE I (EXT.)

SOURCE OF VAR.	SS	df	MS	F
WITHIN S (CONT.)				
AE		1	236.53	33.76*
BE		1	24.50	3.50
CE		1	924.50	131.97*
DE		1	0.78	---
ABE		1	6.12	---
ACE		1	4.50	---
ADE		1	0.78	---
BCE		1	94.53	13.49*
BDE		1	8.00	1.42
CDE		1	0.50	---
ABCE		1	5.28	---
ABDE		1	0.12	---
ACDE		1	0.00	---
BCDE		1	1.53	---
ABCDE		1	16.53	2.36
Ex SUBJ. W. GROUPS	336.25	48	7.00	

* $p<0.001$

TABLE II

ANALYSIS OF VARIANCE SUMMARY TABLE
FOR SWEET WATER CONSUMPTION DATA
ON CONDITIONING DAY

SOURCE	SS	df	MS	F
A (PHYSO. VS. SALINE)	319.52	1	319.51	25.63 *
B (ECS VS. SHAM)	43.89	1	43.89	3.52
C (COND. VS. NONCOND)	5.64	1	5.64	---
AB	0.76	1	0.77	---
AC	0.01	1	0.02	---
BC	11.39	1	11.39	---
ABC	5.64	1	5.64	---
WITHIN CELL	698.12	56	12.47	
TOTAL		63		

* $p<0.001$

TABLE III

ANALYSIS OF VARIANCE SUMMARY TABLE
FOR SWEET WATER CONSUMPTION DATA
ON TEST DAY

SOURCE OF VARIANCE	SS	df	MS	F
A (PHYSO. VS. SALINE)	15.02	1	15.02	3.01
B (ECS VS. SHAM)	185.64	1	185.64	37.14*
C (COND. VS. NONCOND.)	1650.39	1	1650.39	330.22*
AB	19.14	1	19.14	3.83
AC	8.27	1	8.27	1.65
BC	293.27	1	293.27	58.68*
ABC	0.77	1	0.77	---
WITHIN CELL	279.88	56	5.00	
TOTAL		63		

* $p<0.001$

Groups which were conditioned for taste aversion differed significantly from nonconditioned groups (p 0.001, Table 1) and conditioning significantly interacted with days (p 0.001, Table 1). A significant difference between conditioned and nonconditioned groups existed on test day (p 0.001, Table 3) but not on conditioning day (Table 2) which reflects the measurement of sweet water intake prior to injection of the conditioning agent.

A significant triple order interaction between shock, conditioning and day (p 0.001, Table 1) and a significant double order interaction between shock and conditioning were found (p 0.001, Table 1). Shock and conditioning significantly interacted on test day (p 0.001, Table 3) but not on conditioning day (Table 2) which accounts for the triple order interaction. These results indicate that ECS treatment effectively interfered with the conditioned taste aversion.

A summary of multiple individual comparisons between groups on test day, analyzed by Dunnett's t, is presented in Table 4. There were no statistical differences among the nonconditioned control groups on test day which indicates that drug pretreatment and shock treatment, either separately or in combination, had a negligible effect upon sweet water consumption that was unrelated to conditioning. The physostigmine-ECS-LiCl group did not statistically differ from the saline-ECS-LiCl group which suggests that physostigmine was ineffective as a preventive against ECS disruption. The physostigmine-sham-LiCl group drank significantly more sweet water than the saline-sham-LiCl group (p 0.05) which suggests that physostigmine may interfere with the conditioned taste aversion.

TABLE IV

MULTIPLE COMPARISONS SUMMARY TABLES
FOR SWEET WATER CONSUMPTION DATA
ON TEST DAY

TREATMENT	GROUP MEAN	COMPARISON TREATMENT	M_1-M_2	t	p
SAL-SHAM-SAL	17.75	----			
SAL-SHAM-LiCl	2.37	SAL-SHAM-LiCl	15.37	13.76	0.001
PHY-SHAM-LiCl	5.37	" " "	12.37	11.07	0.001
SAL-ECS-LiCl	11.37	" " "	6.37	5.71	0.001
PHY-ECS-LiCl	11.75	" " "	6.00	5.37	0.001
PHY-ECS-SAL	17.12	" " "	0.62	1.01	
SAL-ECS-SAL	17.75	" " "	0.00	0.00	
PHY-SHAM-SAL	18.87	" " "	0.62	0.56	
PHY-SHAM-LiCl		SAL-SHAM-LiCl	3.00	2.68	0.05
PHY-ECS-LiCl		PHY-SHAM-LiCl	6.38	5.70	0.001
SAL-ECS-LiCl		PHY-SHAM-LiCl	6.00	5.37	0.001
PHY-ECS-LiCl		PHY-ECS-SAL	5.75	5.14	0.001

DISCUSSION

The hypothesis that disruptive ECS interference with a conditioned taste aversion could be prevented by pretreatment with physostigmine was not supported by the present data. The data does not support the theoretical postulate of Adams et al., that ECS disrupts learning and memory by an increase in ACHE activity level. Physostigmine, which acts to reduce ACHE activity, should have attenuated any disruptive effects induced by increased ACHE activity. The discrepancy between the present experiment's results and those of Adams et al. and Davis et al. is quite possibly related to differences between the learning tasks which were employed. However, our results coincide with the findings of Lewis and Bregman (1972) which were that physostigmine did not offer protection from ECS disruption of learning even though a passive avoidance task similar to the one used by Davis et al. was employed. Lewis and Bregman found the drug effect on step down latency dissipated within twenty-four hours and therefore the drug effect was acting upon memory. Ray and Barret (1969) had shown that ECS treatment caused an increase in step down latency unrelated to passive avoidance conditioning when the test trial followed the ECS by four hours or less. Since neither Adams et al. nor Davis et al. controlled for proactive ECS effects, their results are possibly confounded and may be related to drug effects on locomotor activity instead of memory. It appears that a mechanism other than increased ACHE activity may be responsible for ECS induced learning and memory disruption.

ECS, in the present experiment, was found to disrupt learning of a conditioned taste aversion which supports the results of Kral (1970), and Kral and Beggarly (1973). Though no support for increased ACHE activity as the mechanism of ECS induced learning disruption was found, the possibility of cholinergic involvement can not be discarded since Richter and Crossland (1949) have shown that ECS administration increased brain ACH levels. It is possible that the increase in ACH level both raises ACHE activity and disrupts learning. It appears plausible that the increase in ACHE activity following ECS administration is not causally related to learning disruption but merely correlated.

The present data indicates that physostigmine alone mildly disrupted conditioned taste aversion. Physostigmine disruption of learning has been shown in active avoidance tasks (Adams et al. 1969, Hamburg, 1967), passive avoidance tasks (Davis et al. 1971, Lewis and Bregman, 1972), and in operant conditioning tasks (Biederman, 1970). A more specific acting anticholinesterase, diisopropyl flurophosphate, has been shown to have effects on learning similar to those of physostigmine (Wiener and Deutsch, 1968) which implicates the involvement of ACHE activity in memory. Specifically suggested by anticholinesterase memory disruption is the necessity of a sufficient level of ACHE activity for the optimal retrieval of memory to occur. The present experiment suggests the possible need for a sufficient level of ACHE activity to exist for optimal formation of associations to occur.

Two alternate possibilities which may explain physostigmine disruption of the conditioned taste aversion are that 1) physostigmine

attenuated the perceived severity of the induced illness or 2) retention was tested in a drug state different from the state in which learning occurred. Since the disruptive effects of physostigmine and ECS on disruption of the taste aversion was nonadditive and equivalent disruption was found in both the saline and physostigmine ECS conditioned groups, a reduced severity of illness explanation appears tenuous. St. Omer and Kral (1972) have shown that the conditioned taste aversion is not readily amenable to drug disruption. An unpublished pilot study by Kral, Bair, and DeBriere has shown physostigmine to be ineffective in inducing state dependent learning of a conditioned taste aversion. Though both alternate explanations for physostigmine disruption appear to be improbable, neither can be eliminated without further investigation.

CONCLUSION

No evidence was found to support the hypothesis that physostigmine pretreatment would protect against ECS induced disruption of a taste-illness association. However, initial evidence was found suggesting that physostigmine pretreatment was capable of disrupting the taste aversion but to a milder degree than ECS. Finally, the effects of physostigmine pretreatment with ECS were not additive.

It is now necessary to begin an investigation of the mechanism by which physostigmine pretreatment caused taste-illness disruption. The procedure will be to:

1) add four state dependent control groups that receive physostigmine injections throughout recovery and on test day. Otherwise, treatment will be similar to the four physostigmine groups used in this experiment.
2) use diisopropyl fluorophosphate instead of physostigmine to further implicate the role of ACHE in associate formation.
3) examine time dependent effects of physostigmine and diisopropyl fluorophosphate on taste-illness disruption. The establishment of time dependency is necessary to determine if physostigmine disruption results from attenuation of the perceived severity of induced toxicosis.

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Appendices

Appendix A

Electrical Circuit Diagram

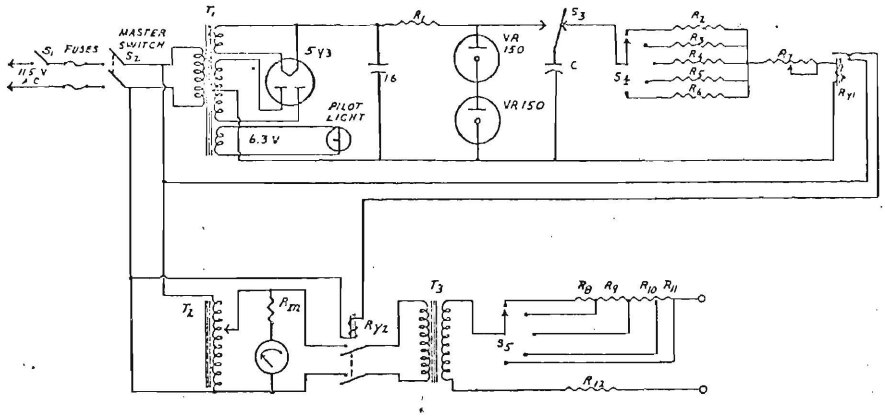

FIG. 1

Circuit diagram and parts for electroshock apparatus

S_1 Interlock switch on cabinet door.
S_2 Master Switch.
S_3 Push button to initiate timer; normally connected to timer.
S_4 Timer switch 5 pole, single circuit wafer type.
S_5 Current range switch, porcelain base, high voltage.
T_1 Small replacement type transformer 350-0-350 volts.
T_2 Variable autotransformer, 3 ampere capacity.
T_3 Plate supply transformer, primary 110 volts, secondary 1000 volts, 250 ma.
C Timer condenser, 5 mfd, 1000 volts.
Ry_1 Sensitive relay, 10,000 ohm winding.
Ry_2 Heavy duty, double pole relay or contactor, 110 volt coil.
R_1 Adjust to give 25-30 ma through regulator tubes; approximately 2,000 ohms 10 watts.
R_2-R_6 Select to give desired time of closure.
R_7 Adjust to give slight changes in timing of all timing positions.
R_8-R_{12} Wire wound, 100 watt variable resistors with sliding taps. Adjust to give desired full scale current. The following values are approximate.
R_8 120,000 ohms
R_9 40,000 ohms
R_{10} 20,000 ohms
R_{11} 16,000 ohms
R_{12} 4,000 ohms
Meter: (Indicated by circle-enclosed arrow in lower left part of diagram.) Any meter capable of reading rms *a-c* volts; scale may be hand-calibrated to give desired current ranges.

Appendix B

Schedule of Operations

SCHEDULE OF OPERATIONS

ΔT = TIME CHANGE

WI = WEIGH AND INJECT

P = PLACE WATER BOTTLE

R = READ WATER BOTTLE

ΔT	Operation		
0	WI 1		
1	WI 2		
2	WI 3		
3	WI 4		
4	WI 5		
5	WI 6		
6	WI 7		
7	WI 8		
8	WI 9		
9	WI 10		
10	WI 11		
11	WI 12		
12	WI 13		
13	WI 14		
14	WI 15		
15	WI 16	P1	
16	WI 17	P2	
17	WI 18	P3	
18	WI 19	P4	
19	WI 20	P5	
20	WI 21	P6	
21	WI 22	P7	

ΔT	Operation		
22	WI 23	P8	
23	WI 24	P9	
24	WI 25	P10	
25	WI 26	P11	
26	WI 27	P12	
27	WI 28	P13	
28	WI 29	P14	
29	WI 30	P15	
30	WI 31	P16	R1
31	WI 32	P17	R2
32	P18		R3
33	P19		R4
34	P20		R5
35	P21		R6
36	P22		R7
37	P23		R8
38	P24		R9
39	P25		R10
40	P26		R11
41	P27		R12
42	P28		R13
43	P29		R14

ΔT	Operation	
44	P30	R15
45	P31	R16
46	P32	R17
47	R18	
48	R19	
49	R20	
50	R21	
51	R22	
52	R23	
53	R24	
54	R25	
55	R26	
56	R27	
57	R28	
58	R29	
59	R30	
60	R31	
61	R32	

ANIMALS	1-4	WERE IN GROUP	PHY - ECS - SAL
"	5-8	" " "	PHY - SHAM - LiCl
"	9-12	" " "	PHY - ECS - LiCl
"	13-16	" " "	PHY - SHAM - SAL
"	17-20	" " "	SAL - ECS - SAL
"	21-24	" " "	SAL - SHAM - LiCl
"	25-28	" " "	SAL - ECS - LiCl
"	29-32	" " "	SAL - SHAM - SAL

Appendix C

Raw Data

RAW DATA OF SWEET WATER CONSUMPTION

IN MILLILITERS FOR CONDITIONED GROUPS

LiCl (CONDITIONED)

	PHYSOSTIGMINE				SALINE			
	ECS		SHAM		ECS		SHAM	
S's	COND. DAY	TEST DAY	COND. DAY	TEST DAY	COND. DAY	TEST DAY	COND. DAY	TEST DAY
1	8	15	10	4	20	13	20	3
2	14	11	13	7	12	15	16	1
3	16	15	17	6	22	11	14	1
4	17	12	10	6	20	11	9	2
5	10	10	7	7	20	10	10	3
6	14	14	9	3	11	9	12	1
7	10	8	12	5	16	12	18	4
8	19	9	7	5	20	10	16	4
SUM	108	94	85	43	141	91	124	19
AVG.	13.5	11.8	10.6	5.4	17.6	11.4	15.5	2.4

RAW DATA OF SWEET WATER CONSUMPTION

IN MILLILITERS FOR NONCONDITIONED GROUPS

SALINE (NONCONDITIONED)

	PHYSOSTIGMINE				SALINE			
	ECS		SHAM		ECS		SHAM	
S's	COND. DAY	TEST DAY	COND. DAY	TEST DAY	COND. DAY	TEST DAY	COND. DAY	TEST DAY
1	12	13	12	19	21	20	15	17
2	12	14	12	16	13	19	17	18
3	9	18	16	23	14	18	17	16
4	10	19	7	21	12	13	13	19
5	10	17	9	18	19	19	12	18
6	14	16	16	22	20	19	15	17
7	11	18	13	16	20	19	14	16
8	14	22	7	16	15	15	18	21
SUM	92	137	92	151	134	142	121	142
AVG.	11.5	17.1	11.5	18.9	16.8	17.8	15.1	17.8