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The Effects of Several Chemical Agents on Short Term Memory

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THE EFFECTS OF SEVERAL CHEMICAL AGENTS ON SHORT TERM MEMORY

by

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Michael J. Bedecs

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INTRODUCTION

Memory consolidation, that is, the ability to receive, transmit and store data, is a phenomenon which, in contemporary literature, is generally hypothesized to be a function of one of two broad explanatory categories. These are the electrical hypothesis and the molecular hypothesis.

Historically the electrical hypothesis of memory consolidation is the earlier of the two. There are two main subapproaches to the study of this phenomenon. The first concerns the parameters around the conditions necessary for establishing the memory trace. This is exemplified by the neural reverberating circuit theory (Hebb, 1949) which postulates that an electrical reverberating circuit is established for a short duration after the initial stimulus and aids in the formation of the more permanent engram.

The second approach evolves around the sequential lesioning of all or parts of the trace. Lashley (1950) was one of the first investigators to propose extensive data on surgical lesioning techniques as well as an analysis of sensory stimulation in his attempt to "trace conditioned reflex paths through the brain or to find the locus of specific memory traces."[1] A great deal of additional data concerning memory destruction techniques has been gleaned from the use of amnesic treatments (Jarvik, 1970; Coons and Miller, 1960; Russel and Nathan, 1946; Chorover and Scheller, 1965). Here memory deficits following electroconvulsive shock, trauma,

etc. are reported.

The molecular approach to memory consolidation was first seriously suggested in the form of proposed "molecular models" built from known concepts of protein organization (Katz and Halstead, 1950). A great deal of work has been done on the possible role of RNA changes during the learning process where significant increases in total RNA and alterations in base ratio composition of both cytoplasmic and nuclear RNA in rat neurons were observed following "trial and error" learning experiences, (Hyden, 1959; Hyden and Egyhazi, 1962). Additional interest was generated by a number of investigators working in the field of "molecular approaches" to memory (see reviews by Smith, 1962 and Byrne, 1970).

There are several avenues of investigation open in this area, one of which is the memory transfer phenomenon first reported in a series of experiments by Jacobsen (1965) and later elaborated upon by Babich (1967). These experiments caused much controversy and ultimately initiated a symposium entitled "Molecular Approaches to Learning and Memory" (1967). Investigations of the memory transfer phenomenon were basically outgrowths of the larger area of research concerning protein synthesis at the neuronal level as a possible facilatory device for memory storage (Hyden, 1959; Hyden and Egyhazi, 1962; Barondes, 1965; Watson and Crick, 1953). Neuronal protein synthesis as a mode for long term memory storage, but not short term storage, has been the theme of many studies where cerebral protein inhibition procedures have been employed (Barondes, 1966, 1967, 1968; Flexner et. al. 1962, 1963, 1964, 1966).

Chamberlain (1967) using 1,1, 3 tricyano - 2 - amino - 1 - propene, a drug which reportedly stimulates nucleic acid (and protein) synthesis (Egyhazi and Hyden, 1962), found a shortening of the interval necessary for memory consolidation.

The investigation and manipulation of the chemical composition at the synapse is yet another aspect of molecular research on learning and memory. Carlton (1963), has reviewed a number of studies on the experimental application of anticholinergics where the general effect is the emission of normally inhibited behavior i.e. the disinhibition of unrewarded responses. This is compatible with the earlier data indicating an interference with maze performance after anticholinergic administration (Macht, 1963; Miles, 1929).

The question of behavior as a function of acetycholine concentration at the synapse has received much attention (see review Carlton, 1969). The data indicate that an increase in acetycholine activity is directly related to increases in behavioral inhibition.

The effects of the systemic application of anticholinergics on memory in single trial learning situations have been reported by several investigators (Buresova et. al. 1964; Bohdanecky and Vogel, 1968) where subjects were presented with painful electric shock immediately following drug application. Subjects receiving controlled injections showed a greater suppression of a trained response than the experimental group.

According to Carlton, "Inhibition of acetylcholinesterase, the

enzyme that inactivates acetylcholine, can augment normal cholinergic activity,"[2] This inhibition can initially increase acetylcholine levels but with further application can lead to a functional blockage at the synapse (McLennan, 1963) resulting in concomitant behavioral defecits. Herz (1959) was shown that anticholinergic drugs will abolish a conditioned avoidance response early in the acquisition process but that they have no effect on a well established response. This phenomenon was also examined with intrahippocampol application of the anticholinerterase disopropylfourophosphate (DFP) during a discrimination task (Liebowitz, 1968) and with the anticholinergic agent scopolamine during a maze training procedure (Pazzagli and Pepeu, 1964).

There are data which propose a possible anatomical relationship between the above chemical effects and the hippocampus. Virtually every behavioral effect observable following hippocampal lesioning (Douglas, 1967), can also be produced by the hippocampal administration of anticholinergics (Carlton, 1969).

Several studies report memory deficits following hippocampal lesions (Milner and Penfield, 1955; Isaacson and Wickelgren, 1962; Kimble, 1963; Thomas and Otis, 1968; Husich, 1970). Additional studies have shown a relationship between the role of the hippocampus in memory and both protein synthesis (Flexner et. al. 1962) and acetlcholinesterase activity (Liebowitz, 1968). It is to these findings that the majority of this investigation is directed.

This study was designed to determine the effects of several drugs on the acquisition of short term memory. The agents used were

the anticholinesterase, tetraethylpyrophosphate (TEPP); acetylcholine (ACH); inhibitors of protein synthesis, puromycin and cycloheximide, and the RNA initiator 1,1, 3, tricyono - 2 - amino - 1 - propene (TAP).

The major problem associated with the examination of short term memory is the choosing of a behavioral paradigm which scrutinizes necessarily small fluctuations in behavior. Single trial avoidance paradigms which examine changes in response rate recovery following an aversive consequence, (Jarvik and Essman, 1960; Jarvik, 1964; Essman and Alpern, 1964) have been succesfully employed. In order to measure subtle variations in the acquisition of short term memory, this study employed a one trial suppression type learning paradigm described by Feldman and Gault (1968 unpublished) and Gault and Hunt (1971, JCPP, in press).

METHOD

Subjects

Subjects were forty Sprague Dawley male rats, approximately ninety days old weighing between 280 and 320 grams. All were experimentally naive at the beginning of the experiment. Subjects were housed individually with free access to water and maintained at 80% of their ad lib weight.

Apparatus

Cannuli were constructed from 10mm lengths of PE 190 polyethylene tubing which were plugged with 10mm lengths of PE 50 tubing, the lumen of which were filled with plexiglas.

Two clear plexiglas "Skinner boxes", each with a lever, food dispenser, and floor shock grid were individually housed in sound attenuated chambers. Each chamber was provided with an exhaust fan which served to provide air circulation and background noise.

The apparatus was programmed and the responses recorded by standard electromechanical equipment. Foot shock was provided by a Grason Stadler (E6070B) shock generator.

Procedure

All subjects underwent either bilateral hippocampal or cortical cannuli implantation using clean but not aseptic surgical techniques. They were anesthetized with sodium

pentobarbital with atropine added to reduce secretion and fixed in a stereotaxic instrument. Cannuli were implanted using De Grott coordinates (Pellegrino and Cushman, 1967, see table 1) and attached to the skull with dental acrylic. Following surgical recovery the animals were placed on food deprivation until they reached 80% *ad lib* weight.

Hippocampal and cortically implanted animals received identical initial training in which they were shaped to lever press for food on a variable interval 30 second schedule of reinforcement. Daily 30 minute sessions were employed for nine days, or until S's had reached a stable baseline of responding (300 $\pm$ 50 responses). Subsequent to stabilization of responding on this schedule the two groups received different treatments.

Hippocampally implanted animals received one 30 minute session of extinction following stabilization. They were then returned to their home cages for 24 hours. On the next session they were allowed to make one lever pressing which triggered a 5.0 milliamp foot shock for 0.75 seconds. They were then immediately removed from the apparatus, received bilateral administration of the specified drug, and returned to their home cages for 24 hours. On the next session, they were placed back on the VI - 30 food reinforced schedule and responses were tabulated either every minute or in the case of animals run later in the sequence, every ten seconds.

Cortically implanted animals were treated similarly to the hippocampal animals with the exception that they were given 3 injections at 3 day intervals (prior to each session) and they did

not receive the 30 minute extinction session. On the session following stabilization of responding, they were allowed to make two lever presses, each triggering a 3.0 milliamp foot shock for 0.75 seconds. On the subsequent day they were returned to the VI - 30 schedule with responses being recorded every minute.

Each subject received 6 microliters of drug. The concentrations of each agent are shown in table 2. Histological examination to verify cannuli placement were done following termination of the final session.

RESULTS

In the present paradigm rats were exposed to several days of training for a particular task followed by a sudden single, novel, noxious stimulus. They were subsequently tested 24 hours later for their ability to remember the single trial and respond appropriately. Thus behavior indicative of short term memory consolidation was examined.

Figures 1 through 6 show the response recovery rate for post shock sessions. Table 3 indicates the results of the analysis of the first 4 minutes of recovery from the examination of differences in post shock response rates between experimental and control groups using the standard "t" test.

The effects of both intrahippocampal and cortical applications of TEPP are shown by figures 1 and 2 respectively. In both cases the data indicate a greater post shock recovery rate of responding for the experimental subjects as compared to each treatments saline control group. Both hippocampal and cortical applications of TEPP yielded response rates significantly higher than their controls ($p < .001$).

The effects of intrahippocampal administration of ACH and puromycin on response rate are displayed in figures 3 and 4 respectively. Again differences in post shock recovery rates between experimental and control groups as a result of drug administration are depicted. These differences in rates are

found to be significant in both the ACH experiment (p .001) and those animals receiving puromycin (p .05).

Figures 5 and 6 show the recovery rates of subjects receiving intrahippocampal injections of TAP and cycloheximide respectively. While the trend of recovery indicates higher rates in the experimental groups (especially the cycloheximide treated animals), neither experiment yields statistical significance between experimental and control rates of responding.

Table 1

Cannuli Implantation Coordinates

Implant Site	Anterior	Lateral	Depth
Hippocampus	+2.18mm	± 3.0mm	+2.5mm
Cortex	+2.18mm	± 3.0mm	Cortex Surface

De Groot stereotoxic coordinate used for implantation procedures (Pellegrino and Cushman, 1967).

Table 2

Drug Doseage In Micrograms

Drug	Micrograms
TEPP (Hippocampus)	1.74×10^{-3}
TEPP (Cortex)	1.74×10^{-5}
ACH	0.96
TAP	54.00
Puromycin	0.54
Cycloheximide	0.12

Table 3

Levels of Statistical Significance

Treatment	P
TEPP (Cortex)	.001
TEPP (Hippo)	.001
PURO (Hippo)	.05
CYCLO (Hippo)	N.S
ACH (Hippo)	.001
TAP (Hippo)	N.S

FIGURE 1

Post shock recovery response rate of subjects receiving hippocampal applications of TEPP.

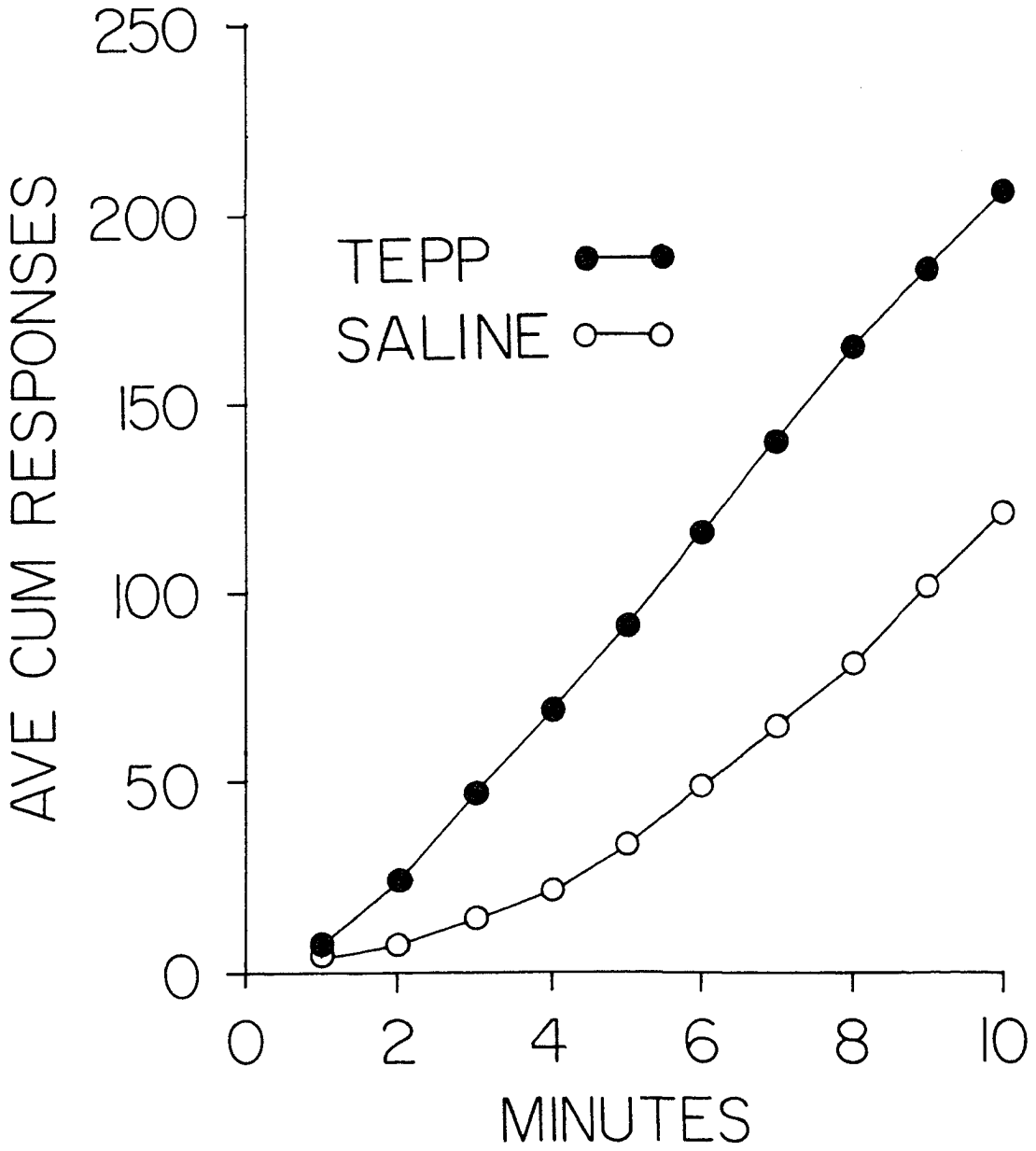


FIGURE 2

Post shock recovery response rate of subjects receiving cortical applications of TEPP.

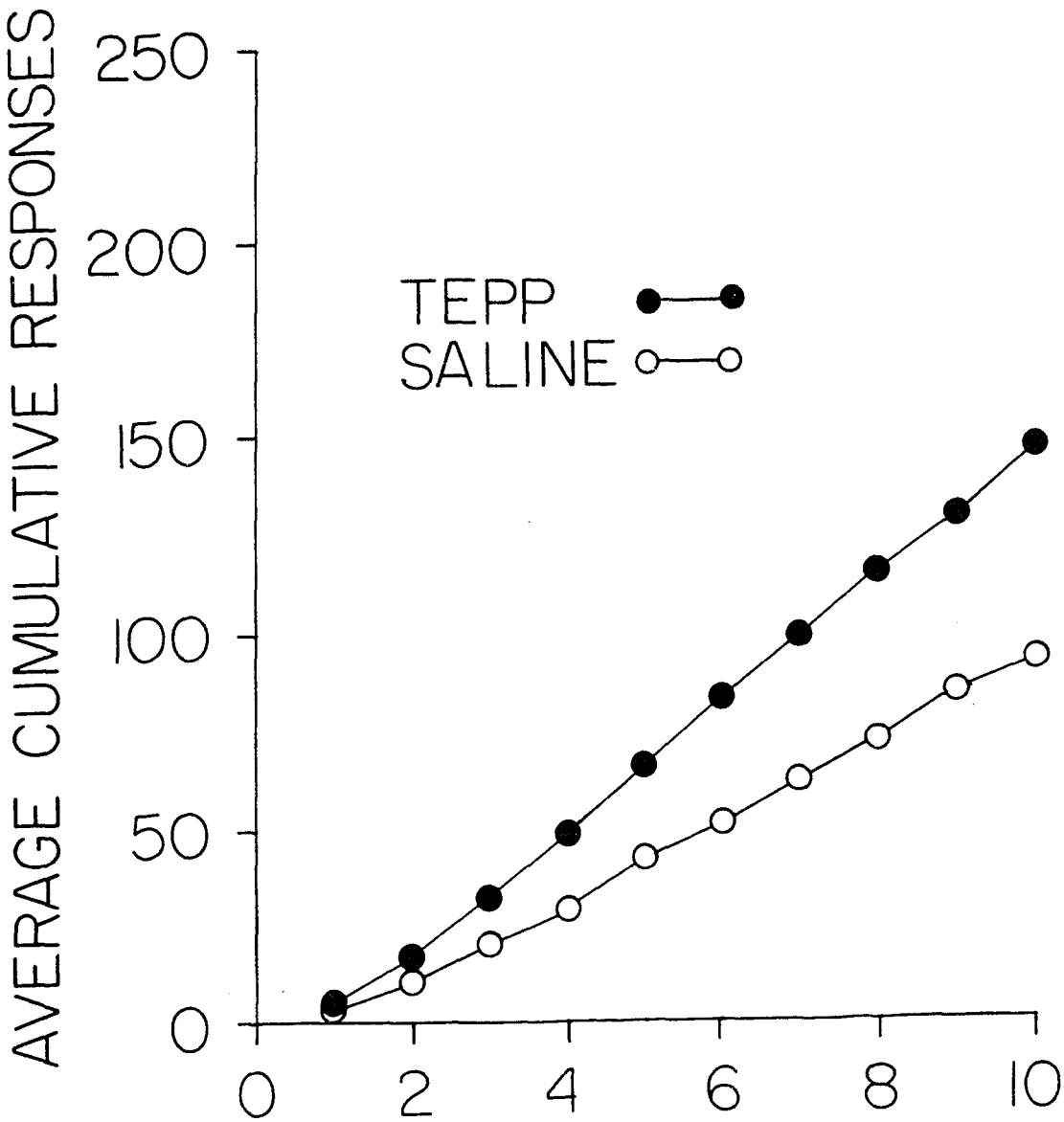


FIGURE 3

Post shock recovery response rate of subjects receiving hippocampal applications of Acetylcholine.

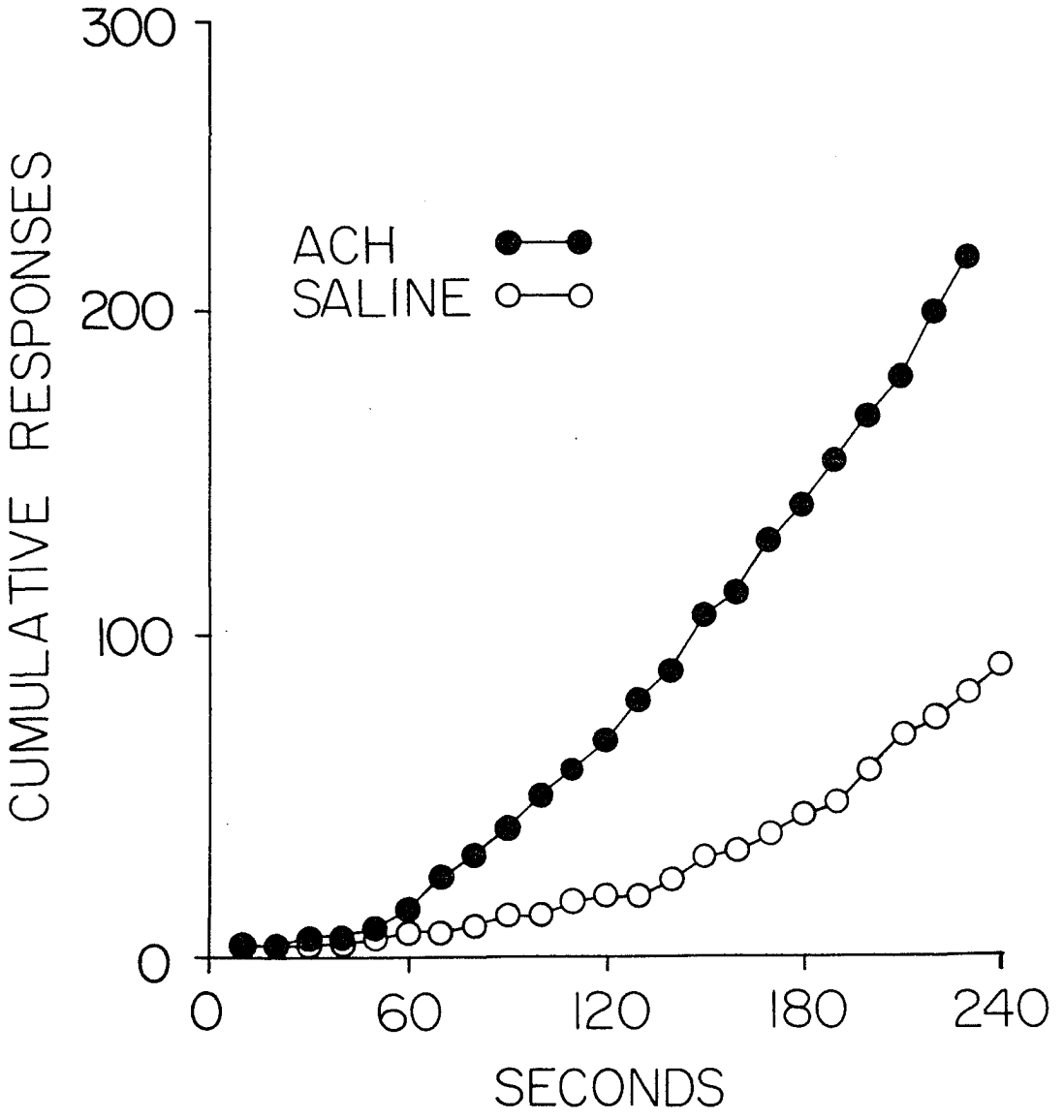


FIGURE 4

Post shock recovery response rate of subjects receiving hippocampal applications of Puromycin.

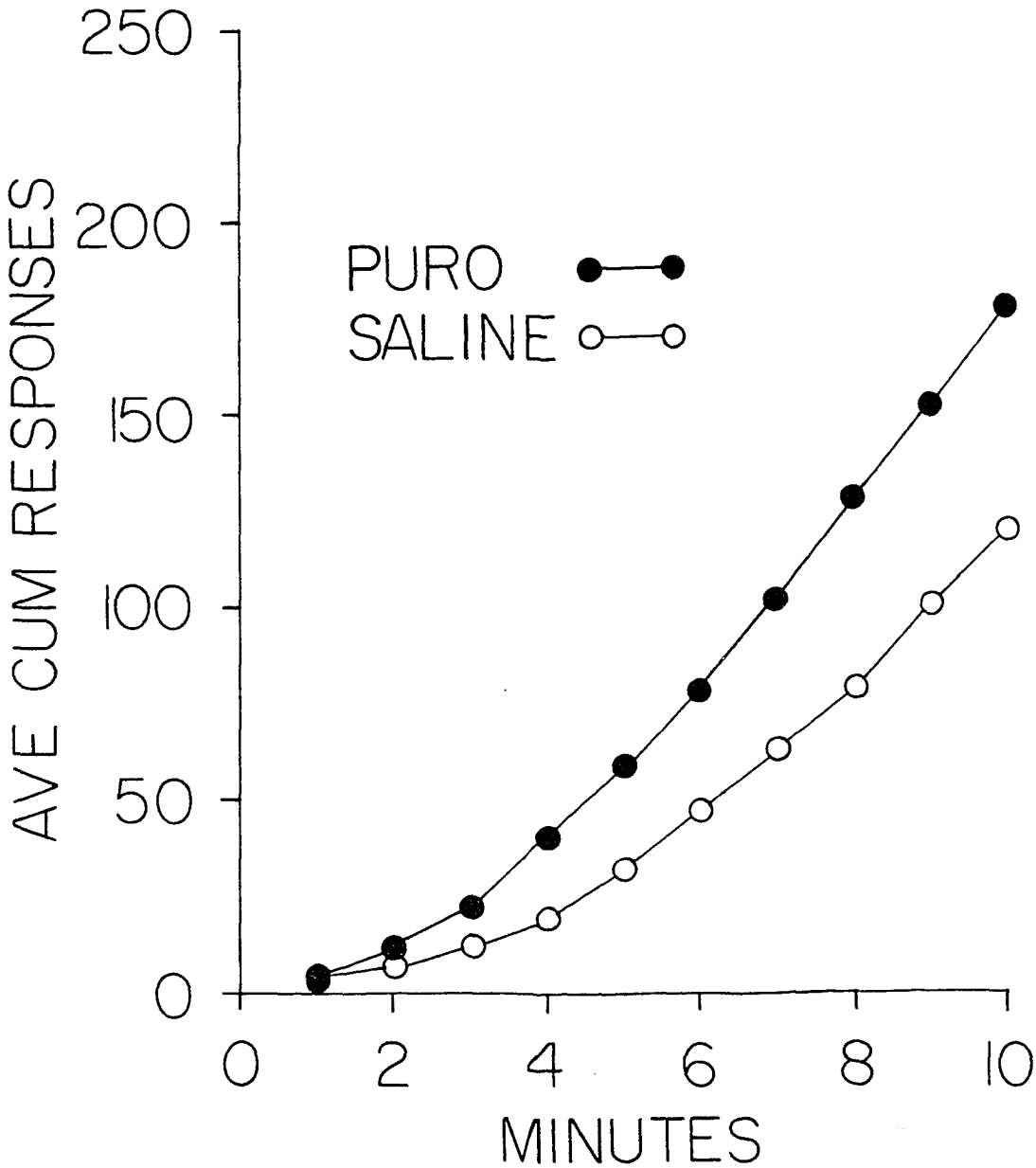


FIGURE 5

Post shock recovery response rate of subjects receiving hippocampal applications of TAP.

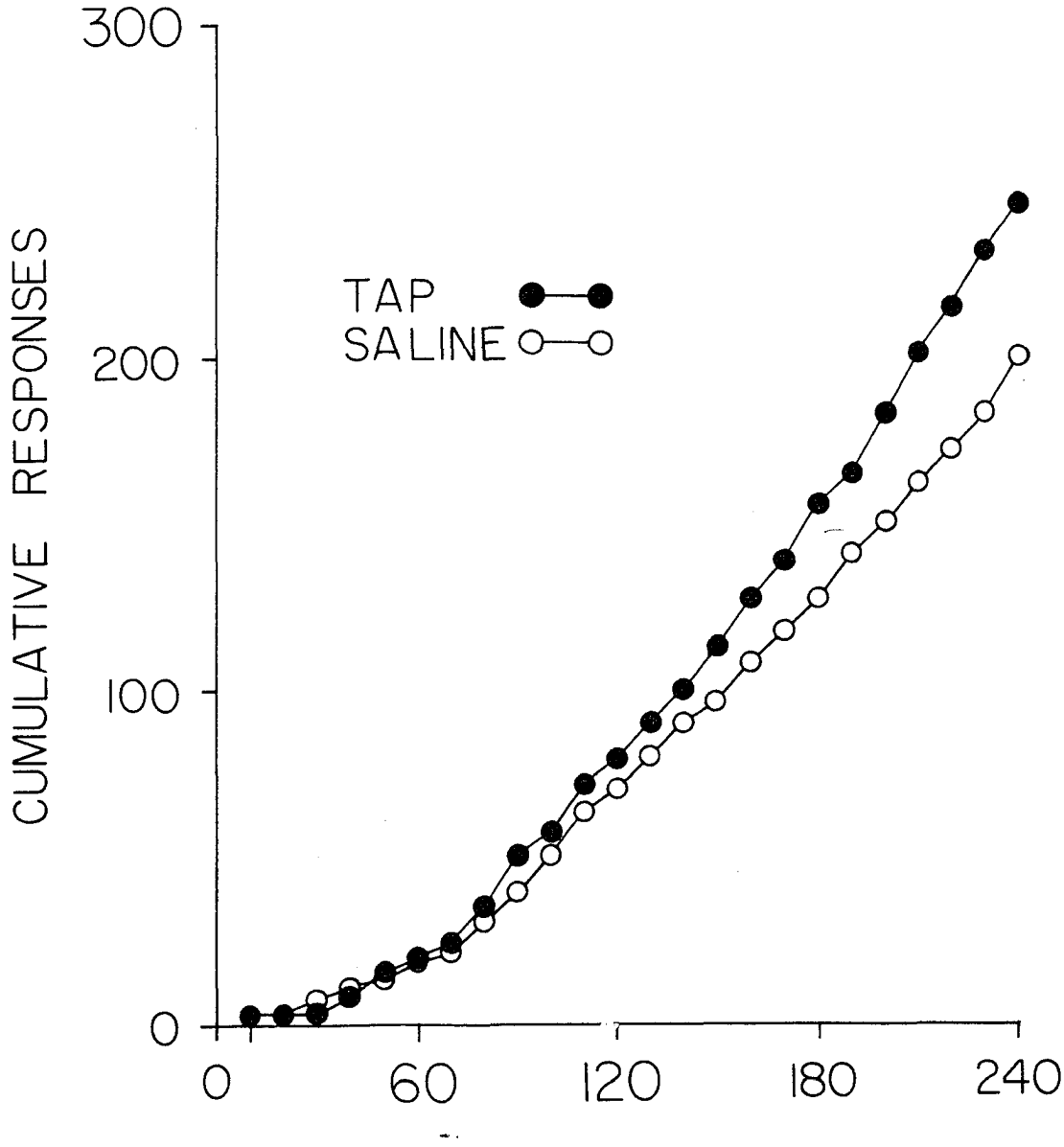
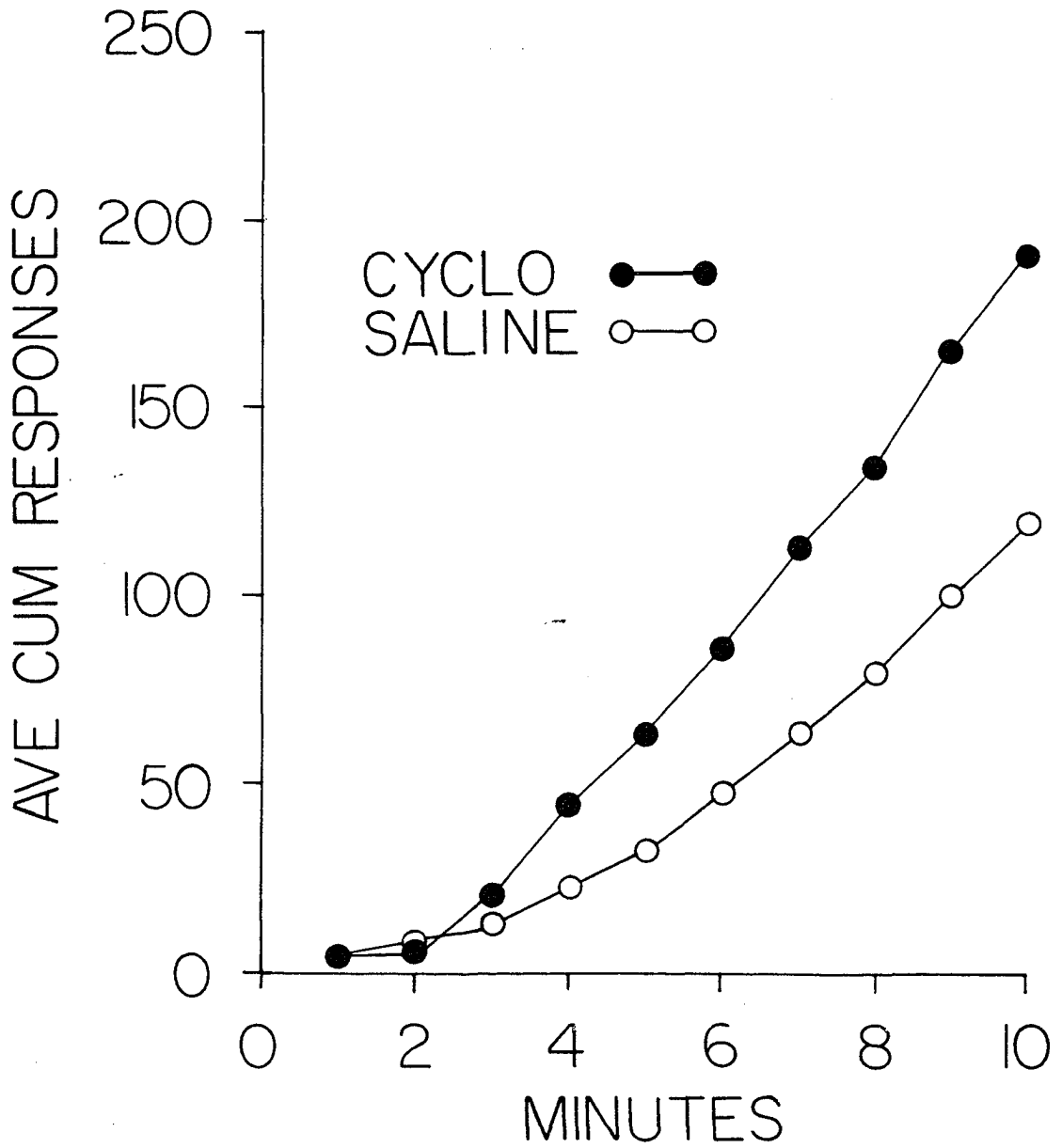


FIGURE 6

Post shock recovery response rate of subjects receiving hippocampal applications of Cycloheximide.



DISCUSSION

The results of these experiments demonstrate the disruption of short term memory consolidation as a function of intrahippocampal and cortical injections of various chemical agents. These data are partially consonant with results obtained with puromycin and cycloheximide (Gault and Hunt, 1971). They also agree with results obtained with DFP, another anticholinesterase, (Liebowitz, 1968), and with those effects reported for hippocampal lesioning (Douglas, 1967; Milner and Penfield, 1955; Isaacson and Wickelgren, 1962; Kimble, 1963; Thomas and Otis, 1968; Leaton, 1963; Husich, 1970).

These investigations were designed to influence the memory consolidation process in several biochemically different dimensions. The application of protein (RNA) inhibitory substances had varying effects. Puromycin was shown to inhibit memory while cycloheximide did not significantly alter consolidation. These data are in partial agreement with studies which have demonstrated significant results following application of both agents (Gault and Hunt, 1971; Flexner et. al. 1962, 1963, 1964, 1965), where both puromycin and cycloheximide treated animals incured memory loss for a specific stimulus.

Several factors may be considered as possible explanations for the discrepancy in the cycloheximide data, i.e. a small sample size (n=4) and the use of 1 minute rather than 10 second data recording intervals. Demonstration of the effects of cycloheximide may in

fact need the additional precision gained by shorter data collection intervals and larger sample size to become apparent.

To test the effects opposite to RNA inhibition at the hippocampus, 1,1, 3 - tricyano - 2 - amino - 1 - propene (TAP), an RNA initiating substance was employed. Both visual inspection and analysis of the data show virtually no influence upon memory consolidation, following administration of this drug. This is not in full agreement with those data indicating a facilitory effect on memory (Chamberlain, 1967) following TAP administration.

The role of the neural transmitter substance acetycholine (ACH) in memory consolidation was also investigated. Intrahippocampal administration of tetraethylpyrophosphate (TEPP), an anti enzymatic agent which blocks ACH activity, was a powerful method for acquisitional disruption of short term memory. These data are in excellent agreement with investigations utilizing a similar drug (DFP), paradigm and injection site, (Liebowitz, 1968).

The logical conclusion that since inhibition of ACH resulted in inhibition of memory, facilitation of the transmitter should enhance memory was not obtained in this investigation. Administration of ACH directly to the hippocampus resulted in a significant disruption of memory. Additional investigations are necessary to scrutinize the significance of these data.

The experiment which examined the results of cortical applications of TEPP over a several day period during task training was designed to test the effects of an anticholinesterase specific drug (at a specified doseage) on memory acquisition. Because the

drug dosage was aimed at the selective inhibition of cholinesterase (CHE) while not affecting acetylcholinesterase (ACHE) activity, a viable speculation in conjunction with Rosenzweig studies (1969) on environmental complexity and cerebral change may be extended. Since CHE is found in glial cells as opposed to the ACHE in neuronal cells, and since the agent was administered over the entire acquisitional span of the study, the possibility of glial cell proliferation in the cortex as a synaptic mode for memory acquisition is postulated. There however exists the possibility that this effect may be related to the above hippocampal effect as cannuli were approximated to cortical surfaces directly above the hippocampus. This again is an area for further research.

FOOTNOTES

[1]Lashley, C. In search of the engram. In Beach, F.A., Hebb, D.O., Morgan, C.T. and Niessen, H.W., The Neuropsychology of Lashley. New York: McGraw Hill, 1960, p. 479.

[2]Carlton, P.L. Reinforcement and Behavior. New York: Academic Press, 1969, p. 317.

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