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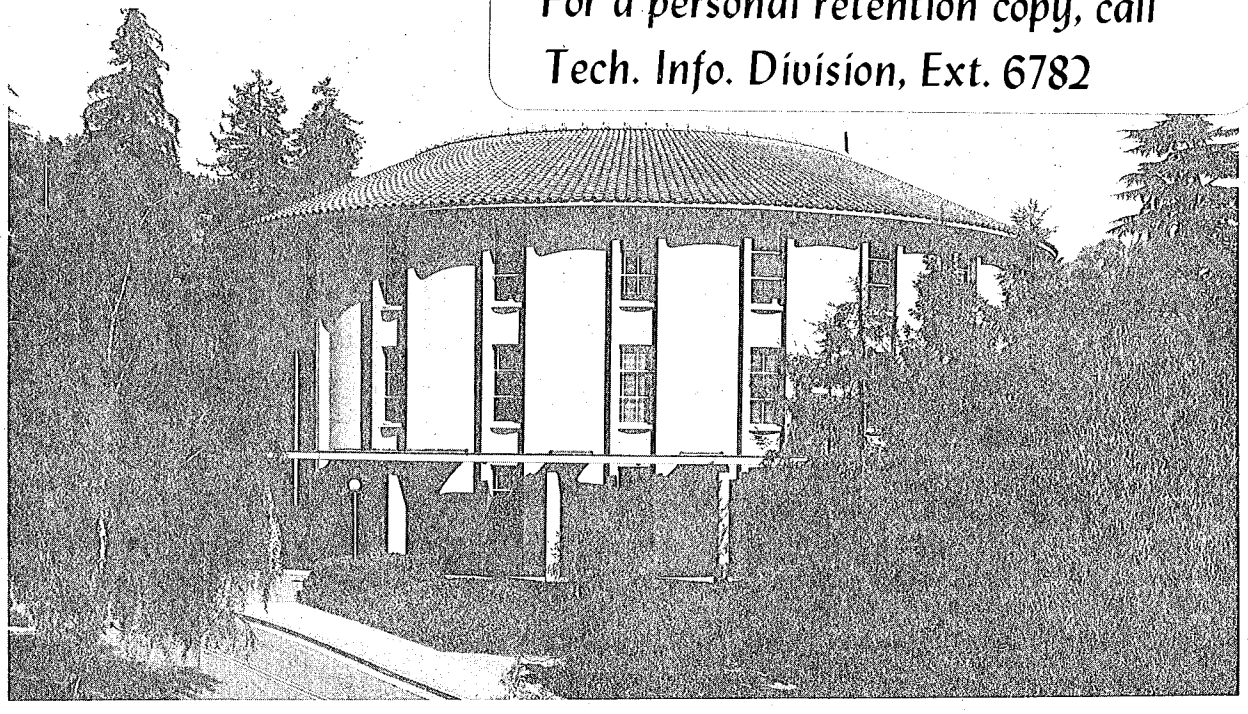

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KEY WORDS

Memory, Passive Avoidance, Active Avoidance, Protein Synthesis Inhibition, Inhibition of Axonal Transport, Anisomycin, Colchicine, Vinblastine, Podophyllotoxin

ABSTRACT

Colchicine (60 µg/kg), an inhibitor of axoplasmic transport, administered subcutaneously to mice had no detectable effect on retention when given before or after active avoidance training. A single pretraining injection of anisomycin (ANI) had no amnesic effect. However, when ANI was administered prior to training and colchicine was administered after training, retention test performance was disrupted. The amnesic effect was dependent on the time and dose at which colchicine was administered. The amnesic effect was also obtained with ANI and vinblastine (6 µg/kg) or podophyllotoxin (3 µg/kg), drugs which inhibit axoplasmic transport. Lumicolchicine (60 µg/kg) did not impair retention in ANI pretreated mice. Intracerebral injections of colchicine (60 ng to 60 pg) in subjects pretreated with ANI yielded amnesia. However, in saline pretreated subjects the intracerebral administration of colchicine did not affect retention. It is proposed that transport of recently synthesized protein into neuronal processes is required for long-term memory storage.

Research has led to the hypothesis that protein synthesis is necessary for long-term memory consolidation. While there is general agreement that inhibitors of brain protein synthesis cause amnesia, opinions differ as to the nature of the protein needed for the formation of new memories. Hypotheses have been advanced which suggest that the important protein is either a "memory molecule", an enzyme, a protein altering DNA expression or a structural protein. Some of these proteins would be transported into the axons, others in dendrites and some would be expected to remain in the cell body of neurons in the central nervous system.

Colchicine, a plant alkaloid, inhibits fast axoplasmic flow by binding to tubulin, the principal structural protein of microtubules (~~Wilson and Friedkin, 1966; McClure, 1972~~[19,34]). Cherfas and Bateson (~~1978~~[3]) found that an injection of colchicine as high as 256 µg/kg slightly impaired overall responding on a chick passive avoidance task but did not clearly impair either acquisition or short term memory. In another study, mice were trained to jump onto a shelf to avoid footshock. When vinblastine was administered one week prior to training either peripherally (50 µg) or intracerebrally (0.5 µg), it impaired acquisition (~~Murakami, 1980~~[21]). Retention test performance was impaired by similar injections given to different groups of mice immediately after training. But most of the impairment of retention was probably due to general disability since subjects that had been previously trained and were then injected with vinblastine also performed poorly on the retention test. These high doses of inhibitors of axoplasmic transport appear to disrupt performance rather than processes specifically involved in learning or memory storage. In comparing vinblastine with colchicine, the 50 µg dose of vinblastine is about equivalent to giving 500 µg of colchicine (~~McClure and Paulson, 1977~~[20]). At a lower

absolute dose, colchicine (60 μg) caused amnesia in goldfish when injected into the cranial cavity prior to shock avoidance training but it did not cause amnesia when injected after training. The pretraining injection of colchicine did not affect acquisition. One hour after colchicine administration, microelectrode recordings from cells in the optic tectum being driven by visual stimuli failed to reveal any gross abnormalities (~~Cronly-Dillon et al., 1974~~)[5].

Based on studies in rodents, these doses seem to be considerably higher than should be necessary, since 40 μg/kg administered peripherally to rodents was reported to inhibit fast axoplasmic flow in the central nervous system (~~Rose and Sinha, 1976; Rose, 1976~~)[28,29]. More importantly, high doses of colchicine are associated with a variety of side effects such as blocking the release of neurotransmitters, inhibiting uptake of dopamine (DA) and norepinephrine (NE), and inhibiting the action of vasopressin. The purpose of this study was to test the hypothesis that the formation of new memories requires the synthesis and transport of protein shortly after training.

Behavioral Experiments

Animals

The subjects, Swiss Webster (CD-1) male mice, 60-80 days old at training, were obtained from Charles River Breeding Laboratories at 6 weeks of age. They were housed singly 24 hr prior to training and until the retention test one week later.

Training Procedures

Step-Through Passive Avoidance

The procedure for training and testing mice for the one-trial, step-through passive avoidance task has been described previously (~~Flood et al., 1972, 1974~~)[9,10]. In brief, the apparatus consisted of a black start compartment

joined to a white shock compartment by a partition containing a mouse hole through which the subjects could enter the white compartment. In the white compartment, footshock was given until the mouse returned to the black compartment. In order to test retention, the mice were again placed into the black compartment and the time required for the subject to enter the white compartment was taken as a measure of retention. A latency-to-enter the white shock compartment on the test day of 20 sec or less was defined as amnesia. Most trained non-amnesic mice did not enter the white compartment within the three-minute test period. Throughout, training and testing were done between the hours of 0730 and 1400.

T-Maze Active Avoidance

The T-maze training and apparatus were previously described (~~Flood et al, 1975a~~)[11]. The training apparatus consisted of a black Plexiglas start alley with a start box at one end and two goal boxes at the other fitted with clear plastic liners used to remove the mouse from the box. The bottoms of these liners went below the shock grid which ran throughout the entire maze. The start box was separated from the rest of the start alley by a black Plexiglas guillotine door which prevented the animals from moving down the start alley until the trial started. A doorbell-type buzzer served as the conditioned stimulus. The intertrial interval was about 45 sec.

Training consisted of placing the mouse in the start box, then raising the guillotine door and simultaneously sounding the buzzer. Mice not moving to the correct goal box within 5 sec received footshock until they did so. The side preference was determined on the first training trial by forcing all mice to go to the side opposite their first response. On subsequent trials, the correct side was the initially non-preferred side for each mouse. At the end

of each trial, the mouse was removed to its home cage by carefully removing the liner and placing it into the mouse cage. As training proceeded, a mouse could make one of two types of response: (a) a response latency longer than 5 sec was an escape; (b) a response latency of 5 sec or less was an avoidance. The retention test was given one week after training and consisted of retraining the mouse to a criterion of 1 avoidance response (~~Flood et al, 1975a~~[11]). Mice requiring more than 3 trials to make an avoidance response were classed as amnesic.

Drugs

All drugs and control saline injections were administered subcutaneously. Anisomycin (ANI) was administered at 20 mg/kg and colchicine at 60 µg/kg. Both drugs were prepared in physiological saline and pH's were about 5-6. ANI was obtained from Pfizer, Inc. and colchicine from Eli Lilly and Co. Lumicolchicine was prepared by the ultraviolet irradiation (Xenon lamp source, $CuSO_4$ filter) of colchicine in absolute ethanol (~~Wilson and Friedkin, 1966~~[34]). Conversion to lumicolchicine was 96% based upon final uv spectrum. The lumicolchicine solution was evaporated to dryness in vacuo and the product was dissolved at a concentration of 25 µg/ml in 0.9% NaCl as a stock solution.

Experiment 1

Effect of Pretraining Colchicine Injection on Retention

The purpose of this experiment was to determine if colchicine (60 µg/kg) administered subcutaneously would impair retention for passive avoidance conditioning. Colchicine or saline was administered 1 hr prior to passive avoidance training using latencies to enter and escape the shock box of 2 sec (to the nearest second) and a footshock intensity of 0.30 ma. Retention tests were given to three sets of saline and colchicine injected subjects at either 1, 3 or 5 weeks. The results showed that forgetting increased from 1 to

5 weeks in both saline and colchicine injected subjects (saline 20%, 40%, 69%; colchicine 29%, 50%, 75% for tests given 1, 3, 5 weeks). While colchicine mice consistently showed poorer retention at each test period, the largest difference between the saline and colchicine groups was only 10%.

Experiment 2

The Effect of ANI and Post-training Colchicine Administration on Retention for T-Maze Active Avoidance

Experiment 1 showed that colchicine had no significant effect on retention. However, colchicine does not completely block cytoplasmic flow (Paulson and McClure, 1975[25]). If a large amount of memory-related protein is synthesized immediately after training, then a partial blockage of axoplasmic or dendritic flow might not be sufficient to prevent long-term memory from forming. We reported previously that as protein synthesis occurs at later intervals after training, the less likely is the synthesis to establish long-term memory (Flood et al, 1975b[12]). Thus in this experiment, anisomycin (ANI) was used to delay protein synthesis for 2 hr until the capacity for protein synthesis to establish long-term memory was reduced. Colchicine was administered immediately after training to test if transport of protein into the processes of neurons was important for long-term memory formation.

The subjects were given 5 training trials on T-maze active avoidance as described above. ANI or saline was administered subcutaneously 15 min prior to training. The saline or ANI injected subjects also received a subcutaneous injection of either colchicine (60 µg/kg) or saline immediately after training. The number of subjects in each group was 20. The retention test was

given one week after training. Forgetting was defined as failing to make an avoidance response on or before the third retention trial. For those who prefer results expressed as means and standard errors, these data are provided in Table 1 as mean trials to first avoidance.

RESULTS

Saline control retention was good (15% forgetting). Neither ANI nor colchicine alone had an effect on retention. However, when ANI was administered prior to training and colchicine was administered after training, significantly more mice were classed as forgetting (70%) than controls (Table 1).

Experiment 3

A Comparison of the Effect of Three Axoplasmic Flow Inhibitors on Retention

This experiment compared the amnesic effect of ANI given in combination with the inhibitors of fast axoplasmic flow (ANI+Colchicine, ANI+Vinblastine, ANI+ Podophyllotoxin). Colchicine and its isomer lumicolchicine have similar effects on the central nervous system (~~Paulson and McClure, 1975; Owellen et al, 1972~~)[25, 24], but lumicolchicine has a low binding affinity for microtubule protein; therefore it is considerably less effective as a blocker of axoplasmic flow (~~McClure and Paulson, 1977; Dahlstrom et al, 1975; Madelon, 1974~~)[8, 18, 20]. ANI, in combination with lumicolchicine, was used to determine if ANI+Colchicine induced amnesia was related to side effects not associated with blocking axoplasmic/dendritic flow.

The training and testing were as in Experiment 2. ANI was administered 15 min prior to training and each of the following was administered immediately after training: colchicine (60 µg/kg), vinblastine (6 µg/kg), podophyllotoxin (3 µg/kg), lumicolchicine (60 µg/kg), or saline. The doses of the three inhibitors were based in part on the work by Paulson and McClure (1975)[25] from

which we estimated the ratio of doses needed to obtain similar degrees of inhibition of axonal transport relative to the dose of colchicine being used. Preliminary tests showed that mice given saline prior to training and colchicine, vinblastine or podophyllotoxin after training did not develop amnesia (see Table 2). The pretraining test and experiment were run double blind with respect to all substances injected. The N's are given in Table 2. Retention was tested one week after training.

RESULTS

The retention of the saline control group was good (22% forgetting). Subjects receiving an injection of saline prior to training and an injection of colchicine, vinblastine, podophyllotoxin or lumicolchicine yielded retention scores that did not differ significantly from the saline controls (Table 2). However, when ANI was administered prior to training and any of the inhibitors of axoplasmic flow were administered after training, significantly more subjects were classed as forgetting than controls given only ANI or saline. Lumicolchicine given in combination with ANI had no impairing effect on retention.

Experiment 4

The Effect of Blocking Transport of Memory-Related Protein on Retention

In a previous study, a "pulse" of protein synthesis could be created by altering the normal schedule under which ANI was administered. Usually, ANI was administered every two hours to maintain continuous inhibition of protein synthesis at 80% or greater, but an additional delay beyond the 2-hr interinjection interval allowed recovery of protein synthesis proportional to

the length of the delay period (Flood et al, 1975b). This pulse of protein synthesis against a background of extensive inhibition prevented amnesia when the delay in the injection schedule was sufficiently long. This suggested that protein needed for long-term memory was synthesized during the pulse period. If colchicine blocks the transport of protein(s) needed for long-term memory, then blocking axoplasmic/dendritic flow before, during and after the pulse should yield a time dependent amnesic effect with respect to the time of colchicine administration.

Mice received three successive injections of ANI or saline at 2 hr intervals beginning 15 min prior to step-through passive avoidance training. Several additional groups received one injection of ANI 15 min prior to training: the second injection was delayed 90 min beyond the usual 2 hour interinjection interval and was given 3.5 hr after the first ANI injection and the third injection 2 hrs after the second ANI injection (ANI-90-ANI+ANI). Pilot data indicated that the additional 90 min delay between the first and second ANI injections prevented amnesia induced by ANI+ANI+ANI treatment. The ANI-90-ANI+ANI subjects were divided into groups that received either colchicine or saline. Colchicine was administered 1, 2, 3 or 4 hrs after the first ANI injection. Saline was administered in place of colchicine either 1 or 4 hrs after the first ANI injection. To determine if colchicine might be blocking the transport of catecholamine transmitters, AMPT was used to inihibit dopamine and norepinephrine synthesis. AMPT was either administered simultaneously with ANI or was administered 2 hr after the first ANI injection (ANI+AMPT-90-ANI+ANI/Saline$_1$, ANI-90-ANI+ANI/AMPT$_1$). The training procedure for passive avoidance was described above. The distribution of latencies to enter and escape from the shock compartment control acquisition of this task. Only subjects with latencies to enter in 2 seconds and to escape in 2 seconds

were used. Latencies were measured to the nearest 10th sec and rounded off to seconds. The N's are given in Figure 1. The retention test was administered 1 week after training and drug treatment.

RESULTS

Three successive injections of ANI at 2 hr intervals yielded a significantly higher percentage of mice classed as forgetting than occurred in saline controls (85% vs 10% forgetting, $p<.001$). An additional 90 min delay in the injection schedule between the first and second ANI injection blocked amnesia (85% vs 6% or 12% forgetting for ANI+ANI+ANI vs ANI-90-ANI+ANI/Saline$_1$ or $_4$; either $p<.001$). Thus protein synthesis needed for long-term memory formation occurred during the 90 min delay period. This replicated previous findings more extensively studied (~~Flood et al., 1975b~~ [12]).

Colchicine was administered prior to the pulse of protein synthesis to determine if transport of the memory related protein was required for memory consolidation. When colchicine was administered either 1 or 2 hrs after the first ANI injection as much forgetting occurred as with subjects receiving three successive injections of ANI at 2 hr intervals (Figure 1). Thus, colchicine prevented the pulse of protein synthesis from establishing long-term memory. The ANI+ANI+ANI group did not differ from either ANI-90-ANI+ANI/Colchicine$_1$ or$_2$ (85%, 73%, 65% forgetting respectively). However, when colchicine was administered 3 or 4 hrs after the first ANI injection, it failed to prevent long-term memory formation since significantly fewer subjects were classed as forgetting (Figure 1). AMPT administered either simultaneously with the first ANI injection or 1 hr after the first ANI injection did not significantly alter the retention of groups having the pulse of protein synthesis (ANI+AMPT-90-ANI+ANI/Saline$_1$ 29%, ANI-90-ANI+ANI/AMPT$_1$ 18% and ANI-90-ANI+ANI/Saline$_1$ 6% forgetting). The slightly higher percent forgetting in the groups receiving AMPT is consistent with reports that pretraining AMPT administration can cause amnesia (~~Quartermain and Botwinick~~[27], ~~1975~~).

Experiment 5

Effect of Central Colchicine Administration on Retention

Colchicine does not readily cross the blood brain barrier (~~Hunter and Klaasen, 1975; Bennett et al, 1980~~[1,14]). This raised the question whether the amnesic effect of colchicine in ANI-treated mice resulted from central or peripheral effects of the drug. We tested for central mediation of the amnesic effect by giving ANI subcutaneously prior to training and by giving colchicine intracerebrally after training. The mice were prepared for the intracerebral

injections by surgery performed 24 to 48 hr prior to training. This was done so that only the shortest possible time was needed to administer colchicine or saline under ether anesthesia in a stereotaxic instrument. Prior to beginning this study an extensive effort was made to verify that the stereotaxic coordinates for bilateral injections into the anterior region of the caudate were correct. Thionin injected into the area showed that a volume of 0.5 µl was reliably found in the anterior, medial portion of the caudate and over a 30 min period did not diffuse into the surrounding structures.

The operation consisted of deflecting the scalp and drilling one hole over each part of the caudate (0.5 mm anterior to bregma, 2 mm left and right of the central suture). The holes were covered with a light application of bone wax and the subject was returned to its cage. Immediately after training, the subjects were anesthetized with ether and returned to the stereotaxic instrument. Colchicine or saline was injected within 3 min after training using a microsyringe fixed with a 31 gauge needle and anchored to the micromanipulator. A volume of 0.5 µl was injected at a depth of 3.2 mm into each caudate. After the injection, bone wax was reapplied, the wound closed and the subject returned to its cage. Operated, injected controls were included in every session. Groups were run double blind.

Mice were trained on T-maze active avoidance 24 to 48 hr after preliminary surgery. Subjects were given 5 training trials as described above. The results of Exp. 2 through 4 indicated that the amnesia was due to an interaction between ANI and colchicine. In a previous study, we found that injections of ANI into each caudate caused amnesia (~~Flood et al, 1980~~[13]). Thus it seemed likely that the caudate was one area that might mediate the interaction. To determine if a relationship between the dose of colchicine and amnesia existed, colchicine was administered at 0.5 µl into each caudate in ANI

pretreated mice at the following concentrations: $6 \times 10^{-2}, ^{-3}, ^{-4}, ^{-5}$ or $^{-6}$ mg/ml. Other groups of subjects were given saline 15 min prior to training and bilateral injections of one of the 5 concentrations of colchicine into the caudate. In addition, two groups received either a pretraining injection of ANI, or saline followed by a 0.5 µl injection of saline. The groups and their N's are shown in Table 3.

Results

Subjects pretreated with saline and given any of the 5 concentrations of colchicine after training did not differ from subjects given saline before training and intracerebral injections of saline after training. However, subjects that received ANI subcutaneously prior to training and colchicine after training forgot significantly more than ANI-injected subjects given saline injections after training. Colchicine was an effective amnesic treatment in combination with ANI at quantities from 30 ng/side to 30 pg/side. Mice given injections of colchicine at 3 pg/side did not differ significantly from ANI subjects given intracerebral injections of saline (Table 3).

Discussion

Three hypotheses may account for the amnesic effect of administering both an inhibitor of protein synthesis and an inhibitor of axoplasmic transport. The inhibitors may aid one or the other's mechanism of action so that the inhibitor of axoplasmic flow also inhibited protein synthesis, or ANI inhibited axoplasmic flow. A second mechanism might involve some of the side effects of colchicine, vinblastine or podophyllotoxin which interacted with ANI to cause amnesia. A third possibility is that ANI prevented the synthesis of

protein(s) needed at synaptic membranes, and colchicine reduced the flow of newly synthesized protein(s) once synthesis recovered.

Synergistic Mechanism of Action

Research results indicated that colchicine did not inhibit the uptake of labeled amino acid into protein as did the protein synthesis inhibitors cycloheximide or puromycin (Karlsson and Sjöstrand, 1969; James et al, 1970; Cronly-Dillon et al, 1974; Rose and Sinha, 1976)[5, 15, 16, 29]. In addition, Rose and Sinha (1976)[29] studied the effect of cycloheximide on axoplasmic flow and concluded that cycloheximide had no greater effect on transport of labeled amino acid than would be expected from its inhibitory effect on protein synthesis. Thus it seems unlikely that ANI inhibited axoplasmic flow or that colchicine inhibited protein synthesis.

Side Effects as a Mechanism of Action

Colchicine, vinblastine and podophyllotoxin block fast and slow axoplasmic transport by binding to the protein in microtubules. A variety of side effects of colchicine or vinblastine administration have been reported which might in part account for the amnesic effect. These inhibitors are known to block the transport of NE (Sorimachi et al, 1977; Dahlström, 1968[7, 31]) or its release (Thoa et al, 1972; Berl and Nichlas, 1975[2,32]). The uptake of NE, DA, gamma aminobutyric acid and glutamate in a rat brain synaptosomal fraction was inhibited by vinblastine (.05 to .25 mM) and colchicine (.1 to 1.0 mM); the lowest concentrations were clearly less effective at blocking uptake than the high concentrations (Nicklas et al, 1973[22]). Serotonin release was decreased by vinblastine in response to potassium ions, but colchicine did not affect serotonin release. Sodium-potassium dependent ATPase activity was not affected by either colchicine or vinblastine (Segawa et al, 1978[30]). Colchicine (1 mM) and vinblastine (.1 mM) blocked acetylcholine transmission when perfused into the superior cervical ganglion in cat (Trifaró et al, 1972[33]). Colchicine (40 µg) injected intraocularly in pigeons depressed evoked potentials in the optic tectum (Perišić and Cuénod, 1972[26]). RNA synthesis can be inhibited by vinblastine with the high dose of 2 mg/kg (Creasey, 1968[4]). Certain changes in the ultrastructure of neurons in the supraoptic nucleus occurred after administration of colchicine (40 µg) into the subarachnoid space in rat: (a) the golgi complex was enlarged; (b) the number of mitochondria, lysosomes and phagosomes increased; (c) the nuclear membrane was deeply folded; and (d) the granular endoplasmic reticulum was distended by reticular and filamentous material (Norström et al, 1971[23]). Colchicine administration intraocularly at 100 to 500 µg induced an enlargement of the synaptic vesicles, abnormal mitochondria and glycogen granules (Cuénod et al,[6]

1972). While we cannot rule out side effects such as those above as part of the cause of the amnesia obtained with ANI and any of the inhibitors of axoplasmic flow, the dosages or concentrations used to demonstrate these effects are considerably greater than used in our experiments.

The Role of Protein Synthesis and Cytoplasmic Flow in Memory Processing

The interpretation of these results depends on several findings relating to the effect of ANI on protein synthesis and retention. First, ANI inhibits protein synthesis for 2 hrs at 80% or greater and, when injections are given at 2 hr intervals, each injection extends this level of protein synthesis inhibition by 2 more hours. Second, as the number of ANI injections increases, so does the amnesic effect. If one increases the interval between successive ANI injections beyond the 2 hr interinjection interval, then a pulse of protein synthesis occurs (Flood et al[12], 1975b). A pulse of protein synthesis blocked the amnesic effect of the total inhibition time more effectively when it occurred at 2 hr than the same duration pulse at 4 hr or 6 hrs. From this, we concluded that the probability that protein synthesis leads to long term memory consolidation decreased as the time since training increased. Fourth, when ANI or cycloheximide were injected into various brain regions, the caudate was one of three areas for which amnesia resulted (Flood et al[13], 1980). One finding about colchicine of particular importance is that it only inhibits axoplasmic flow by about 50% and the same is true for vinblastine and podophyllotoxin (Paulson and McClure[25], 1975).

In Exp. 1, colchicine was administered prior to training and failed to affect retention but it would have allowed half the protein synthesized to

reach synaptic areas. Inhibiting protein synthesis by 50% does not cause amnesia. In Exp. 2, ANI was administered prior to training to prevent protein synthesis during and after training until the inhibition of cytoplasmic flow was maximal, and to maintain the level of protein synthesis inhibition until the capacity for protein synthesis to establish long-term memory had diminished slightly. Even so, the amount of protein synthesized was sufficient to establish what appeared to be normal retention, as mice receiving only the single ANI injection performed as well as the saline control. But in Exp. 2 and 3, when colchicine, vinblastine or podophyllotoxin were administered after ANI, they, in effect, reduced the amount of protein reaching synaptic areas by 50%; thus the amnesia resulted from both a decreased likelihood that protein synthesis would establish memory 2 hrs after training and reduced transport of the protein synthesized. In Exp. 4, a 90 min additional delay between the first and second ANI injection allowed some recovery of protein synthesis which prevented amnesia resulting from three successive injections of ANI at 2 hr intervals. The study found that if colchicine were administered prior to the pulse of protein synthesis, amnesia occurred. This was presumably because not enough protein was able to reach the synaptic areas. Injections during and after the pulse reduced colchicine's amnesic effect because some protein had already been transported to synaptic areas.

We suggest that the protein was transported to recently activated receptors which were altered by neurotransmitter action. If the protein does not convert the short-term change in receptor structure into a more stable form, the receptor reverts to its original structure and amnesia results. We injected colchicine into the caudate in our test for the central mechanism of action because we found that this was one area of the brain where ANI could be

injected and cause amnesia (~~Flood et al, 1980~~[13]). Assuming that ANI reaches the caudate when ANI is administered peripherally, then colchicine and ANI are affecting the same population of neurons. The caudate contains 95% interneurons (~~Kemp and Powell, 1971~~[17]); therefore, it is likely that the synaptic changes occurring in support of memory take place at the receptor sites of these interneurons. This should not be construed as meaning that memories are stored in the caudate. This is only one of three areas we have found so far where both protein synthesis inhibitors and drugs which alter receptor activity can affect retention. Parts of the memory may be stored in different areas of the brain, but changes in the synaptic receptors within each area may be the fundamental event underlying memory processing. Thus, normal memory processing is hypothesized to involve (a) transmitters acting on receptors, (b) protein(s) being synthesized, and (c) protein(s) being transported to the structurally altered receptors before they return to their original form.

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TABLE 1

Effect of ANI and Colchicine on Retention

TREATMENT GROUPS	% Forgetting	Mean*	S.E.M.
ANI/Colchicine	70	4.10	± .32
ANI/Saline	10	2.65	± .24
Saline/Colchicine	15	2.35	± .29
Saline/Saline	15	2.65	± .30

Table 1. ANI/Colchicine differed significantly from ANI/Saline (χ^2=15.0, P<.001), and Saline/Colchicine (χ^2=12.38, P <.001)

* Mean to trials to make first avoidance response.

TABLE 2

TREATMENT GROUPS	(N)	% Forgetting	Mean S.E.
ANI/Colchicine	23	78	4.43 ± .24
ANI/Vinblastine	24	83	4.38 ± .27
ANI/Podophyllotoxin	25	88	4.16 ± .23
ANI/Saline	26	19	2.65 ± .21
ANI/Lumicolchicine	20	20	2.75 ± .29
Saline/Saline	23	22	2.69 ± .31

Table 2. ANI/Saline differed significantly from ANI/Colchicine ($\chi^2 = 16.9$), ANI/Vinblastine ($\chi^2 = 22.32$) and ANI/Podophyllotoxin ($\chi^2 = 24.19$) and with P values greater than .001. ANI/Colchicine also differed significantly from ANI/Lumicolchicine ($\chi^2 = 17.08$, $P < .001$). Preliminary tests of Saline/Colchicine, Saline/Vinblastine, Saline/Podophyllotoxin, and Saline/Saline yielded no amnesic effects (% forgetting and N respectively were: 19%,16; 25%,16; 17%,18; and 20%,15).

TABLE 3

Effect of Colchicine Injection into the Caudate on Retention

ANISOMYCIN PRETREATED MICE

Colchicine*	(N)	% Forgetting	Mean ± S.E.M.
Quantity Injected			
30 ng	29	69	4.03 ± .32
3 ng	28	67	4.28 ± .32
300 pg	25	68	4.36 ± .41
30 pg	26	53	3.50 ± .35
3 pg	23	21	2.43 ± .33

SALINE PRETREATED MICE

Quantity injected	(N)	% Forgetting	Mean ± S.E.M.
30 ng	29	10	2.48 ± .15
3 ng	27	11	2.26 ± .25
300 pg	23	13	2.21 ± .27
30 pg	26	15	2.42 ± .26
3 pg	25	8	2.44 ± .19
ANI with Saline intracerebrally	24	21	2.83 ± .34
Saline with Saline intracerebrally	22	18	2.55 ± .18

Table 3. Colchicine administration in ANI pretreated subjects yielded significant amnesia with a dose as low as 30 pg (ANI/Saline vs ANI/Colchicine$_{30\ pg}$; $\chi^2 = 5.71$, $P<.02$). The ANI/Colchicine groups all differed at $P<.001$ from their respective Saline/Colchicine controls except for the 3 pg dose.

* The colchicine was administered in 0.5 µl bilaterally at a concentration of 6×10^{-2} mg/ml for 30 ng and was reduced by a factor of 10 for each smaller dose.

RETENTION DEPENDENT ON AXOPLASMIC/DENDRITIC FLOW OF MEMORY RELATED PROTEIN(S)

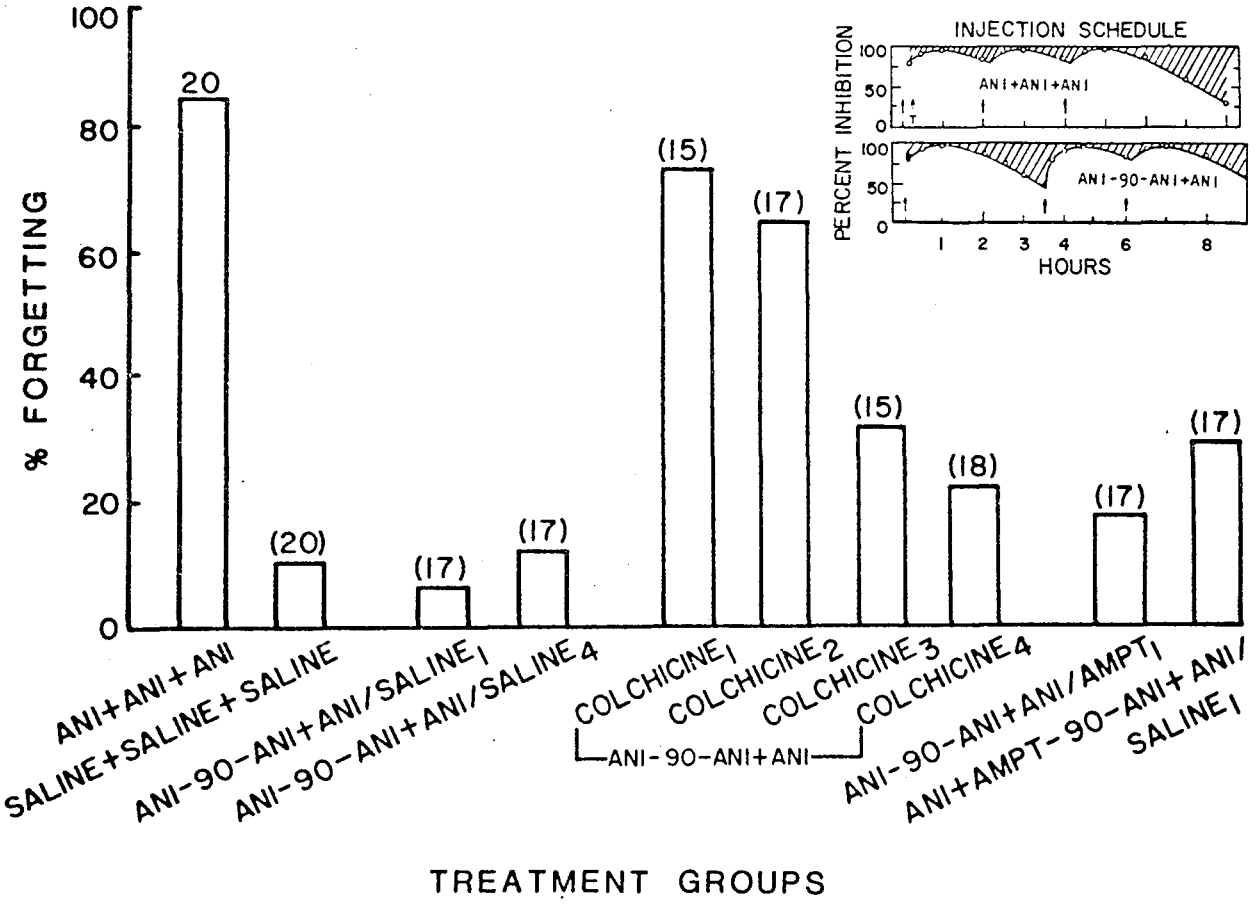

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Fig. 1

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