Pharmacology Biochemistry & Behavior, Vol. 12, pp. 377–384. Printed in the U.S.A.

Paradoxical Sleep and Memory: Long-term Disruptive Effects of Anisomycin[1]

BARUCH M. GUTWEIN,[2] PRIYATTAM J. SHIROMANI AND WILLIAM FISHBEIN

Psychobiology Laboratory, Department of Psychology
The City College of The City University of New York

Received 5 July 1979

GUTWEIN, B. M., P. J. SHIROMANI AND W. FISHBEIN. *Paradoxical sleep and memory: Long-term disruptive effects of anisomycin.* PHARMAC. BIOCHEM. BEHAV. 12(3) 377–384, 1980.—The effects of the protein synthesis inhibitor Anisomycin (ANI) on Paradoxical Sleep (PS or REM sleep), slow wave sleep (SWS), and retention of one-trial inhibitory avoidance training was examined in mice in three separate experiments. In Experiment 1, mice injected with ANI 120 mg/kg and 210 mg/kg exhibited reductions in PS for 9 consecutive hours and ANI 40 mg/kg treated mice for 6 consecutive hours with no PS rebound in all three groups. ANI increased SWS commencing 3 hr postinjection, continuing for 9 consecutive hours and then returning to saline control levels. There were no significant differences between ANI-treated groups in the degree of SWS augmentation. In Experiment 2, Part A, ANI 120 mg/kg and 210 mg/kg but not ANI 40 mg/kg impaired retention measured 72 hr after training. In Experiment 2, Part B, ANI 120 mg/kg and 210 mg/kg induced amnesia from 3 to 9 hr post-training but ANI 40 mg/kg was effective only from 3 to 6 hr. In Experiment 3, the gradient of memory trace susceptibility to disruption by ECS was extended to 3 hr post-training in mice given immediate post-training injections of ANI 40 mg/kg. ANI 20 mg/kg and ANI 10 mg/kg alone or in combination with ECS was ineffective in extending the lability of the memory trace. The results of this study indicate that PS in the 3 hr period after aversively motivated training is not essential for memory processing. We suggest that memory stability and maintenance is dependent on PS occurring over a protracted time period.

Paradoxical (REM) sleep Anisomycin Amnesia Slow-wave sleep Inhibitory avoidance
Short-term memory Long-term memory Memory lability Protein synthesis ECS

AN issue of significance demanding further clarification is the importance of the 3 hr sleep period immediately after learning to enhance memory. For example, in a recent series of reports, Bloch and his colleagues have demonstrated the existence of paradoxical sleep (PS or REM sleep) augmentation during the first 3 hr following two-way conditioned avoidance learning; they have suggested that this PS augmentation is essential for unimpaired retention [17, 18, 19]. Thus, if sleep onset is delayed for 3 hr, retention of the learned response is impaired and PS augmentation is not observed. Studies by Pearlman and Greenberg [12, 20, 21, 22] have supported the notion that a requisite element for memory fixation is the presence of either PS or conditions compatible with PS occurrence during the hours immediately after learning. They have shown that selective PSD (PS deprivation) via water-tank procedure or drugs for 3 hr immediately after two-way conditioned avoidance or discrimination learning in the rat produces marked retention deficits whereas no amnesia is observed if PSD is delayed until 3 hr after training.

On the other hand, data recently obtained in our laboratory clearly demonstrate that the first 3 hr of sleep immediately after aversively motivated training is not essential for unimpaired memory processing. We examined the effects of 3 hr PSD via the water-tank procedure to produce retrograde amnesia of two-way and one-way active avoidance and one-trial inhibitory avoidance learning in mice [27]. Our results indicated no memory impairment in experimentally treated groups. We then attempted to induce amnesia by administering ECS immediately after 3 hr PSD, thereby increasing the susceptibility of the memory trace to disruption. This procedure, however, also resulted in good retention. A correlative EEG study showed that placing mice on 3 cm pedestals for 3 hr resulted in a selective and almost total loss of PS compared to animals placed on 8 cm pedestals or dry cage controls. We concluded that PS immediately after aversively motivated training is not essential for subsequent development of learning and memory.

The objective of the present series of experiments is to confirm and extend these findings through the pharmacological inhibition of PS. We employ Anisomycin (ANI), a potent inhibitor of cerebral protein synthesis and examine its effects on sleep, long-term memory (LTM), and short-term memory (STM) of one-trial inhibitory avoidance training. The

[1]Supported in part by grants from Sigma-Xi to B.M.G. and by CUNY Faculty Research Award No. 11382 to W.F. We thank David Bern for expert technical assistance and Dr. N. Belcher of Pfizer Pharmaceuticals for the generous supply of Anisomycin which is now commercially available through Pfizer Diagnostics, Brighton Road, Clifton, NJ 07012.

[2]This report forms a part of a doctoral dissertation submitted by the first author to the Department of Psychology, The City University of New York in partial fulfillment of the requirements for the Ph.D. degree.

glutarimide antibiotic cycloheximide (CYC) and the pyrrolidine antibiotic ANI have been previously employed to elucidate the pharmacological basis of sleep [23,26] and memory [3]. CYC interferes with the interaction between the enzyme peptide synthetase and the ribosomes and may inhibit the translocation of the ribosomes along the RNA strand. ANI inhibits protein synthesis at the translational level by blocking peptide bond formation [13]; its inhibition of cerebral protein synthesis is of shorter duration and less toxic compared to CYC. In our first experiment, we examine the long-term effects of ANI on PS and SWS.

EXPERIMENT 1

METHOD

Subjects

Male and female mice of the CF-1 (Carworth Farms, NY) strain, 50–70 days of age, obtained from our laboratory breeding stock are used in all experiments described in this study.

Surgery and EEG Recording Procedure

Mice are surgically implanted under light anesthesia (Nembutal, IP) with extra-dural cortical and neck muscle electrodes. Following surgery, animals are individually housed in high-walled open-top plastic cages, measuring 53.2×16.5×12.7 cm which are placed in a sound-attenuated electrically isolated recording chambers for 7 days. On Day 8, an electrode cable is connected to the animal beginning at 0700 for 48 hr adaptation. The electrode cable consists of a ball-bearing commutator mounted on a counter-balanced arm permitting the animal unrestricted activity. On Day 10, mice are injected subcutaneously on the back of the neck at 0700 hr with either Saline (SAL, n=10), ANI 40 mg/kg (n=8), ANI 120 mg/kg (n=8), or ANI 210 mg/kg (n=7) and 24 hr recordings are obtained. Food and water are available ad lib and a 12:12 (0700–1900) light-dark cycle is maintained. The influence of seasonal variations on drug-induced alterations of sleep is controlled by the random administration of SAL and ANI.

RESULTS

Measures of PS

The effects of SAL and dose-response ANI on the latency to onset of the first PS and SWS episodes are shown in Fig. 1. Forty mg and 120 mg delay the onset of the first PS episode by 4 hr and 210 mg by 7 hr. Latency to the onset of the first SWS episode in ANI-treated mice is not significantly different from that of SAL controls, F(3/29)=1.53.

Mice treated with 120 mg and 210 mg exhibit highly significant decreases in the number of PS episodes, mean duration of PS episodes, Mean PS time, and % PS/hr for 9 consecutive hours after injection compared to SAL. Percent PS of total recording time per hour and mean PS time are shown in Fig. 2 and measures of PS are summarized in Table 1. Levels of PS return to SAL baseline by 4 p.m. in the 120 mg and 210 mg treated groups. On the other hand, mice injected with 40 mg exhibit significant decreases on measures of PS for only 6 consecutive hours following injection with PS values returning to SAL baseline by 1 p.m. There is no subsequent PS rebound in any of the drug-treated groups.

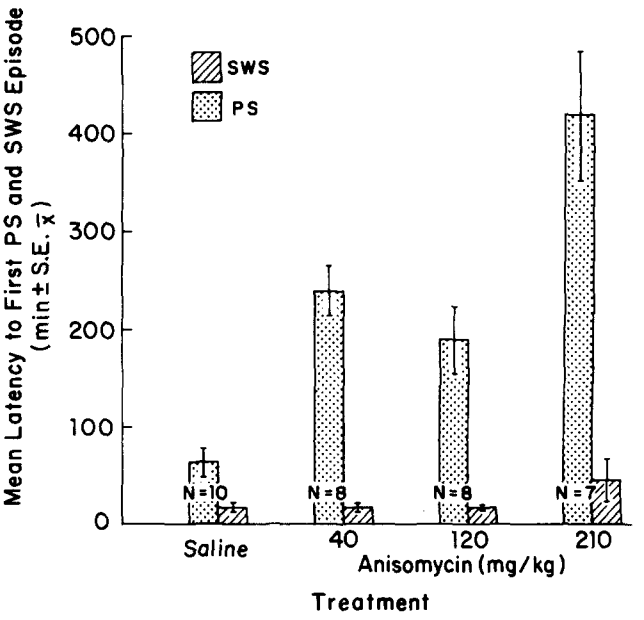

FIG. 1. Anisomycin effects on EEG recorded sleep patterns. Alteration of the latency to appearance of the first PS and SWS period. Separate groups of mice were (1) implanted with chronic indwelling EEG and ENG electrodes, (2) adjusted to recording chamber for 7 days and electrode cable for 2 days, (3) subcutaneously injected with drug (40, 120, or 210 mg/kg or saline) and 24 hr recording obtained. Note the significant delay to onset of the first PS episode in all drug-tested animals and particularly the delay in the 40 mg/kg group.

Measures of SWS

All three doses of ANI significantly increase SWS. This SWS augmentation first appears 3 hr after injection and persists for the next 9 hr. Levels of SWS in ANI-treated mice return to SAL baseline by 7 p.m. There are no significant differences between ANI-treated groups in the degree of SWS augmentation. Percent SWS of total recording time per hour and mean SWS time are shown in Fig. 3; measures of SWS are summarized in Table 2.

DISCUSSION

The results of this experiment indicate that ANI-induced reductions of PS are dose-dependent, whereas SWS is generally augmented by the drug. PS inhibition occurs immediately following injection but SWS augmentation is delayed for 3 hr and then persists for 9 hr. This is the first demonstration that ANI decreases not only PS frequency but also PS duration with a concomitant increase in SWS time. Other investigators [23,26], assessing the effects of CYC and ANI on sleep have reported a decrease only in PS frequency with no alteration in SWS. Our results may be a function of higher dosages employed in this study. Of particular interest is our finding of no PS rebound in ANI 120 and ANI 210 mg treated mice.

EXPERIMENT 2a

The following experiment is designed to correlate the effects of dose-response ANI on PS with its effects on LTM of one-trial inhibitory avoidance training.

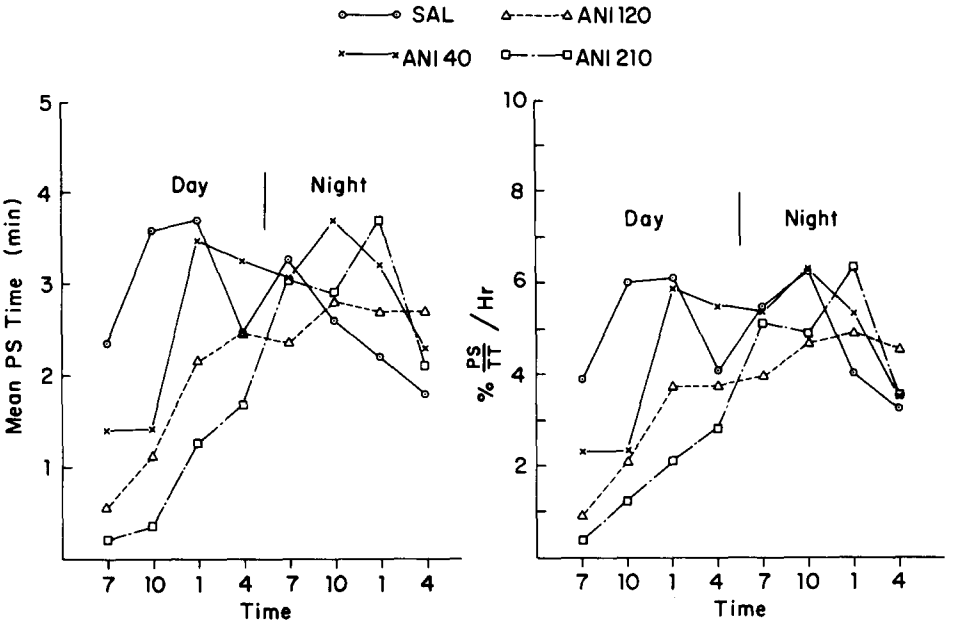

FIG. 2. Effects of dose-response ANI on measures of PS: Left Panel: Mean of total PS time. Right Panel: Percent PS per hour of total recording time. Each data point represents the mean for 3, 1-hr blocks of recording.

METHOD

Housing

In all behavioral experiments in this study mice are individually housed in standard size clear plastic cages (27.7×16.5×12.7 cm) in our vivarium commencing one week prior to training and throughout the retention interval.

Apparatus

The apparatus is a two-compartment inhibitory avoidance task. The entry, trough-shaped translucent compartment measures 10.8×4.5×12. 1 cm and is illuminated by a high-intensity tensor lamp that opens to a rectangular compartment constructed of black Plexiglas measuring 22.9×16.6×8.3 cm with a stainless steel grid floor. A stainless steel rod gate separates the two compartments. The entire apparatus is covered with a clear Plexiglas top. Electric current is delivered to the grid floor from a combination AC shock source scrambler (Grason-Stadler E 700) and the latency to enter the darkened compartment is automatically recorded to the nearest 0.1 sec.

Procedure

On the training day, mice are placed into the trough-shaped compartment; 2 sec later the connecting door is opened and the animals' step-through latency (STL) is recorded. Upon entering the large compartment, the connecting door is closed and a 500 msec at 0.5 mA footshock is automatically delivered through the bars. Animals are then removed immediately from the apparatus and injected with either SAL, ANI 40 mg/kg, ANI 120 mg/kg, or ANI 210

TABLE 1

COMPARISON BETWEEN ANI AND SAL TREATED MICE ON MEASURES OF PS (7 a.m.–4 p.m.)

	Number of episodes			Mean duration PS			Mean time PS			% PS/hr		
	F	df	Significance level	F	df	Significance level	F	df	Significance level	F	df	Significance level
ANI 40 mg* vs SAL	13.5	1/105	$p<0.001$	9.9	1/105	$p<0.002$	12.9	1/105	$p<0.0001$	12.9	1/105	$p<0.0001$
ANI 120 mg vs Sal	36.0	1/155	$p<0.0001$	18.2	1/155	$p<0.0001$	33.6	1/155	$p<0.0001$	30.0	1/155	$p<0.0001$
ANI 210 mg vs SAL	70.1	1/146	$p<0.0001$	45.0	1/146	$p<0.0001$	65.4	1/146	$p<0.0001$	49.3	1/146	$p<0.0001$

*Significant only 7 a.m.–1 p.m. Return to SAL levels at 1 p.m.

TABLE 2

COMPARISON BETWEEN ANI AND SAL TREATED MICE ON MEASURES OF SWS (7 a.m.–7 p.m.)

	Number SWS episodes			Mean duration SWS			Mean time SWS			% SWS/hr		
	F	df	Significance level	F	df	Significance level	F	df	Significance level	F	df	Significance level
ANI 40 mg vs SAL	0.58	1/209	NS	13.8	1/209	$p<0.0001$	31.4	1/209	$p<0.0001$	32.6	1/209	$p<0.0001$
ANI 120 mg vs SAL	12.6	1/207	$p<0.0001$	26.5	1/207	$p<0.0001$	19.9	1/207	$p<0.0001$	25.9	1/207	$p<0.0001$
ANI 210 mg vs SAL	15.9	1/195	$p<0.0001$	29.5	1/195	$p<0.0001$	25.5	1/195	$p<0.0001$	29.7	1/195	$p<0.0001$

mg/kg at one of the following times: (a) 1 min; (b) 15 min) (c) 30 min; (d) 45 min; (e) 1 hr; or (f) 3 hr after training. For the non-contingent shock (NCS) treatment, the rectangular shock compartment is fitted with a white Plexiglas insert; mice are placed directly into the shock compartment and given a 500 msec, 0.5 mA footshock, then injected with either SAL or dose-response ANI at the same times as experimentally treated mice. Retention for all groups is tested 72 hr after training by placing animals into the start compartment and allowing them a maximum of 300 sec to cross into the shock compartment. No footshock is delivered on the retention test. Training and testing is carried out between 0700 and 1500 hr. n=16/group/data point.

RESULTS

Results of this experiment are shown in Fig. 4. STL's are initially transformed to $\log_e$ scale in order to reduce heterogeneity of variance. Training trial STL's for experimental groups are equivalent, $F(3/380)=0.86$. A fixed effects ANOVA is employed in the analysis of all the behavioral data of this study. The effects of ANI on LTM are dose and time dependent. One hundred twenty mg and 210 mg significantly impair LTM (120 mg vs SAL; $F(1/299)=29.1$, $p<0.001$; 210 mg vs SAL; $F(1/299)=48.9$, $p<0.001$, whereas mice injected with ANI 40 mg/kg show good retention, ANI 40 mg vs SAL; $F(1/301)=4.1$. Memory trace lability is significantly extended with delayed post-training ANI injections. ANI 120 induces amnesia up to 15 min post-training, ANI 120 mg vs SAL; $F(1/47)=5.4$, $p<0.01$, and ANI 210 mg is effective up to 30 min after training, ANI 210 mg vs SAL; $F(1/48)=22.7$, $p<0.001$.

DISCUSSION

The results of Experiment 1 and 2a indicate that ANI 40

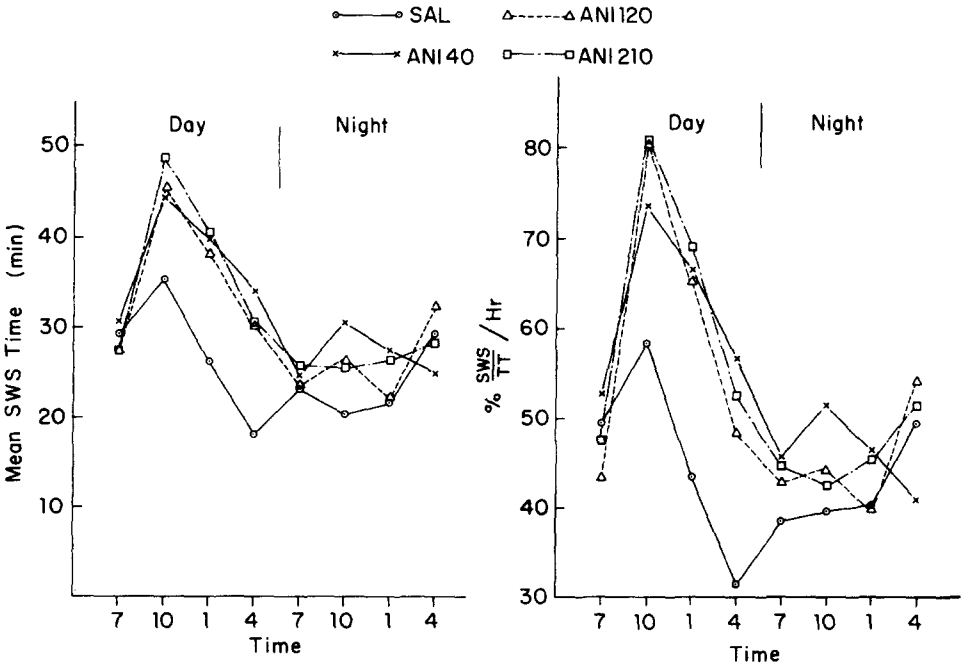

Fig. 3. Effects of dose-response ANI on measures of SWS: Left Panel: Mean of total SWS time. Right Panel: Percent SWS per hour of total recording time. Each data point represents the mean for 3, 1-hr blocks of recording.

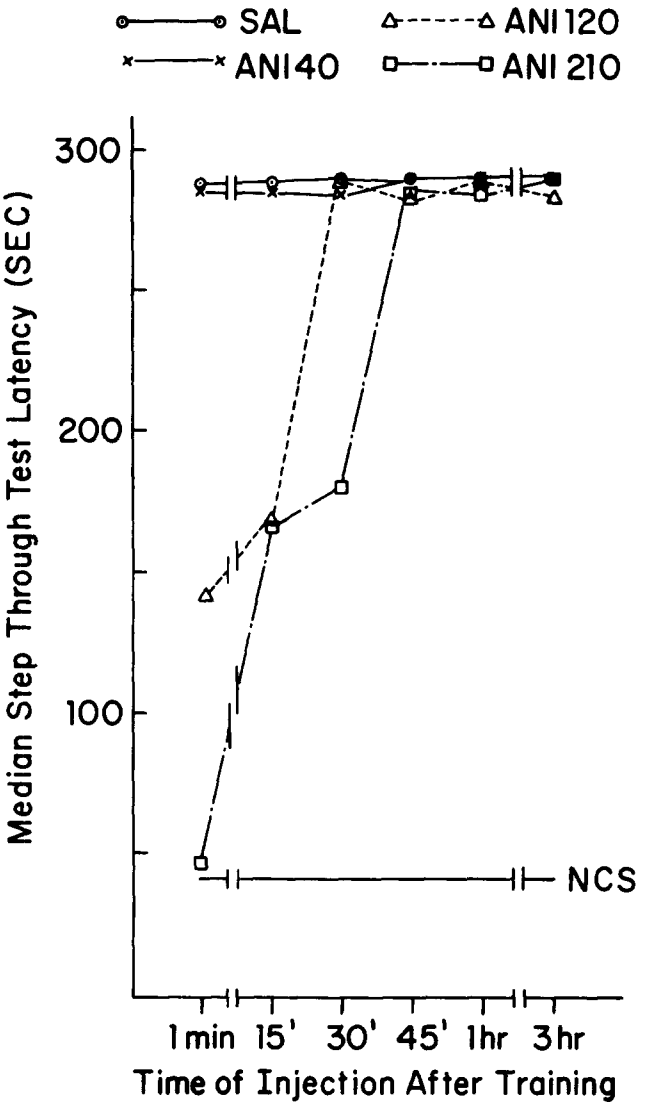

FIG. 4. Long-term retrograde amnesic effects of anisomycin on one-trial inhibitory avoidance retention. Median test latencies: Independent groups of mice are (1) trained (or noncontingent shock, NCS), (2) subcutaneously injected with anisomycin (40, 120, or 210 mg/kg or saline), and (3) tested for retention 72 hr after training. Each point represents the median latency (seconds) of 16 animals. Note the failure to produce amnesia in the 40 mg/kg injected group.

selectively decreases PS for 6 consecutive hours following administration but does not impair retention of one-trial inhibitory avoidance measured 72 hr after training. These findings in conjunction with our recent data of learning and memory development following 3 hr of PS deprivation via the water tank technique [27] provide clear evidence that PS in the 3 hr period after aversively motivated training is not a requisite element for unimpaired LTM. These data do suggest, however, that the protracted inhibition of PS induced by low dosage ANI may correlate with impaired STM. For example, a number of studies have shown that CYC produces amnesia within minutes to hours after training [16, 24, 28]. The objective of Experiment 2b is to examine the effects of ANI on STM of one-trial inhibitory avoidance training.

EXPERIMENT 2b

METHOD

Procedure

Training for experimental and NCS groups is as described in Experiment 2a. Immediately following training, independent groups of mice are injected with either SAL, ANI 40 mg/kg, ANI 120 mg/kg, or ANI 210 mg/kg and tested for retention at one of the following times: (a) 1 min, (b) 15 min, (c) 30 min, (d) 45 min, (e) 1 hr, (f) 3 hr, (g) 6 hr, or (h) 9 hr after training. Retention is tested by allowing mice a maximum of 300 sec to cross over into the shock compartment. No footshock is delivered on the retention test. n=16/group/data point.

RESULTS

Results in this experiment are shown in Fig. 5. An ANOVA performed on $\log_e$ transformed data reveals that training STL's for experimental groups are equivalent, $F(3/504)=0.73$, and good retention in ANI-treated mice from 15 min to 1 hr post-training, $F(3/300)=0.47$. At 3 and 6 hr post-training all ANI-treated mice exhibit amnesia relative to SAL controls, 3 hr; $F(3/61)=8.34$, $p<0.005$; 6 hr; $F(3/62)=25.5$, $p<0.0001$. At 9 hr mice injected with ANI 40 are not significantly different from SAL, $F(1/29)=0.07$, but 120 mg and 210 mg injected mice are amnesic, 120 mg and 210 mg vs SAL; $F(1/61)=14.4$, $p<0.001$. There are no significant differences between the 120 mg and 210 mg groups at this time, $F(1/30)=0.10$. The short-step through latencies exhibited by all ANI-treated mice at 1 min may be a function of drug-induced increases in locomotor activity. For example, one of us [16] has recently demonstrated that CYC-treated mice exhibit significantly shorter step-through latencies in the inhibitory avoidance task within minutes of injection. In addition, data from this laboratory (Gutwein and Fishbein, unpublished data) also show that dose-response ANI significantly increases locomotor activity within minutes of administration.

DISCUSSION

Data from Experiment 2b suggests that the amnesia observed between 3 and 9 hr after training may result from ANI's influence on the conversion of the STM trace into LTM. For example, Daniels [4] has also reported a similar time course of development of amnesia in one-trial appetitive learning after CYC administration. The importance of PS in the conversion of STM to LTM is suggested by our finding that ANI 120 and ANI 210 significantly decreases PS for 9 consecutive hours following injection, impairs STM commencing at 3 and up to 9 hr post-training and maintains this loss of memory to 72 hr post-training. On the other hand, ANI 40 decreases PS for 6 hr after training and does not induce LTM loss.

These data suggest that inhibition of PS by ANI 40 mg may effectively increase the susceptibility of a labile STM trace to long-term disruption by the subsequent administration of an additional memory impairing agent such as ECS. For example, pre- or post-training PS deprivation induced by 48–72 hr exposure to the water-tank technique [5, 6, 9] or immediate post-training LC lesions [29] has been shown to extend memory trace susceptibility to disruption with delayed post-training ECS. This effect has also been obtained

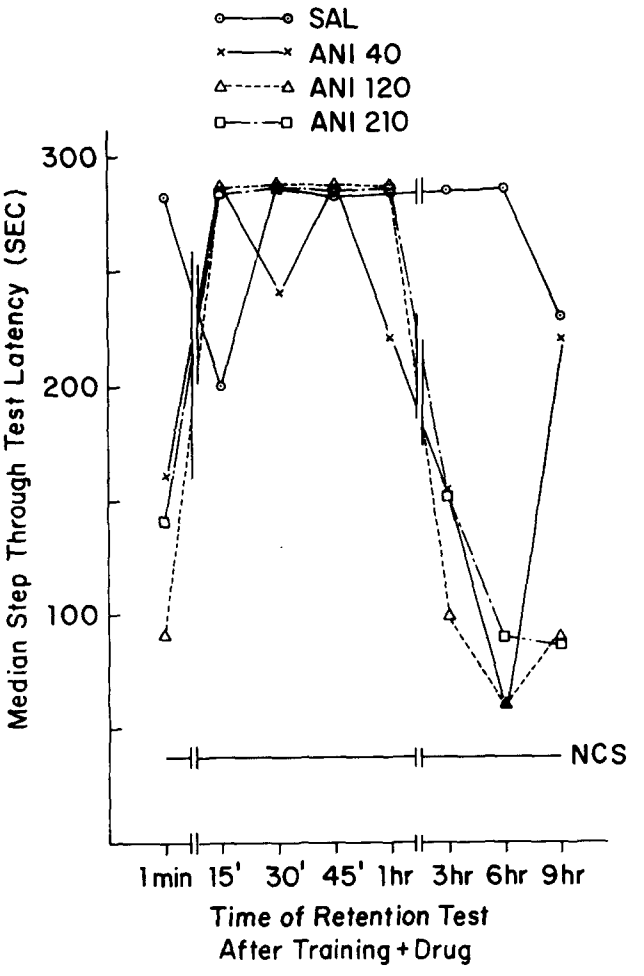

FIG. 5. Short-term retrograde amnesic effects of amisomycin one-trial inhibitory avoidance retention. Median test latencies. Independent groups of mice are (1) trained (or noncontingent shock, NCS), (2) subcutaneously injected with anisomycin (40, 120, or 210 mg/kg or saline), and (3) tested for retention at one of several intervals after training. Each point represents the median latency (seconds) of 16 animals. Note the failure to produce amnesia in the 40 mg/kg injected group at the 9 hr retention interval.

by coupling pre- or immediate post-training subamnesic doses of CYC or ANI with delayed post-training administration of either ECS, carbon dioxide anesthesia, or several additional doses of the antibiotic inhibitor [1, 2, 10, 25]. Flood, *et al.* [10] maintain, however, that PS is unrelated to these effects because the temporal parameters of functional and lesion PS deprivation studies and retention testing are not comparable to the time course of the inhibitor experiments. The objective of Experiment 3 is to assess the relationship between PS and memory trace lability by examining the effects of 3 low ANI doses administered alone or in combination with ECS on the time course of LTM development following one-trial inhibitory avoidance training.

Procedure

Training for experimental and NCS groups is as described in Experiment 2a. Immediately following training, independent groups of mice are injected with either SAL, ANI 10 mg/kg, ANI 20 mg/kg, or ANI 40 mg/kg and then administered transcorneal ECS (800 msec @ 15 mA, Lafayette Instruments Company A-615B shocker) or sham ECS (SECS; mice receiving SECS are handled in the same way as the ECS group but no current is delivered) at one of the following times: (a) 1 min, (b) 15 min, (c) 30 min, (d) 45 min, (e) 60 min, (f) 3 hr, (g) 6 hr, (h) 9 hr, or (i) 24 hr after training. Retention is measured 3 days after training with animals allowed a maximum of 300 sec to cross into the shock compartment. No footshock is delivered on the retention test. n=16/group/data point.

RESULTS

Results of this experiment are shown in Fig. 6. Training STL's for experimental groups are equivalent, $F(7/1059)=1.2$. An ANOVA performed on these data indicates that mice administered SAL+ECS are amnesic compared to SAL+SECS controls, $F(1/270)=38.2, p<0.001$, and mice given ANI 40+ECS are significantly impaired relative to the SAL+ECS group, $F(1/270)=23.7, p<0.001$. Administration of ANI 10 mg/kg or ANI 20 mg/kg in combination with ECS does not produce amnesia compared SAL+ECS controls, 10 mg: $F(1/270)=1.2$, 20 mg: $F(1/270)=0.35$. Furthermore, ANI 40 mg prolongs the lability of the memory trace so that ECS administered up to 3 hr after training is still effective in producing amnesia (ANI 40+ECS vs SAL+ECS at 3 hr: $F(1/30)=9.3, p<0.004$.

GENERAL DISCUSSION

The results of this study provide clear evidence in support of our contention that PS in the 3 hr period immediately after learning is not essential for the development of LTM. Data from Experiments 1 and 2a indicate that PS inhibition for 9 consecutive hours following injection of either ANI 120 mg or ANI 210 mg correlates with LTM loss. Mice treated with ANI 40 mg, however, exhibit a reduction of PS for 6 consecutive hours after injection but their long-term retention is unimpaired. ANI's specificity is evidenced by its dissociable effects on PS and SWS; PS inhibition commences immediately following injection and is dose-dependent whereas SWS augmentation is delayed until 3 hr postinjection and then persists for 9 consecutive hours. There were no significant differences between ANI-treated groups in the degree of SWS augmentation. The neurochemical mode of action for ANI's dissociable effects on sleep are presently unknown, but this unique finding merits further investigation.

This finding of ANI-induced SWS augmentation with concomitant amnesia does not support the findings of Fowler, *et al.* [11] who reported induction of amnesia following SWS but not PS deprivation.

Of particular interest is our finding that ANI 40, 120, and 210 mg/kg *decreases PS duration* in addition to *PS frequency* with *no subsequent PS rebound*.

Data from Experiments 2b and 3 demonstrate that the labile period of memory formation is extended by delaying ECS administration up to 3 hr after training in ANI 40 mg treated mice. ANI 10 mg and ANI 20 mg, do not, however, extend the period of memory trace susceptibility to disruption even when followed by immediate post-training ECS. These results confirm recent reports [1, 2, 10, 25] that prolonging the duration of protein synthesis inhibition by subamnesic doses of an inhibitor extends the period of memory

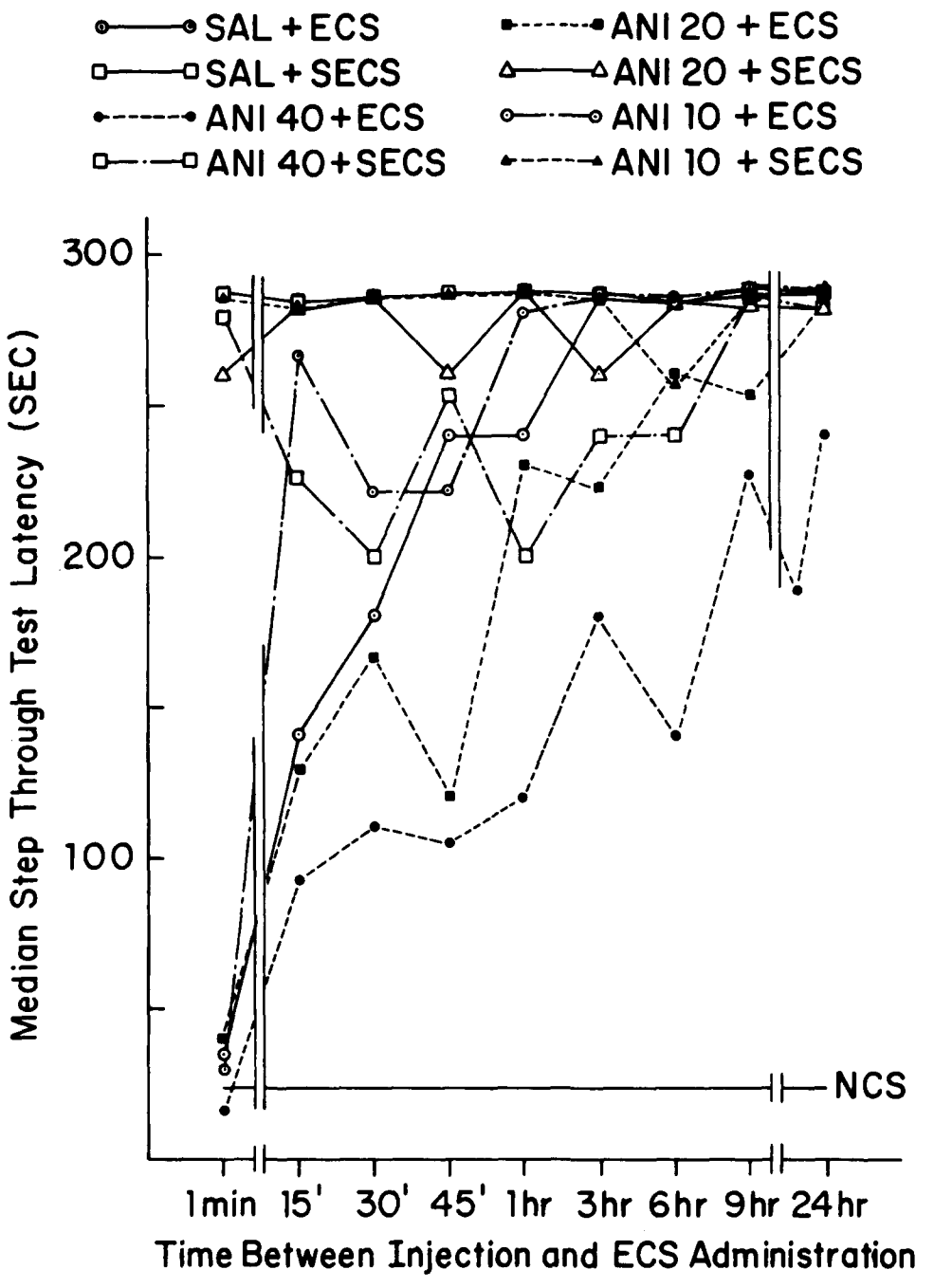

FIG. 6. Retrograde amnesia: Prolonging the fixation phase of memory consolidation by low dosage anisomycin. Inhibition avoidance median test latencies: Mice (1) trained (or non-contingent shock, NCS), (2) injected with low dosages of anisomycin (10, 20, or 40 mg/kg or saline), (3) treated with ECS at one of several intervals after training, and (4) tested for retention 72 hr post-training. Each data point is based on 16 animals. Note the prolonged retrograde amnesia gradient in the ANI 40+ECS group.

trace susceptibility to disruption by a variety of amnestic agents, and now also show that PS is a requisite element for this stability and maintenance of LTM.

The present data in conjunction with our recent study [27] in which we demonstrated unimpaired learning and retention following 3 hr of PS deprivation via the water-tank technique does not support the contention of Bloch; Pearlman and Greenberg; who maintain that memory processing is dependent on PS occurrence in the 3 hr immediately after training. Our review [7] and investigations of the relationship between PS and memory storage processes now indicate that only protracted PS inhibition induced either by the water tank technique [5, 6, 9, 27], ANI, or 30 days of environmental isolation [14,15] impairs LTM; conversely protracted

PS augmentation induced either by formal training [8] or 30 days of environmental enrichment [14,15] enhances long-term retention. These data provide considerable support for our hypothesis that PS occurring over a prolonged time period is a requisite neurobiological mechanism for the processing, maintenance, and storage of long-term memory.

REFERENCES

1. Andry, D. K. and M. W. Luttges. Experimental separation by cycloheximide and electroconvulsive shock. *Science* **172:** 518–520, 1972.
2. Andry, M. L. and M. W. Luttges. Time variables affecting the permanence of amnesia produced by combined cycloheximide and electroconvulsive shock treatments. *Pharmac. Biochem. Behav.* **1:** 301–306, 1973.
3. Barraco, R. A. and L. J. Stettner. Antibiotics and memory. *Psych. Bull.* **83**(2): 242–302, 1976.
4. Daniels, D. Acquisition, storage, and recall of memory for brightness discrimination by rats following intracerebral infusion of acetoxy cycloheximide. *J. comp. physiol. Psychol.* **76:** 110–118, 1971.
5. Fishbein, W. Interference with conversion of memory from short-term to long-term storage by partial sleep deprivation. *Communs behav. Biol.* **5:** 171–175, 1970.
6. Fishbein, W. Disruptive effects of rapid eye movement sleep deprivation on long-term memory. *Physiol. Behav.* **6:** 279–282, 1971.
7. Fishbein, W. and B. M. Gutwein. Paradoxical sleep and memory storage processes. *Behav. Biol.* **19:** 425–464, 1977.
8. Fishbein, W., C. Kastaniotis and D. Chattman. Paradoxical sleep: Prolonged augmentation following learning. *Brain Res.* **79:** 61–75, 1974.
9. Fishbein, W., J. L. McGaugh and J. R. Swarz. Retrograde amnesia: Electroconvulsive shock effects after termination of rapid eye movement sleep deprivation. *Science* **172:** 80–82, 1971.
10. Flood, J. F., E. L. Bennett, A. E. Orme and M. E. Jarvik. Protein synthesis dependent gradient of ECS retrograde amnesia. *Behav. Biol.* **21:** 307–328, 1977.
11. Fowler, M. J., M. J. Sullivan and B. R. Ekstrand. Sleep and memory. *Science* **179:** 302–304, 1973.
12. Greenberg, R. A. and C. A. Pearlman. Cutting the REM nerve: An approach to the adaptive role of REM sleep. *Perspect. Biol. Med.* (Summer), 513–521, 1974.
13. Grollman, A. P. and M. T. Huang. Inhibitors of protein synthesis in eukaryotes: Tools in cell research. *Fedn Proc.* **32:** 1673–1678, 1973.
14. Gutwein, B. M. and W. Fishbein. Paradoxical sleep and memory (I): Selective alterations following enriched and impoverished environmental rearing. *Brain Res. Bull.* (in press), 1980.
15. Gutwein, B. M. and W. Fishbein. Paradoxical sleep and memory (II): Sleep circadian rhythmicity following enriched and impoverished environmental rearing. *Brain Res. Bull.* (in press), 1980.
16. Gutwein, B. M., D. Quartermain and B. S. McEwen. Dissociation of cycloheximide's effects on activity from its effects on memory. *Pharmac. Biochem. Behav.* **2:** 753–756, 1974.
17. Hennevin, E., P. Leconte and V. Bloch. Effet de niveau d'acquisition sue l'augmentation de la durée de sommeil paradoxal consécutive á un condionnement d'evitement chez le rat. *C. r. hebd. Séanc. Acad. Sci., Paris* **273:** 2595–2598, 1971.
18. Leconte, P. and E. Hennevin. Augmentation de la durée de "Sommeil Paradoxal" consécutive á un apprentissage chez le rat. *C. r. hebd. Séanc. Acad. Sci., Paris* **273:** 86–88, 1971.
19. Leconte, P., E. Hennevin and V. Bloch. Analyse de effets d'un apprentissage et de son niveau d'acquisition sur le sommeil paradoxal consecutif. *Brain Res.* **49:** 367–379, 1973.
20. Pearlman, C. A. and M. Becker. Brief posttrial REM sleep deprivation impairs discrimination learning in rats. *Physiol. Psychol.* **1:** 373–376, 1973.
21. Pearlman, C. A. and M. Becker. REM sleep deprivation impairs bar press acquisition in rats. *Physiol. Behav.* **13:** 813–817, 1974.
22. Pearlman, C. A. and R. A. Greenberg. Posttrial REM sleep: A critical period for consolidation of shuttle-box avoidance. *Anim. Learn. Behav.* **1:** 49–51, 1973.
23. Pegram, V., D. Hammond and W. Bridgers. The effects of protein synthesis inhibition on sleep in mice. *Behav. Biol.* **9:** 377–382, 1973.
24. Quartermain, D. and B. S. McEwen. Temporal characteristics of amnesia reduced by inhibition of protein synthesis. *Nature* **228:** 677–678, 1970.
25. Quinton, E. E. Subamnesia cycloheximide treatment delays consolidation in mice. *J. comp. physiol. Psychol.* **92:** 742–748, 1978.
26. Rojas-Ramirez, J. A., E. Aguilar-Jiménez, A. Posada-Andrews, J. G. Bernal-Pedraza and R. R. Drucker-Colin. The effects of various protein synthesis inhibitors on the sleep-wake cycle of rats. *Psychopharmacology* **53:** 147–150, 1977.
27. Shiromani, P. J., B. M. Gutwein and W. Fishbein. Development of learning and memory in mice after brief paradoxical sleep deprivation. *Physiol. Behav.* **22:** 971–978, 1979.
28. Squire, L. R., G. A. Smith and S. H. Barondes. Cycloheximide effects memory within minutes after the onset of training. *Nature* **242:** 201–202, 1973.
29. Zornetzer, S. F. and M. S. Gold. The locus coeruleus: Its possible role in memory consolidation. *Physiol. Behav.* **16:** 331–336, 1976.