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PARADOXICAL SLEEP AND MEMORY: ELECTROPHYSIOLOGICAL AND BEHAVIORAL CORRELATES OF INFORMATION PROCESSING FOLLOWING ANISOMYCIN OR ENRICHED AND IMPOVERISHED ENVIRONMENTAL REARING

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by

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Abstract

PARADOXICAL SLEEP AND MEMORY: ELECTROPHYSIOLOGICAL AND BEHAVIORAL CORRELATES OF INFORMATION PROCESSING FOLLOWING ANISOMYCIN OR ENRICHED AND IMPOVERISHED ENVIRONMENTAL REARING

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Adviser: Professor William Fishbein

The experiments described in this study are designed to examine the relationship between PS and memory storage processes. Two complementary paradigms; induction of PS deprivation and induction of PS augmentation are employed. In Chapter I, a hypothesis proposed by two sleep research laboratories that the 3 hr sleep period immediately after learning (specifically PS) is necessary to consolidate learning and enhance long-term memory is examined with Anisomycin, a pyrrolidine antibiotic drug and potent inhibitor of cerebral protein synthesis. The results show that prolonged PS inhibition by Anisomycin correlates with Anisomycin-induced amnesia of short-term and long-term memory. A low-dosage of the drug inhibits PS for 6 consecutive hours but does not impair long-term memory; it is sufficiently

potent, however, to increase the memory's susceptibility to disruption with the additional administration of electroconvulsive shock. These data are interpreted as providing clear evidence that PS in the 3 hr period immediately after aversively motivated learning is not essential for the development of LTM.

The experiments described in Chapter III examine the relationship between enriched and impoverished environmental rearing, PS, and long-term memory. Environmental enrichment results in a selective and significant increase of PS persisting for at least two weeks and also enhances long-term recall of an aversively motivated Y-maze brightness-discrimination task. Conversely, impoverished reared mice exhibit a selective decrease in PS and impaired task performance relative to controls. In a subsequent experiment, the effects of enriched and impoverished rearing on sleep circadian rhythmicity and its relationship to memory storage processes is evaluated.

I interpret the results of these experiments as providing considerable support for the hypothesis that PS occurring over a prolonged time period is a requisite neurobiological mechanism for the processing, maintenance, and storage of long-term memory.

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CHAPTER I

INTRODUCTION

Sleep in most mammals can generally be classified into two distinct states, a paradoxical sleep (PS) phase, more commonly known as rapid eye movement or REM sleep in man, and a nonparadoxical sleep (NPS) phase, with NPS frequently subdivided into additional stages based upon the degree of cortical electroencephalographic (EEG) synchronization (Rechtshaffen and Kales, 1968; Sterman, Knauss, Lehmann, and Clemente, 1965; Ursin, 1968). PS is characterized primarily by activation of the cortical EEG, hippocampal theta rhythm, spiking activity in the pons, lateral geniculate nuclei, and occipital cortices (PGO spikes), postural muscle atonia, and bursts of eye movements. It is indeed a paradox that while PS is behaviorally defined as a part of sleep, the neural activity of the brain during PS is qualitatively and quantitatively more similar to that of aroused waking than to NPS (Evarts, 1969; Hobson and McCarley, 1971). Numerous theories have been formulated about the function of sleep and, most specifically, of PS (Adler, 1936; Berger, 1969; Dement, 1964; Ephron and Carrington, 1966; Fisher, 1965; French, 1954; Freud, 1950; Hartmann, 1970; Roffwarg, Muzio, and Dement, 1966; Snyder,

1966; Ullman, 1959). Many of these theories have been concisely reviewed elsewhere (Hennevin and Leconte, 1971; Dallett, 1973).

In recent years there has been a notable increase in the number of reports suggesting that the PS phase provides conditions which facilitate learning and memory. This section provides a selective review of the literature in a way that is consistent with the view of this author and other theorists who maintain that during PS the brain sets in motion a "chain-of-events" that is particularly conducive to learning and memory (Breger, 1967; Bloch and Fishbein, 1975; Bryson and Schacher, 1969; Dewan, 1970; Feinberg and Evarts, 1969; Fishbein and Gutwein, 1977; Fiss, 1969; Greenberg, Pearlman, Fingar, Kantrowitz, and Kawliche, 1970; Hennevin and Leconte, 1971; McGrath and Cohen, 1978; Newman and Evans, 1965; Shapiro, 1967; Stern and Morgane, 1974). Recent animal research, however, points to a more specific role which PS plays in learning and memory storage processes. It is my view that these studies indicate that PS is important for memory in two ways: First, the PS phase provides conditions which facilitate the conversion of a labile short-term memory trace, requiring extensive adaptational changes in problem solving strategies, into a stable long-term memory; second, following initial fixation, the conditions of PS activity serve to promote the maintenance of a stable memory in long-term storage.

A. Pretraining PSD Effects on Memory

The majority of basic research in memory has concentrated on the problem of elucidating the fixation process of memory consolidation. Therefore, the first pertinent question is whether PS is involved in the fixation phase of the consolidation of a long-term memory (LTM) trace.

It is important to make clear a distinction between experiments that focus on the role of PS in acquisition and experiments that are concerned with the importance of PS in the conversion phase of a labile short-term memory (STM) into a stable LTM.

The majority of investigators who have examined the relationship between PS and memory have primarily used the "water tank" technique of depriving the animal subjects of PS. This PSD (PS deprivation) procedure involves placing the animals on small pedestals in the middle of a pool of water. Since postural muscle tonus persists during NPS, animals can learn to obtain this sleep phase by crouching or sitting on the small pedestals. As the animals enter PS, the tonus of antigravity muscles of the entire body and particularly of the head and neck diminishes, causing the animals to slip into the water and awaken. Thus, PS is interrupted before it fully develops.

There has been a variety of studies that have investigated the effects of PSD on acquisition. Several

investigators (Hartmann and Stern, 1972; Stern, 1971) have demonstrated that PSD impairs the acquisition of active avoidance conditioning, while others (Albert et al., 1970; Joy and Prinz, 1969) failed to find such impairments. Hicks and Paulus (1973) report that PSD alters latency, but not accuracy in T-maze learning. Plumer et al. (1974) and Greenberg and Pearlman (1974) have suggested that the lack of consistent results regarding the effects of PSD on avoidance conditioning are related to: (1) the nature of the training tasks that are employed; (2) the size of the control pedestal; (3) the size of the animals in relation to the size of the PSD pedestals; and perhaps (4) the animal species, age, and strain.

The experiments designed for the purpose of examining the effects of PSD on the conversion phase of memory consolidation have all demonstrated that acquisition remains unaffected. Indeed, it is imperative to demonstrate in these experiments that subjects learn and remember normally for at least a brief period before amnesia sets in, otherwise the experiments would be difficult to interpret because there would be no way to distinguish acquisition from retention impairments. To make it very clear then, the experiments described in this section do not focus on the role of PS in registration of new information; rather, I am concerned with experiments that focus on the formation and maintenance of the LTM trace.

In one of the earlier studies designed for the purpose of examining the role of PS in the memory conversion process, mice were deprived of PS before one-trial inhibitory (passive) avoidance training (Fishbein, 1970). The pretraining effects of PSD on acquisition, STM (up to 1 hr), and LTM (1-7 days) were examined.

Fishbein found that the PSD procedure produced a robust amnesic effect on long-term retention without impairing acquisition or short-term retention. The number of days of the PSD procedure appeared to be a critical factor. One day of PSD had no effect on acquisition, STM, or LTM. In addition, 3 days of the PSD procedure did not affect acquisition or STM, i.e., retention was not significantly different from controls up to 1 hr post-training. On the other hand, 3-day PSD animals tested for LTM exhibited a well-developed amnesia compared to controls. The step-through training latencies for experimental and control animals were not significantly different. Thus, the results indicated that: (1) an accumulation of PSD is necessary to produce LTM impairment, and (2) acquisition and STM are not affected by the PSD procedure. Three alternative interpretations of these data were considered and rejected: (1) The amnesia seen in the experimental animals is the result of the PSD effect on acquisition rather than on retention. This interpretation was rejected because the step-through training latencies of the PSD group and

controls were identical; (2) amnesia in the PSD group was due to a state-dependent effect, i.e., the animals learned in the PSD state but were tested for retention in the NPSD state. A control group which was trained and tested under PSD was, however, also found to be amnesic; (3) altered locomotor activity was not a contributing factor to the amnesia since mice that underwent the PSD procedure for 3 days did not have significantly different step-through latencies as compared to controls.

The data from this study suggest that PS is actively involved in the fixation process of LTM storage.

Sagales and Domino (1973) have recently replicated these general findings and have drawn the same conclusion. Mice deprived of PS prior to avoidance conditioning in an automated shuttle-box exhibit significant impairment of LTM with no impairment in acquisition or STM, compared to large pedestal or dry cage controls. Miller, et al. (1971) failed to replicate the findings in rats, but their experiment employed a very long footshock experience, inadequate controls, and so few animals that interpretation of the results is, at best, tenuous.

In a subsequent experiment by Linden et al. (1975), the effects of pretraining PSD on learning and memory were further defined. They reasoned that if the PSD procedure impairs the consolidation of a LTM trace, perhaps the PSD treatment induces its effect by way of altering the

fixation gradient of the memory consolidation process. In this experiment the same experimental design was employed as in the previous experiment (Fishbein, 1971). PSD animals, however, were administered ECS at various intervals after inhibitory avoidance training and were then tested for retention 3 days after recovery from the effects of the PSD procedure and ECS treatment. Once again the results supported the hypothesis.

It was found that the combined PSD + ECS treatment produced a protracted retrograde amnesia gradient, whereas the ECS alone produced only a sharp gradient. In short, the memory fixation gradient appeared to be extended far beyond its normal limits in the animals that had undergone the PSD procedure before training. In my view this finding provides considerable evidence that PSD produces a central-neural change within the brains of PSD subjects, during which time the brain is considerably more susceptible to agents that produce amnesia (Handwerker and Fishbein, 1975). Thus, PS may provide the conditions which play a very important role in facilitating the fixation of a LTM trace.

B. Post-Training PSD Effects on Memory

One other pertinent question; how is memory "maintained"? Maintenance is an exceedingly important problem in the study of memory. Not only does the memory process develop over some period of time (Barondes and Squire, 1972), but it persists for years. Fishbein (1971) and

Pearlman (1973) have provided evidence suggesting that PS promotes the maintenance of a stable memory trace in long-term storage. However, the most direct evidence that PS plays a role in the maintenance of a memory trace comes from experiments (Fishbein et al., 1971; Wolfowitz and Holdstock, 1971) which suggest that the memory trace continues in a labile form as a result of PSD, and therefore it remains susceptible to permanent disruption by a massive brain assault. In the experiment performed by Fishbein et al. (1971), mice were trained in an inhibitory avoidance task, deprived of PS for 48 hr, and then administered ECS (or sham ECS). An ECS has the standard amnesic effect only if administered within a very brief period after training (McGaugh, 1966; Mah and Albert, 1973). The results of this experiment indicated that mice administered an ECS immediately following 48 hr of the PSD procedure and tested after 1 day of recovery sleep were completely amnesic. On the other hand, mice receiving sham ECS displayed normal retention, demonstrating that the memory trace had certainly been established in LTM. In addition, mice given ECS several hours after PSD termination, during which time PS rebounded, showed normal retention much like the sham ECS animals. A well-developed time-dependent retrograde amnesia was apparent in these delayed PSD + ECS groups. The findings parallel the results described by McGaugh (1966) when the ECS is applied after training, with the exception

that in the present study ECS is applied 2 days after training. It appears therefore, that the memory trace remains in a labile state after prolonged PSD and is similar to a memory trace immediately after learning, i.e., highly susceptible to disruption. The data suggest that PS may be directly involved in the temporal course of the susceptible period of a recently established memory trace. It is also important to note that Wolfowitz and Holdstock (1971) have replicated these observations in a rat study employing shuttle-box avoidance learning.

Pearlman (1971) has provided additional evidence that lends support to this conclusion. Exposure to certain pre-extinction experiences has been shown to speed up the extinction of a stable bar-pressing response; Pearlman reports that PSD following the pre-extinction experience nullifies this facilitation. In this experiment the influence of stress was minimized as in the above experiment by allowing 24 hr for recovery from the PSD procedure before retention testing. Thus, following acquisition of a stable bar-pressing response, rats were exposed to two types of pre-extinction: (1) passage of several days without the accustomed daily bar-pressing sessions with accompanying normal sleep, or (2) daily exposure for several days to clicks from an empty food reward mechanism with PSD interpolated during the daily pre-extinction experience. The results indicated that the rats administered the PSD

procedure during the pre-extinction period displayed little effect of the pre-extinction experience on the rate of extinction, and behaved like rats extinguished after original learning in the non-PSD condition. In short, PSD has disrupted the time course of the consolidation of the pre-extinction experience.

More recently Zornetzer and Gold (1976) have provided evidence linking the neural triggering mechanism, which has been implicated in the production of PS (Jouvet, 1972), with memory storage processes. Their report, that lesions of the locus coeruleus (LC), located in the lateral pontine tegmentum, produce transitory and reversible loss of PS, is similar to observations made by Jones, Harper, and Hilaris (1975) in the cat. In addition, Zornetzer and Gold report that the LC lesions prolong the period during which an established memory remains susceptible to disruption. Their experiments were designed after the one described above (Fishbein et al., 1971).

The experiments follow a general experimental design in which LC lesions are made in mice through previously implanted bilateral electrodes, immediately following training in a one-trial inhibitory avoidance task. They report that retention is normal in animals which are only LC lesioned. However, mice administered transcorneal ECS 40 hr after initial training and unilateral LC lesioning are found to be amnesic when tested 8 or 24 hr after ECS.

Zornetzer and Gold interpret their data as indicating that the LC (and by implication, PS) is directly involved in the temporal delineation of susceptibility to the disruption period of an established yet labile memory.

C. Discussion

These data, taken together, suggest that PS may perform two important memory storage functions. First, PSD prior to training affects only LTM and not acquisition or STM. I believe that the data provide a strong case for the view that PS may be necessary for conversion of a learned response from STM to LTM storage, possibly by way of modulating the fixation phase of the memory consolidation gradient. Second, PS may be necessary for the normal active maintenance of the stability of a consolidated memory trace, since PSD following learning results in a significant prolongation of the period during which an established memory remains susceptible to disruption.

I consider the present group of experiments as only a starting point. Additional research must be done before we fully understand the role of PS in memory storage processes. However, the augmentation of PS that has been observed to occur after learning has further clarified the relationship between PS and memory. While this work is still relatively new, the studies that have been published have all reported consistent results: An increase of PS

time occurs during the sleeping hours following the integration of a learned response.

Augmentation Of Paradoxical Sleep And Memory

A. Short-Term Augmentation

Thus far I have discussed experiments showing that pretraining and post-training PSD impair LTM storage. One may therefore reason that an increase of PS might be expected to follow learning, a prediction Oswald (1969) made some years ago. This hypothesis has been examined with very interesting results.

Lucero (1970) was the first investigator to demonstrate an augmentation of PS following learning. He trained rats in a labyrinth-like maze for 1.5 to 2 hr and demonstrated a significant increase in PS, with respect to controls (exposed to the labyrinth but without a learning situation) during the 3 hr of recording immediately after learning. No change in total sleep time or NPS was observed.

Hennevin, Leconte, and Bloch (1971), Leconte and Hennevin (1971, 1973), and Leconte, Hennevin, and Bloch (1973) have also performed studies investigating the PS augmentation phenomena. Their interests have centered on the relationship between the level of acquisition of a conditioned avoidance response and the subsequent time spent in PS. These investigators have shown that in the course of distributed learning, each learning session is followed

by an immediate and short-lasting (first 0.5 hr of sleep) augmentation of PS. Of considerable interest is that on the day after the animals reach the asymptote of the learning curve (when the integration of newly acquired information is complete), PS augmentation ceases and PS returns to baseline levels; on all training days NPS is completely unaffected. Smith, Kitahama, Valatx, and Jouvet (1974) have replicated these findings in mice trained in a multiple-trial discrimination task. They also report that the PS augmentation phenomenon dissipates as the learning curve approaches the point of maximum learning.

Kitahama (1973), however, has provided evidence suggesting that the augmentation phenomenon is not as great when animals are trained in an easier to learn single-compartment Y-maze. Perhaps the difficulty of the multiple Y-maze sets in motion certain problem solving strategies that are not required by the simple Y-maze. Certainly maze complexity as well as the training criteria considerably influence the degree of learning and retention achieved (Quinton, 1974; Flood, Rosenzweig, Bennett, and Orme, 1973). Perhaps PS augmentation is similarly affected by these variables.

In addition, the immediate and short-lasting PS augmentation is a relative phenomenon, since Leconte and Hennevin (1973) have artifically retarded sleep onset by

90 min following learning without altering the PS augmentation effect or producing a learning deficit. However, if sleep onset is delayed for 3 hr, the retention of the learned response is impaired and PS augmentation is not observed. Leconte, Hennevin, and Bloch (1974) suggest that the first 60-90 min of sleep which follow each conditioned avoidance session are essential for subsequent unimpaired retention. From their observations it would appear that one of the elements necessary for memory fixation is the presence of either PS or conditions compatible with PS occurrence during the hours immediately after learning. The recent studies of Pearlman (Pearlman and Greenberg, 1973; Pearlman and Becker, 1973, 1974a) support this contention. He has shown that selective PSD (by drugs or pedestal procedure) for 3 hr immediately following two-way conditioned avoidance (or discrimination) learning in the rat produces a significant retention deficit, whereas no amnesia is observed if PSD is delayed until 3 hr after training.

B. Long-Term Augmentation

In addition to the immediate and short-lasting PS augmentation, Delacour and Brenot (1975) have noted a relationship between learning and the duration of PS recorded over a 24-hr period. Their results suggest a direct involvement of PS in memory processes. Smith et al. (1974) have further noted that there may be a protracted

augmentation of PS following learning. Recently Fishbein, Kastaniotis, and Chattman (1974) extended the findings of Smith et al. The results of this experiment also indicate a prolonged augmentation of PS following a learning experience.

In this experiment, mice were used as their own baseline control and yoke control. The animals were trained for 1 hr in an active-avoidance task in which the experimental animals learned to avoid footshock while the yoked controls received an equal number of shocks but were unable to escape. One important finding in this study was that in every experimental animal the post-training PS augmentation emerged 6 hr after the termination of the training session, and then the elevation of PS remained throughout the next 15 hr of sleep-awake activity. The PS time of the yoke-control animals was unaltered during the 24-hr period subsequent to the training experience; their sleep-awake cycles were identical to baseline recordings obtained before and after the training sessions. Contrary to the observations of Lucero (1970), Leconte et al., and Smith et al., NPS time was also elevated in both experimental and yoke-control animals. This increase did not become apparent until 3-6 hr after training. Thus, while total sleep (NPS + PS) time for experimental and yoke-control animals was significantly increased above baseline recordings, only PS time was augmented in the experimental animals.

It appears that NPS augmentation is related to the general increase in activity produced by the CS-footshock contingency, since the number and duration of footshocks are exactly the same for both experimentals and controls. This interpretation is consistent with previous observations that NPS is influenced by motor activity (Hobson, 1968) and by information processing of non-emotionally labeled information (Fowler, Sullivan, and Ekstrand, 1973), whereas PS may be associated with newly learned information requiring an extensive change in problem solving strategy (Greiser et al., 1972). An examination of the results confirms this hypothesis. In the first training session, naive mice learned to avoid shock rather slowly, and both PS and NPS were augmented above baseline levels. In the second session, mice which had served as yoked controls in the first session learned the avoidance rather rapidly, resulting in a significant augmentation of NPS, together with only a slight augmentation of PS.

Of further interest is the finding that the major portion of PS augmentation was primarily accounted for by a "slow"-learning group of animals. This observation is consistent with the previously discussed finding that each learning session was followed by an augmentation (processing incomplete) of PS (in the course of distributed learning), to the point where the animals reached the asymptote of the learning curve, after which the augmentation ceased

to occur (processing complete). The authors interpret these findings as suggesting that incomplete learning sets in motion a chain-of-events that actuates the PS augmentation. At the termination of the training session the fast learners were performing at 90% avoidances, thus PS augmentation was minimal. The slow learners, however, were performing at 25% avoidances, thereby triggering the events which led to an augmentation of PS, which in turn may have served to complete the learning.

These latter findings are of particular interest because they lend support to the views of a variety of dream theorists, who attribute to dreams (PS) the function of facilitating problem solving and/or information processing aspects of a dreamer's adaptive mastery over his environment. Actually this problem solving-information processing view of dream function is not a new conceptualization, as it was part of the theories of some of Freud's early followers (Maeder, 1913; Adler, 1936). Contemporary theorists have developed the idea that dreams constitute a mode of continuing a series of memories that have been set in motion during wakefulness, and then push for completion during sleep (Ullman, 1959; Klein, 1967; Fiss, 1969; Pearlman, 1970; Breger, 1967; French, 1954; French and Fromm, 1964; Greenberg, 1970; Shapiro, 1967; Hawkins, 1966). The results of the present animal experiments are in direct support of these contemporary theorists.

C. Discussion

The data presented in these sections are in concordance with the hypothesis (Fishbein and Gutwein, 1977) relating PS to memory storage processes. For example, the PS augmentation following learning is consistent with the interpretation of the PSD + ECS studies that PSD delays the fixation phase of memory storage. These experiments suggest that the PS phase provides conditions which facilitate the conversion of a labile STM trace into a stable LTM. The prolonged augmentation of PS seen after massed learning lends support to the interpretation of the experiments showing the retrograde amnesic effects of ECS after PSD; PS serves to maintain the stability of the consolidated memory trace. In addition, the observation that PS augmentation is greatest in slow-learning animals suggests that incomplete (unfinished or unresolved) "cognitive" tasks constitute problems which continue toward completion (consolidation) during PS. It is also interesting that the temporal parameters of the deprivation and augmentation studies appear to be almost identical. In sum, the converging results of the various experiments support the general view that PS is important for the fixation and maintenance of memory in long-term storage.

CHAPTER II

PARADOXICAL SLEEP AND MEMORY: (I) LONG-TERM DISRUPTIVE EFFECTS OF ANISOMYCIN

Introduction

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 (Hennevin, Leconte, and Bloch, 1971; Leconte, and Hennevin, 1971; Leconte, Hennevin, and Bloch, 1973). 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 (Greenberg and Pearlman, 1974; Pearlman and Becker, 1973; Pearlman and Becker, 1974a; Pearlman and Greenberg, 1973) 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 (Shiromani, Gutwein and Fishbein, 1979). 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 pharmacological inhibition of PS. I employ Anisomycin (ANI) a potent

inhibitor of cerebral protein synthesis and examine its effects on sleep, long-term memory (LTM) and short-term (STM) of one-trial inhibitory avoidance training. The glutarimide antibiotic, cycloheximide (CYC), and ANI, been previously employed to elucidate the pharmacological basis of sleep (Pegram, Hammond, Bridgers, 1973; Rojas-Ramirez, et al., 1977) and memory (cf. review by Barraco and Stettner, 1976). 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 (Grollman and Huang, 1973); its inhibition of cerebral protein synthesis is of shorter duration and less toxic compared to CYC. In the first experiment, I examine the long-term effects of ANI on PS and SWS.

Experiment 1

Subjects

Male and female mice of the CF-1 (Carworth Farms, New York) 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, i.p.) with extra-dural cortical and neck

muscle electrodes. Following surgery, animals are individually housed in high-walled open-top plastic cages, measuring 53.2 x 16.5 x 12.7 cm, which are placed in a sound-attenuated, electrically isolated recording chamber for 7 days. On day 8, an electrode cable is connected to the animal beginning at 0700 for 48 hr of adaptation. The electrode cable consists of a ballbearing 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 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 libitum 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 random administration of SAL and ANI (Reich, Geyer, and Karnovsky, 1972).

Results:

Measures of PS

The effects of SAL and dose-response ANI on the latency to onset of the first PS and SWS episodes is shown in Figure 1. 40 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 ($F = 1,53$, $df = 3/29$).

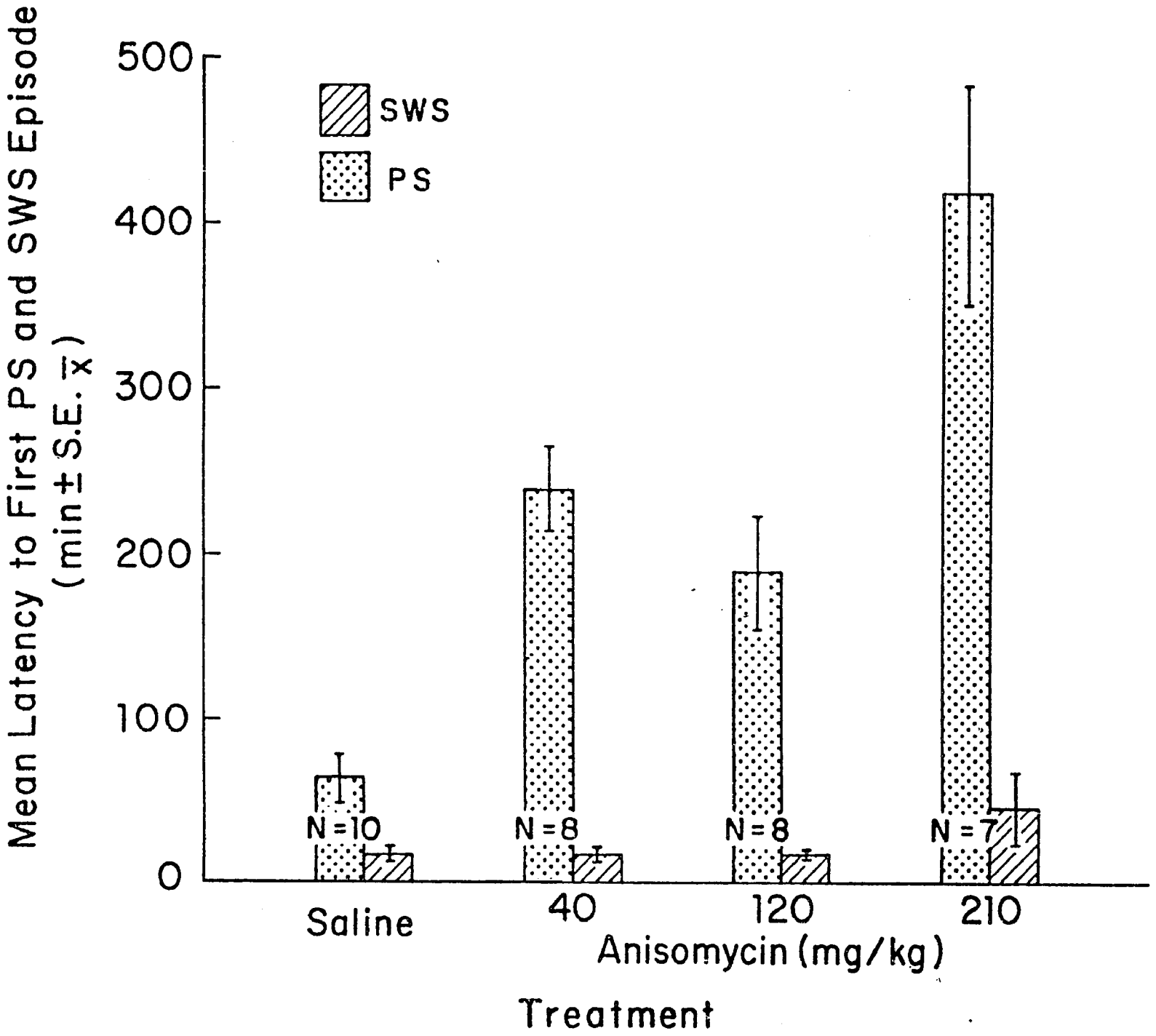
Mean Latency to First PS and SWS Episode
(min ± S.E. $\bar{x}$)
500
400
300
200
100
0
SWS
PS
N = 10
N = 8
N = 8
N = 7
Saline
40
120
210
Anisomycin (mg/kg)
Treatment

Figure 1

Anisomycin effects on EEG recorded sleep patterns: Alteration of the latency to appearance of the first PS and SWS episode. Separate groups of mice were (i) implanted with chronic indwelling EEG and EMG electrodes, (ii) adjusted to recording chamber for 7 days and electrode cable for 2 days, (iii) 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-treated animals and particularly the delay in the 40 mg/kg group.

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 hr after injection compared to SAL. Percent PS of total recording time per hr and mean PS time are shown in Figure 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 hr 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.

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 hr and mean SWS time are shown in Figure 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

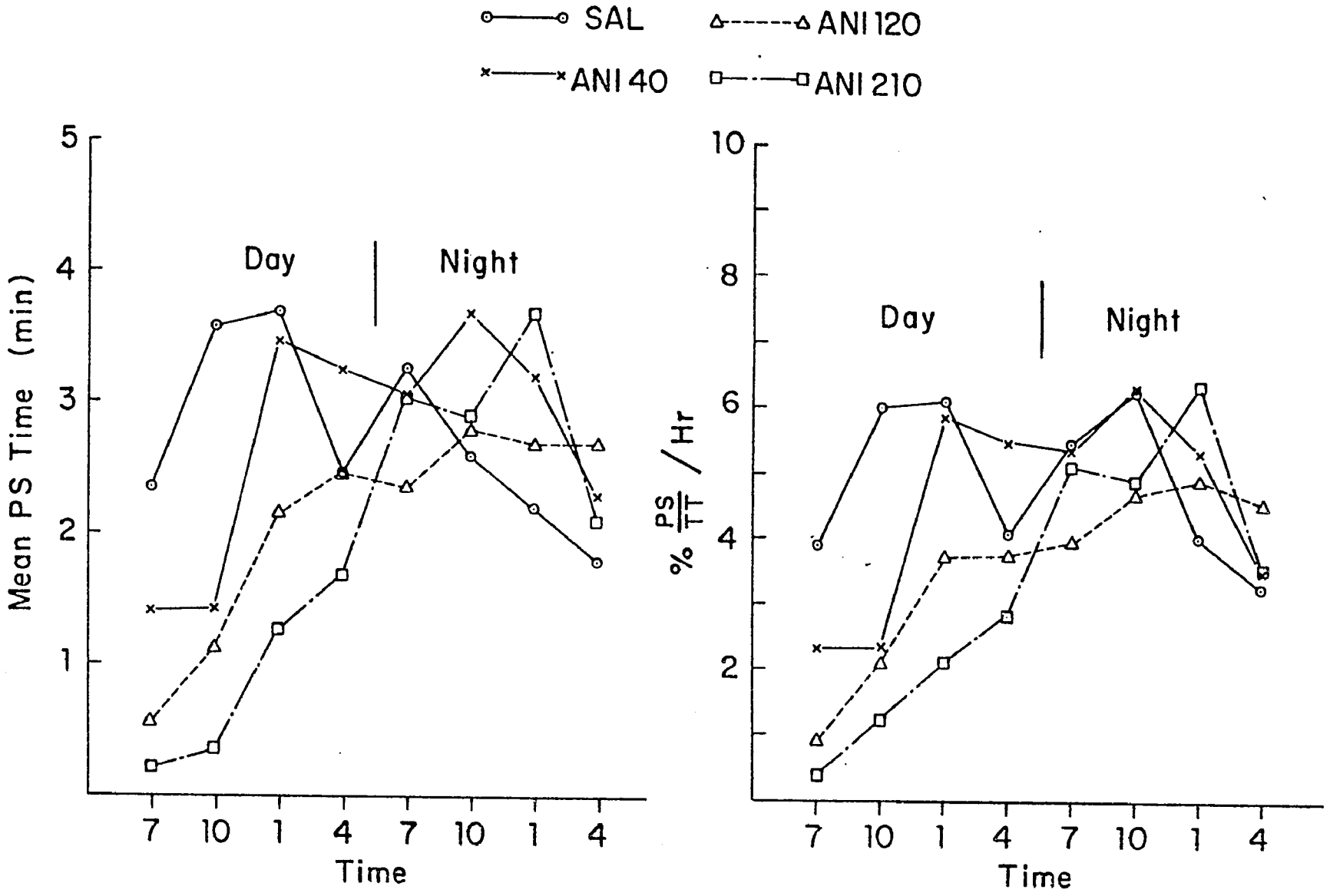
SAL
ANI 120
ANI 40
ANI 210
Mean PS Time (min)
% PS/TT /Hr
Day
Night
Time
7
10
1
4
7
10
1
4

Figure 2

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

TABLE I

COMPARISON BETWEEN ANI AND SAL TREATED MICE ON
(7 A.M. - 4 P.M.)

	Number PS episodes			Mean duration PS			
	F	df	Significance level	F	df	Significance level	F
ANI 40 mg* vs SAL	13.5	1/105	P < .001	9.9	1/105	P < .002	12.9
ANI 120 mg vs SAL	36.0	1/155	P < .0001	18.2	1/115	P < .0001	33.6
ANI 210 mg vs SAL	70.1	1/146	P < .0001	45.0	1/146	P < .0001	65.4

* Significant only

7 A.M. - 1 P.M. Return to SAL levels at 1 P.M.

TABLE I

ISON BETWEEN ANI AND SAL TREATED MICE ON MEASURES OF PS
(7 A.M. - 4 P.M.)

;	Mean duration PS			Mean Time PS			% PS/HR		
ınce	F	df	Significance level	F	df	Significance level	F	df	Significance level
	9.9	1/105	P <.002	12.9	1/105	P <.0001	12.9	1/105	P <.0001
1	18.2	1/115	P <.0001	33.6	1/155	P <.0001	30.0	1/155	P <.0001
1	45.0	1/146	P <.0001	65.4	1/146	P <.0001	49.3	1/146	P <.0001

vels at 1 P.M.

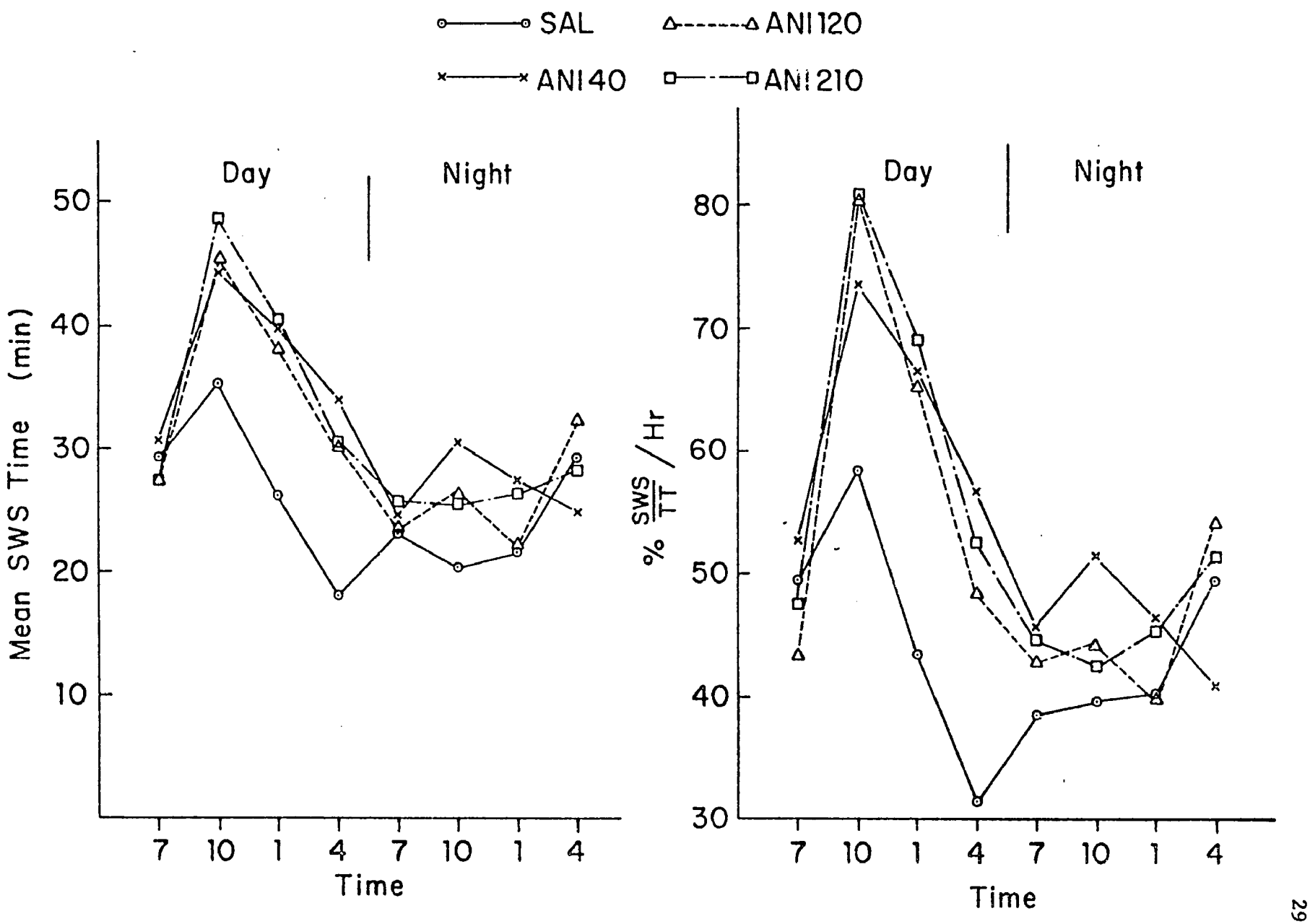
SAL
ANI120
ANI40
ANI210
Day
Night
Mean SWS Time (min)
50
40
30
20
10
7
10
1
4
7
10
1
4
Time
% SWS/TT /Hr
80
70
60
50
40
30
Day
Night
7
10
1
4
7
10
1
4
Time

Figure 3

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

TABLE II

COMPARISON BETWEEN ANI AND SAL TREATED MICE ON M
(7 A.M. - 7 P.M.)

	Number SWS episodes			Mean duration SWS			
	F	df	Significance level	F	df	Significance level	F
ANI 40 mg vs SAL	0.58	1/209	NS	13.8	1/209	P < .0001	31.4
ANI 120 mg vs SAL	12.6	1/207	P < .0001	26.5	1/207	P < .0001	19.9
ANI 210 mg vs SAL	15.9	1/195	P < .0001	29.5	1/195	P < .0001	25.5

TABLE II

BETWEEN ANI AND SAL TREATED MICE ON MEASURES OF SWS
(7 A.M. - 7 P.M.)

Mean duration SWS			Mean Time SWS			% SWS/HR		
F	df	Significance level	F	df	Significance level	F	df	Significance level
13.8	1/209	$P < .0001$	31.4	1/209	$P < .0001$	32.6	1/209	$P < .0001$
26.5	1/207	$P < .0001$	19.9	1/207	$P < .0001$	25.9	1/207	$P < .0001$
29.5	1/195	$P < .0001$	25.5	1/195	$P < .0001$	29.7	1/195	$P < .0001$

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 assessing the effects of CYC (Pegram, et al., 1973) and ANI (Rojas-Ramirez, et al., 1977) on sleep have reported a decrease only in PS frequency with no alteration in SWS. These results may be a function of higher dosages employed in this study. Of particular interest is the 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.

Housing:

In all behavioral experiments in this study mice are individually housed in standard size clear plastic cages (27.7 x 16.5 x 12.7 cm) in our temperature controlled 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 x 4.5 x 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 x 16.6 x 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 .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 animal's 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 mg/kg at one of the following times: (a) 1 min, (b) 15 min, (c) 30 min, (d) 45 min, (e) 1 hr, (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 into this 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 Figure 4. STLs are initially transformed to $\log_e$ scale in order to reduce heterogeneity of variance. Training trial STLs for experimental groups are equivalent ($F = 0.86$, $df = 3/380$). 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. 120 mg and 210 mg significantly impair LTM (120 mg vs SAL; $F = 29.1$, $df = 1/299$, $p < .001$; 210 mg vs SAL; $F = 48.9$, $df = 1/299$, $p < .001$) whereas mice injected with ANI 40 mg show good retention (40 mg vs SAL; $F = 4.1$, $df = 1/301$). Memory trace lability is significantly extended with delayed post-training ANI injections. ANI 120 induces amnesia up to 15 min post-training (120 mg vs SAL; $F = 5.4$, $df = 1/47$, $p < .02$) and ANI 210 is effective up to 30 min after training (210 mg vs SAL; $F = 22.7$, $df = 1/48$, $p < .001$).

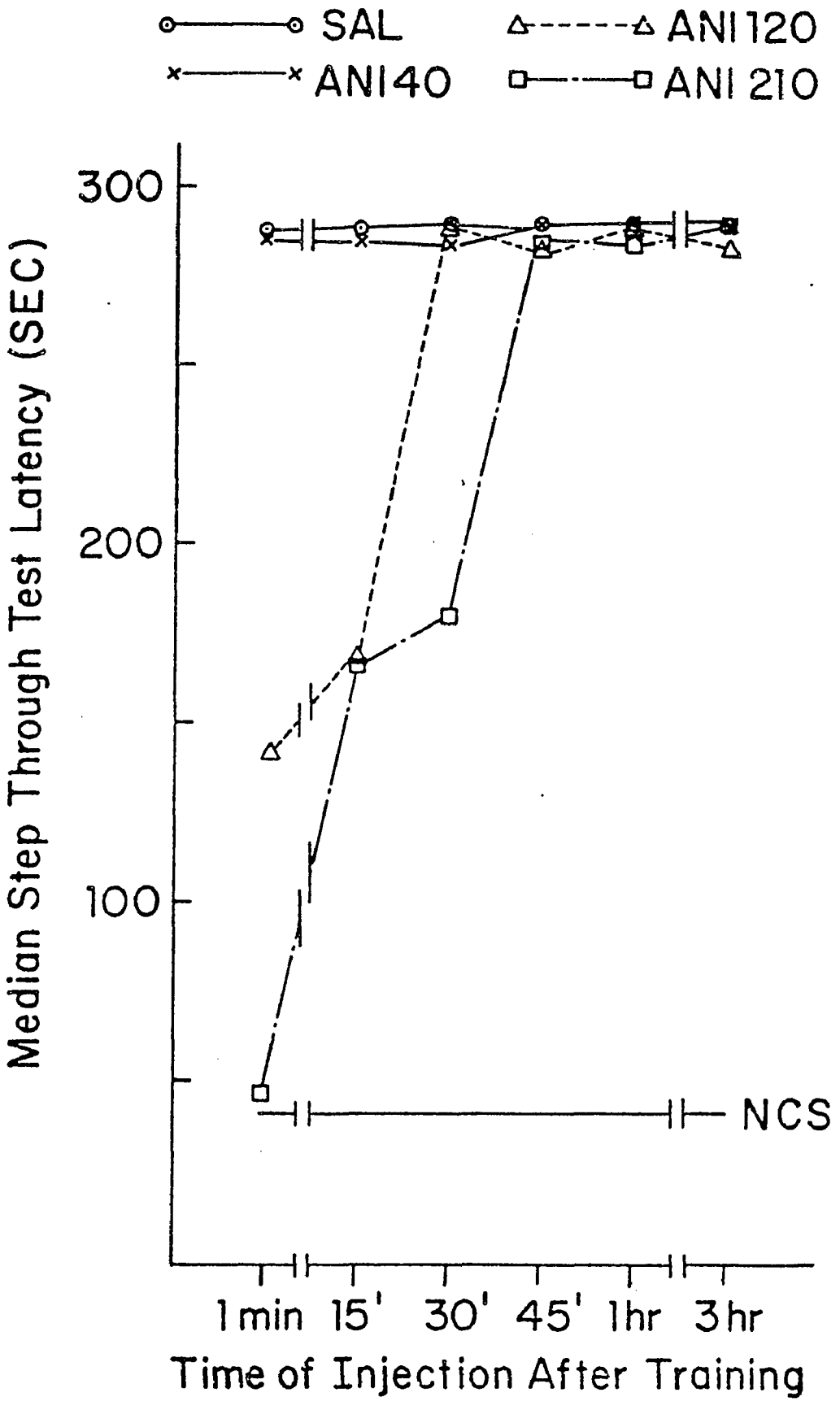
SAL
ANI 120
ANI 40
ANI 210
300
200
100
Median Step Through Test Latency (SEC)
NCS
1 min
15'
30'
45'
1 hr
3 hr
Time of Injection After Training

Figure 4

Long-term retrograde amnesic effects of Anisomycin on one-trial inhibitory avoidance retention. Median test latencies: Independent groups of mice are (i) trained (or administered noncontingent shock, NCS), (ii) subcutaneously injected with anisomycin (40, 120, or 210 mg/kg or Saline), and (iii) 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.

Discussion

The results of Experiments 1 and 2a indicate that ANI 40 selectively decreases PS for 6 consecutive hr 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 (Shiromani, Gutwein, and Fishbein, 1979) 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 (Gutwein, Quartermain, and McEwen, 1974; Quartermain and McEwen, 1970; Squire, Smith, and Barondes, 1973). The objective of Experiment 2b is to examine the effects of ANI on STM of one-trial inhibitory avoidance training.

Experiment 2b

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 of this experiment are shown in Figure 5.

An ANOVA performed on $\log_e$ transformed data reveals that training trial STLs for experimental groups are equivalent ($F = .73$, $df = 3/504$) and good retention in ANI-treated mice up to 1 hr post-training ($F = .47$, $df = 3/300$). At 3 and 6 hr post-training all ANI-treated mice exhibit amnesia relative to SAL controls (3 hr; $F = 8.34$, $df = 3/61$, $p < .005$; 6 hr; $F = 25.5$, $df = 3/62$, $p < .0001$). At 9 hr, mice injected with ANI 40 are not significantly different from SAL ($F = .07$, $df = 1/29$) but 120 mg and 210 mg injected mice are amnesic (120 mg and 210 mg vs SAL; $F = 14.4$, $df = 1/61$, $p < .001$). There are no significant differences between the 120 and 210 mg groups at this time ($F = .10$, $df = 1/30$).

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 (1971a) has also reported a

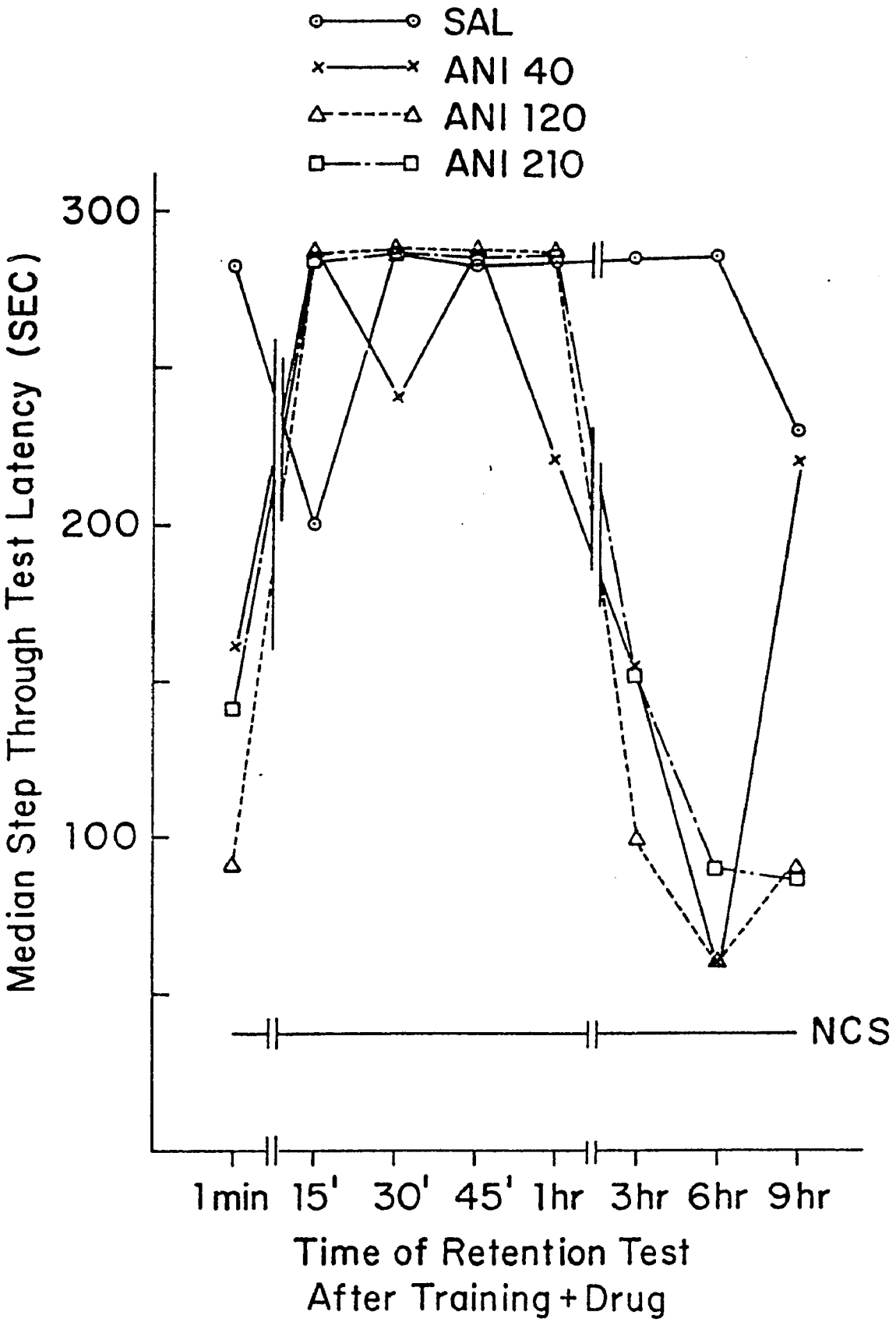
SAL
ANI 40
ANI 120
ANI 210
300
200
100
Median Step Through Test Latency (SEC)
NCS
1min
15'
30'
45'
1hr
3hr
6hr
9hr
Time of Retention Test
After Training + Drug

Figure 5

Short-term retrograde amnesic effects of Anisomycin on one-trial inhibitory avoidance retention. Median test latencies. Independent groups of mice are (i) trained (or administered noncontingent shock, NCS), (ii) subcutaneously injected with Anisomycin (40, 120, or 210 mg/kg or Saline), and (iii) 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.

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 my finding that ANI 120 and ANI 210 significantly decrease PS for 9 consecutive hr 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 consecutive hr after injection, impairs STM only between 3 and 6 hr after training, and does not induce LTM loss.

These data suggest that inhibition of PS by ANI 40 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 (Fishbein, 1970; Fishbein, 1971; Fishbein, McGaugh, and Swarz, 1971) or immediate post-training LC lesions (Zornetzer and Gold, 1976), has been shown to extend memory trace susceptibility to disruption with delayed post-training ECS. This effect has also been obtained 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 (Andry and Luttges, 1972; Andry and Luttges, 1973; Flood et al., 1977; Quinton, 1978). Flood, et al.,

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.

Experiment 3

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 Figure 6. Training STL's for experimental groups are equivalent ($F = 1.2$, $df = 7/1059$). An ANOVA performed on these data reveals that mice administered SAL + ECS are amnesic compared to SAL + SECS controls ($F = 38.2$, $df = 1/270$, $p < .001$) and mice given ANI 40 + ECS are significantly impaired relative to the SAL + ECS group ($F = 23.7$, $df = 1/270$, $p < .001$). Administration of ANI 10 mg/kg or ANI 20 mg/kg in combination with ECS does not produce amnesia compared to SAL + ECS controls (10 mg: $F = 1.2$, $df = 1/270$; 20 mg: $F = 0.35$, $df = 1/270$). 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: $F = 9.3$, $df = 1/30$, $p < .004$).

General Discussion

The results of this study provide clear evidence in support of the contention (Shiromani, Gutwein, and Fishbein, 1979) 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 hr 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 hr after injection but their long-term retention is

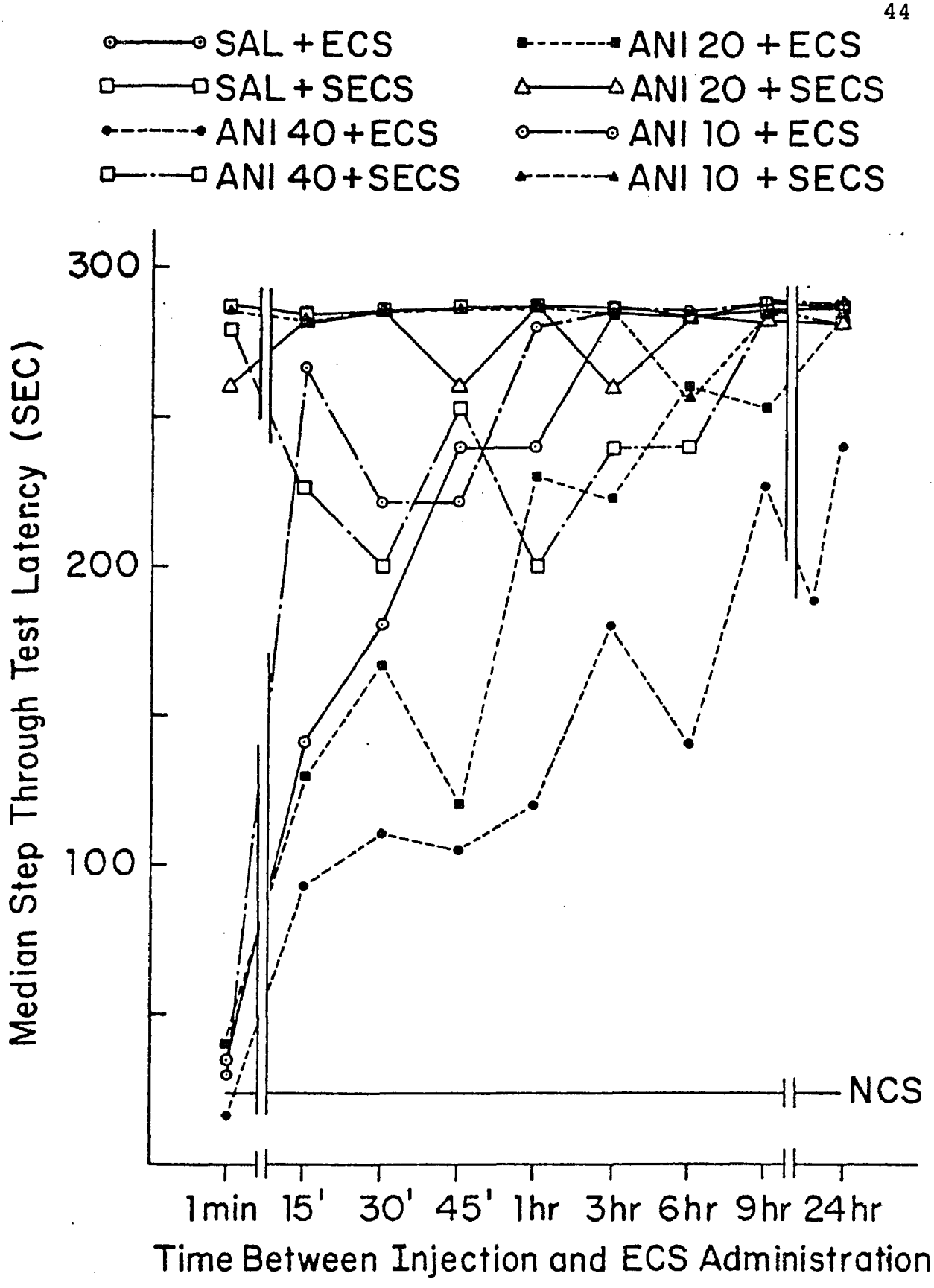
SAL + ECS
SAL + SECS
ANI 40 + ECS
ANI 40 + SECS
ANI 20 + ECS
ANI 20 + SECS
ANI 10 + ECS
ANI 10 + SECS
300
200
100
Median Step Through Test Latency (SEC)
NCS
1min
15'
30'
45'
1hr
3hr
6hr
9hr
24hr
Time Between Injection and ECS Administration

Figure 6

Retrograde amnesia: Prolonging the fixation phase of memory consolidation by low-dosage Anisomycin. Inhibitory avoidance test latencies: Mice (i) trained (or administered noncontingent shock, NCS), (ii) injected with low dosages of Anisomycin (10, 20, or 40 mg/kg or Saline), (iii) treated with ECS at one of several intervals after training, and (iv) 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.

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 post-injection, then persists for 9 consecutive hr, and is not dose-dependent. The neurochemical mode of action for ANI's dissociable effects on sleep are presently unknown, but this unique finding merits further investigation.

ANI-induced SWS augmentation with concomitant amnesia does not support the results of Fowler et al., (1973) who reported induction of amnesia following SWS but not PS deprivation.

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

Data from experiments 2b and 3 demonstrate that the labile period of memory formation can be 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 (Andry and Luttges, 1972; Andry and Luttges, 1973; Flood, et al., 1977; Quinton, 1978) that prolonging the duration of protein synthesis inhibition by subamnesic dosages of an inhibitor extends the period of memory trace susceptibility to

disruption by a variety of amnestic agents, and now also show that PS is an integral element for this stability and maintenance of LTM.

The present data in conjunction with our recent study (Shiromani, Gutwein, and Fishbein, 1979) in which we demonstrated 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 following training. Our review (Fishbein and Gutwein, 1977) and investigations into the relationship between PS and memory storage processes now indicate that only protracted PS inhibition induced either by the water tank procedure (Fishbein, 1970; Fishbein, 1971; Fishbein, McGaugh, and Swarz, 1971; Shiromani, Gutwein, and Fishbein, 1979) drug (ANI, experiments 1-3) or 30 days of environmental isolation (Chapter III,Experiment 4) impairs LTM; conversely protracted PS augmentation induced either by formal training (Fishbein, Kastaniotis, and Chattman, 1974) or 30 days of environmental enrichment (Chapter III, Experiment 4) correlates with enhancement of long term memory. These data provide considerable support for my hypothesis that PS occurring over a prolonged time period is a requisite neurobiological mechanism for the processing, maintenance, and storage of long-term memory.

CHAPTER III

PARADOXICAL SLEEP AND MEMORY (II): SELECTIVE ALTERATIONS FOLLOWING ENRICHED AND IMPOVERISHED ENVIRONMENTAL REARING

Introduction

Interest in the effects of environmental enrichment on brain and behavior has stimulated a considerable amount of research. One specific objective in carrying out these studies is that they will eventually enhance our understanding of the mechanisms underlying sleep, learning, and memory.

A number of investigators have employed animal models for the study of environment related changes in learning and memory in order to obtain more precise experimental control of variables that may influence acquisition and retention. Enrichment has usually been defined as an environment that is more complex than the standard laboratory cage. The degree of environmental complexity has ranged from isolation to social housing to "enriched" rearing (toys, mazes, etc.), conditions that have been defined and relatively standardized by Rosenzweig and colleagues. Numerous studies have focused on the effects of early environmental enrichment upon the acquisition of appetitive and aversively motivated behaviors and various temperment

measures, e.g., locomotor activity, emotionality, and exploration. There is now considerable evidence to indicate that environmental enrichment results in enhanced performance of appetitive and aversively motivated behavior (Greenough, 1976; Bennett, et al., 1970; Gluck and Harlow, 1971; Rosenzweig, 1971; Rosenzweig, et al., 1972a; Rosenzweig and Bennett, 1976).

In related studies, the relationship between PS and quality of environmental rearing has been recently demonstrated; enriched rearing results in PS augmentation (Tagney, 1973; McGinty, 1971; Kiyono and Seo, 1978). For example, Kiyono and Seo, report that Sprague-Dawley rats reared in an enriched environment for 30 days exhibit increase in percent PS and in mean duration of PS episodes during the day cycle and in mean duration of PS episodes during the night cycle compared to impoverished reared animals. These results are difficult to evaluate, however, because (1) no socially-reared control group was included for comparison, and (2) no performance measures were assessed, thus precluding an interpretation of these findings with regard to memory storage processes. It is therefore of interest to clarify the relationship between PS, enriched and impoverished rearing, and memory storage processes. This is the objective of Experiment 4 which examines the effects of enriched and impoverished rearing on PS and long-term memory.

As investigators have shown that PS is selectively and significantly augmented following either appetitive or aversively motivated training (Chapter I, pp.12-17),a second objective of this study is to determine whether differential rearing affects the expression of PS augmentation in the same manner as formal training. Experiment 6 examines the effects of enriched and impoverished environmental rearing on sleep circadian rhythmicity.

Finally, Experiments 7a and 7b in Chapter V are preliminary investigations on the behavioral and physiological effects of differential rearing on open-field activity and body weight respectively.

The objective of Experiment 4 is to correlate the effects of enriched and impoverished rearing on PS with the long-term recall of a multiple-trial, shock motivated, brightness-discrimination task.

Experiment 4

Subjects and Environments:

Subjects are male mice of the CF-1 (Carworth Farms, New York) strain from our laboratory breeding stock. They are weaned at 21 ± 2 days and then placed into either enriched (EE), social (SE), or impoverished (IE) environments for 30 days. The EE was constructed of translucent Plexiglas and measured 30.5 x 81.3 x 28.6 cm. Animals (10-12 per cage) were exposed to numerous toys which were all replaced every 2 days. Food and water were directly accessible. SE mice

were housed 5-7 in 27.7 x 16.5 x 12.7 cm standard clear plastic cages, while IE mice were singly housed in white opaque Plexiglas cages measuring 64 cu cm with clear Plexiglas tops. All groups were maintained in our temperature controlled vivarium on a 12:12 light-dark cycle.

Surgery and EEG Recording Procedure

Mice were removed from their respective environments at 51 days of age and individually housed in high-walled clear plastic cages measuring 53.2 x 16.5 x 12.7 cm for the next 3 days. On day 54, randomly selected mice from each environment were lightly anesthetized with Nembutal, then surgically implanted with extra-dural cortical and dorsal neck muscle electrodes as previously described (Fishbein, Kastaniotis, and Chattman, 1974). Following surgery, animals were placed in a sound-attenuated electrically isolated recording chamber for the next 7 days. On day 61, an electrode cable was connected to the animal beginning at 0900 hr for 72 hr of adaptation. On day 64, animals sleep-wakefulness cycles were recorded beginning at 0900 hr and monitored continuously over the next 48 hr with standard polygraphic techniques. I provide for a considerable period of adaptation between the end of environmental rearing and the start of EEG recording because Reich, Geyer, and Karnovsky (1972) have demonstrated that the nature of the experimental design and treatment conditions in studies investigating sleep-wakefulness relationships must be properly controlled. PS

may be influenced by such factors as temperature, time of day, state of the endocrine system, muscular fatigue, and, most importantly, the acclimatization of the animals to the experimental apparatus.

Results

The number of episodes, average length of episode, and percent episode of total sleep time (TST) was computed for both slow-wave sleep (SWS) and PS (Bern and Fishbein, 1976). Data were analyzed with a two-way fixed effects ANOVA (Winer, 1962).

Results of this experiment are summarized in Table III. There were no significant differences between groups in either (i) number of SWS episodes ($F = 2.18$, $df = 2/761$), (ii) average length of SWS episode ($F = .55$, $df = 2/761$), or (iii) percent SWS/TST ($F = .51$, $df = 2/761$). On the other hand, highly significant differences were observed between groups on measures of PS. EE mice exhibited a greater number of PS episodes ($F = 9.6$, $df = 1/653$, $p < .002$), longer average length of PS episode ($F = 25.4$, $df = 1/653$, $p < .001$), and increased percent PS/TST ($F = 6.9$, $df = 1/653$, $p < .009$) compared to SE controls. IE mice showed shorter average length PS episodes ($F = 6.9$, $df = 1/314$, $p < .009$) compared to SE controls and exhibited a significant reduction in the number of PS episodes ($F = 6.0$, $df = 1/555$, $p < .01$), average length of PS episodes ($F = 45.9$, df 1/555, $p < .001$), and percent PS/TST

TABLE III

MEAN PERCENT SLEEP VALUES (± S.E.M.) PER HOUR (48 H
IN ENRICHED (EE), SOCIAL CONTROL (SE), AND IMPOVERI

	EE (N = 19)		SE (N = 9)	
# SWS episodes	3.13± .19	-NS-	2.87± .32	-NS
Average length SWS episodes (min)	9.47± .77	-NS-	9.10± 1.41	-NS
% SWS/TST	87.80± 1.55	-NS-	87.80± 3.0	-NS
# PS episodes	1.94± .18	-p < .002-	1.59± .24	-NS
Average length PS (min)	1.46± .09	-p < .001-	1.11± .12	-p
% PS/TST	10.78± 1.07	-p < .009-	8.90± 1.6	-NS

[a] $p < .01$ vs EE — EE vs SE, ANOVA df = 1/653

[b] $p < .001$ vs EE — EE vs IE, ANOVA df = 1/555

[c] $p < .002$ vs EE — SE vs IE, ANOVA df = 1/314

TABLE III

SLEEP VALUES (± S.E.M.) PER HOUR (48 HR RECORDING SESSION) (EE), SOCIAL CONTROL (SE), AND IMPOVERISHED (IE). REARED MICE

(N = 19)		SE (N = 9)		IE (N = 5)
.19	-NS-	2.87± .32	-NS-	3.06± .31
.77	-NS-	9.10± 1.41	-NS-	7.80± 1.46
.55	-NS-	87.80± 3.0	-NS-	89:40± 3.41
.18	-p < .002-	1.59± .24	-NS-	1.58± .37[a]
.09	-p < .001-	1.11± .12	-p < .009-	.87± .19[b]
.07	-p < .009-	8.90± 1.6	-NS-	7.90± 2.2[c]

OVA df = 1/653

OVA df = 1/555

OVA df = 1/314

($F = 9.4$, $df = 1/555$, $p < .002$) compared to EE mice.

Experiment 5

The objective of Experiment 5 is to correlate the effects of differential rearing on PS with its effects on the acquisition and recall of a multiple-trial aversively motivated brightness discrimination task.

Subjects and Environments

Subjects and environmental rearing conditions are as described in Experiment 4.

Apparatus

The apparatus is a Y-maze constructed of black lucite and covered with a clear Plexiglas top. Each arm is 22 cm long, 10.2 cm wide, and 18 cm high. The floor consists of stainless steel rods set 1.3 cm apart and 3.2 cm off the ground. The arms of the maze contain lights set 9 cm above the floor. A 12 volt logic system automatically programs the presentation and duration of the CS, UCS, and intertrial interval and randomly positions the S^D for every trial. Each trial is begun by the simultaneous presentation of a tone and the discriminative stimulus; 5 sec later electronic current is automatically delivered to the two incorrect arms of the maze.

Training Procedure

Training procedure is as follows: Commencing at

54 days of age, independent groups of differentially reared mice are administered 25 trials per day for 3 consecutive days at 0.5 mA footshock intensity with a 10 sec intertrial interval. Correct discrimination of the dark limb of the maze served as the S^D on the basis of preliminary studies in which no preference was found for either the lighted or darkened limbs of the maze. Retention of discrimination was measured with a recall procedure in independent groups, either 1 or 28 days following the last training day. Animals were singly housed throughout this acquisition and recall phase. In this test procedure (25 trials) footshock is not administered in the choice limbs. McAllister and McAllister (1971) and Spear and Parsons (1976) have demonstrated this technique to permit a clearer evaluation of the rate of forgetting.

Results

Results of this experiment are summarized in Table IV. There are no differences in the rate of acquisition between EE and SE mice ($F = 1.38$, $df = 1/72$,) but IEs are slower to learn compared to SE controls ($F = 4.98$, $df = 1/72$, $p < .03$). EEs and SEs significantly improved their performance across days (linear trend EE; $F = 4.11$, $df = 1/111$, $p < .04$; linear trend SE; $F = 10.8$, $df = 1/54$, $p < .001$) with IEs exhibiting a trend towards improvement (linear trend IE; $F = 3.6$, $df = 1/51$, $p < .06$).

TABLE IV

RATE OF ACQUISITION AND RECALL OF Y-MAZE TRAINING
(MEAN PERCENT DISCRIMINATION ± S.E.M.)
IN ENRICHED (EE), SOCIAL CONTROL (SE),
AND IMPOVERISHED (IE) REARED MICE

	Acquisition		
	Day 1	Day 2	Day 3
EE (N = 38)	41.8±2.28	45.7±2.51	48.6±2.35
	a ———	p < .04	——— ↑ NS ↓
SE (N = 19)	40.6±2.14	48.6±3.31	54.3±3.20
	a ———	p < .001	——— ↑ p < .03 ↓
IE (N = 18)	36.0±2.41	41.5±2.72	43.1±2.79
	a ———	p < .06	———

	Recall		
	Day 1		Day 28
EE	54.6±2.6	-NS-	50.3±2.79
	(N = 19)		(N = 19)
	↑		↑

			NS ↓
SE	40.6±2.14	48.6±3.31	54.3±3.20
(N = 19)	^{a} ____	$p < .001$	____
			↑ $p < .03$ ↓
IE	36.0±2.41	41.5±2.72	43.1±2.79
(N = 18)	^{a} ____	$p < .06$	____

Recall

	Day 1		Day 28
EE	54.6±2.6	-NS-	50.3±2.79
	(N = 19)		(N = 19)
	↑ NS ↓		↑ $^b p < .05$ ↓
SE	61.3±2.98	-$p < .001$-	37.2±5.13
	(N = 9)		(N = 10)
	↑ $^b p < .05$ ↓		↑ NS ↓
IE	45.3±3.39	-NS-	40.8±2.28
	(N = 9)		(N = 9)

^{a}ANOVA, linear trend component for acquisition

^{b}Duncan Range Test

Analysis of day 1 recall test scores indicates that SEs are significantly better than IEs ($p < .05$, Duncan Range Test); there are no differences between EE and SE groups. At 28 days, EEs demonstrate significantly better recall than SEs ($p < .05$ Duncan Range Test) but recall in SE mice is now as poor as that of IEs. In fact, recall of discrimination in EE mice is as good at 28 days as at 1 day, but SE controls show a significant decline in recall ($F = 15.6$, $df = 1/17$, $p < .001$). IE performance is uniformly poor at both test periods.

Discussion

The results of Experiments 4 and 5 provide considerable support for my contention that PS or conditions compatible with PS occurrence are a requisite neurobiological process for the maintenance and stability of long-term memory. Data from Experiment 4 demonstrating EE induced increases in the number and average duration of PS episodes and increased percent PS/TST for a period of 2 weeks following enriched rearing is of particular interest since many of the CNS alterations associated with environmental enrichment regress after 2 weeks of either social or impoverished rearing (Davenport, 1976; Greenough, 1976). IE mice show significant reductions in PS. Measures of SWS are unaltered in both EE and IE groups. Data from Experiment 5 showing enhanced long-term recall of Y-maze discrimination learning by EE mice and impaired recall by IE mice relative to

socially housed controls are notable in view of the massed practice conditions (10 sec intertrial interval) employed during training. The persistence of this weak habit in EE mice 28 days after training confirms and extends recently reported findings of the positive relationship between amount of PS and level of performance after formal training (Fishbein, et al., 1974; Smith, Kitahama, Valatx, and Jouvet, 1974). In addition, this is the first demonstration that enriched rearing can attenuate the rate of forgetting.

The present findings may reflect extensive neurochemical alterations in cortical cholinergic systems following the prolonged and extensive environmental exposures. For example, increased acetylcholinesterase (AchE) activity in the neocortex of EE rats with concomitant PS augmentation has been previously demonstrated (Gadea-Ciria, Stadler, Lloyd, and Bartholini, 1973; Rosenzweig, Bennett, and Diamond, 1972a) and investigators have also confirmed that a considerable increase in free AchE in hippocampus is essential to the development of unimpaired retention of a multiple trial, aversively motivated Y-maze discrimination task (Matthies, Ott, and Kammerer, 1975). Deutsch (1971), has also implicated the cholinergic system in memory storage processes. In addition, Sitaram, et al., (1976) have recently demonstrated a significant reduction in latency to the onset of the first PS episode following physostigmine infusion during SWS in normal volunteers; this suggests the

involvement of brainstem cholinergic systems in PS. Although the present experimental design does not permit one to determine whether increased PS and the attenuated rate of forgetting in EE mice is mediated by the facilitation of a memory retrieval mechanism, these results do provide considerable support to my contention that PS is a requisite neurobiological mechanism for the processing, maintenance, and stability of long-term memory.

CHAPTER IV

PARADOXICAL SLEEP AND MEMORY (III): SLEEP CIRCADIAN RHYTHMICITY FOLLOWING ENRICHED AND IMPOVERISHED REARING

Introduction

Rhythmic fluctuations in a variety of physiological and behavioral functions have been observed in both man and animals (Halberg, 1969; Mitler, et al., 1977). These circadian rhythms are synchronized with periodic alternations of the light-dark cycle (Aschoff, 1960). For example, it has been shown that sleep circadian rhythmicity may be altered by varying the onset and duration of the light-dark phase (Borbely, 1976).

One related variable shown to affect the circadian sleep-wakefulness cycle is the quality of environmental rearing. There are several reports of environmental enrichment selectively increasing PS (paradoxical sleep or REM sleep) and impoverished rearing inducing a selective decrease in PS. Kiyono and Seo (1978) report that Sprague-Dawley rats reared in an enriched environment for 30 days show increases in percent PS and in mean duration of PS episodes during the day cycle and in mean duration of PS episodes during the night cycle compared to impoverished reared animals. These data are difficult to evaluate,

however, because of the absence of a socially-reared control group.

In Experiments 4 and 5 I demonstrated that environmental enrichment selectively augments PS and attenuates the rate of forgetting of a multiple-trial aversively motivated Y-Maze discrimination task. Impoverished reared mice exhibit a decrease in PS and impaired task retention relative to socially reared controls.

These data provide additional support to previously reported findings of the positive relationship between PS and memory storage processes (Fishbein and Gutwein, 1977; Fishbein, Kastaniotis, and Chattman, 1974; Leconte, Hennevin and Bloch, 1974; Lucero, 1970; McGrath and Cohen, 1978; Shiromani, Gutwein, and Fishbein, 1979; Smith, Kitahama, Valatx, Jouvet, 1974). Since rodents spend the majority of their sleep time in the diurnal phase of the circadian rhythm and investigators studying the neurobiological basis of memory routinely manipulate training parameters and environmental housing conditions during the light cycle, it is pertinent to detail sleep circadian rhythmicity in animals reared in qualitatively distinct environments and to relate these effects to memory storage processes. This is the objective of Experiment 6.

Experiment 6

Description of Subjects and Environments

Male mice of the CF-1 (Carworth Farms, New York) strain are weaned at 21 ± 2 days and then placed into either super-enriched (SEE), regular-enriched (REE), social control (SC) or isolate (IE) environments for 30 days. The SEE environment is constructed of translucent Plexiglas and measures 30.5 x 81.3 x 28.6 cm. In order to obtain food and water, animals (10-12/cage) must solve single, double, and triple tier mazes and tunnels which are introduced on day 5 and whose complexity is gradually increased every 48 hours (environment changed at 1400 hr). The REE environment is of the same dimensions as the SEE environment, but here animals (10-12/cage) are only exposed to various toys and gadgets which are replaced at the same time as the SEE environment. Food and water are easily accessible. SC mice are housed 5-7 in a 27.7 x 16.5 x 12.7 cm clear plastic cage, while IE mice are housed singly in white opaque Plexiglas cages measuring 64 cu cm with clear Plexiglas tops. All groups (with the exception of the IE) are briefly handled while their environments are undergoing the appropriate changes. All groups are maintained in our temperature controlled vivarium which is on a 12:12 (0700-1900) light-dark cycle.

Surgery and EEG Recording Procedure

Mice are removed from their respective environments at 51 days of age and individually housed in high-walled clear plastic cages measuring 53.2 x 16.5 x 12.7 cm for the next 3 days. On day 54, randomly selected mice from each environment are lightly anesthetized with Nembutal, then surgically implanted with extra-dural cortical and neck muscle electrodes as previously described (Experiments 1 and 4). Following surgery, animals are placed in a sound-attenuated electrically isolated recording chamber for the next 7 days. On day 61, an electrode cable is connected to the animal beginning at 0900 hr for 72 hr of adaptation. On day 64, animal's sleep-wakefulness cycles are recorded beginning at 0900 hr and monitored continuously over the next 48 hr with standard polygraphic techniques (Fishbein, et al., 1974).

The 48 hr data block for each animal is pooled and averaged into a single 24 hr period for analysis. The number of episodes, mean duration of episode, total amount, and percent episode of total sleep time (TST) is automatically computed for both SWS and PS (Bern and Fishbein, 1976). Data are analyzed with a two-way fixed effects analysis of variance.

Results

Measures of SWS: 24 hr Cycle

Results of the entire study are summarized in Tables V and VI and Figure 7. Comparisons of values for total SWS between differentially reared groups are shown in Figure 7. SC reared animals spend 89.8 ± 3.0% (mean percent ± S.E.M.) of their TST time in SWS; 90.5 ± 1.4% in the day cycle and 85.2 ± 4.6% in the night cycle. These findings are consistent with data for socially treated CF-1 mice reported by Fishbein *et al.* (1974).

SEE, REE, and IE mice show increased total amounts of SWS compared to SCs (SEE vs SC; $F = 10.1$, $df = 1/438$, $p < .002$; REE vs SC; $F = 5.5$, $df = 1/413$, $p < .01$; IE vs SC; $F = 6.5$, $df = 1/314$, $p < .01$).

Separate within group ANOVAs reveal that REE, SC, and IE mice demonstrate significant increases in % SWS/TST in the Night cycle compared to the Day cycle (REE; $F = 11.6$, $df = 1/213$, $p < .008$; SC; $F = 4.25$, $df = 1/212$, $p < .04$; IE; $F = 5.3$, $df = 1/114$, $p < .02$), whereas SEE rearing induces a uniform distribution of % SWS/TST over the 24 hr period ($F = 0.24$, $df = 1/238$).

Day Cycle

There are no significant differences between groups on either the number of SWS episodes or in % SWS/TST. IE mice show shorter duration of SWS episodes compared to SCs ($F = 4.3$, $df = 1/160$, $p < .04$).

SWS SLEEP MEASURES PER HOUR, DAY CYC
IN ENVIRONMENTALLY ENRICHED (SEE AND REE)

	SEE (N=10)			REE (N	
	24 hr.	Day	Night	24 hr.	Day
Number SWS episodes	3.1±.09	3.3±.1	3.0±.1	3.1±.07	3.2±
Duration SWS episodes (min)	10.0±.982	11.0±.859	9.0±1.1	7.6±.89	8.0±
Percent SWS/TST	88.9±1.92	89.3±1.26	88.5±2.58	86.6±2.26	89.9±1
Amount of SWS (min)	28.0±1.6	30.4±1.3	25.5±2.0	27.3±2.1	31.1±1

TABLE V

R HOUR, DAY CYCLE (0700-1900), AND NIGHT CYCLE (1900-0700)
D (SEE AND REE), SOCIAL CONTROL (SC), AND IMPOVERISHED (IE) MICE

	REE (N=9)			SC (N=9)			IE (N=5)	
24 hr.	Day	Night	24 hr.	Day	Night	24 hr.	Day	Night
3.1±.07	3.2±.08	3.1±.07	2.9±.1	3.2±.1	2.5±.1	3.1±.1	2.9±.1	3.2±.1
7.6±.89	8.0±.975	7.2±.806	9.1±1.41	10.1±1.46	8.1±1.36	7.8±1.46	8.7±1.53	6.9±1.4
5.6±2.26	89.9±1.1	83.4±3.43	87.8±3.0	90.3±1.4	85.2±4.6	89.4±3.41	93.1±2.46	85.7±4.57
7.3±2.1	31.1±1.6	23.4±2.5	24.6±2.4	28.4±2.2	20.8±2.6	28.3±3.2	33.4±3.0	23.1±3.4

PS SLEEP MEASURES PER HOUR, DAY
IN ENVIRONMENTALLY ENRICHED (SEE AND

	SEE (N=10)			REE	
	24 hr.	Day	Night	24 hr.	D
Number PS episodes	1.8±.08	2.0±.08	1.6±.08	2.1±.08	2.0
Duration PS episodes (min)	1.4±.162	1.6±.15	1.3±.168	1.5±.148	1.6
Percent PS/TST	9.8±1.3	10.6±1.26	9.0±1.34	11.9±1.66	10.0
Amount of PS (min)	3.1±.71	3.6±.99	2.6±.43	3.3±.42	3.4

TABLE VI

PER HOUR, DAY CYCLE (0700-1900), AND NIGHT CYCLE (1900-0700),
ICHED (SEE AND REE), SOCIAL CONTROL (SC), AND IMPOVERISHED (IE) MICE

REE (N=9)			SC (N=9)			IE. (N=5)		
24 hr.	Day	Night	24 hr.	Day	Night	24 hr.	Day	Night
2.1±.08	2.0±.07	2.1±.1	1.6±.08	1.8±.07	1.4±.1	1.6±.1	1.3±.1	1.9±.1
1.5±.148	1.6±.152	1.4±.144	1.1±.132	1.3±.149	.89±.116	.88±.189	.8±.188	.96±.190
1.9±1.66	10.0±1.1	13.8±2.23	8.9±1.6	9.6±1.4	8.2±1.8	7.9±2.2	5.0±1.5	10.9±2.9
3.3±.42	3.4±.39	3.3±.45	2.3±.39	2.8±.41	1.8±.38	2.0±.55	1.6±.58	2.4±.56

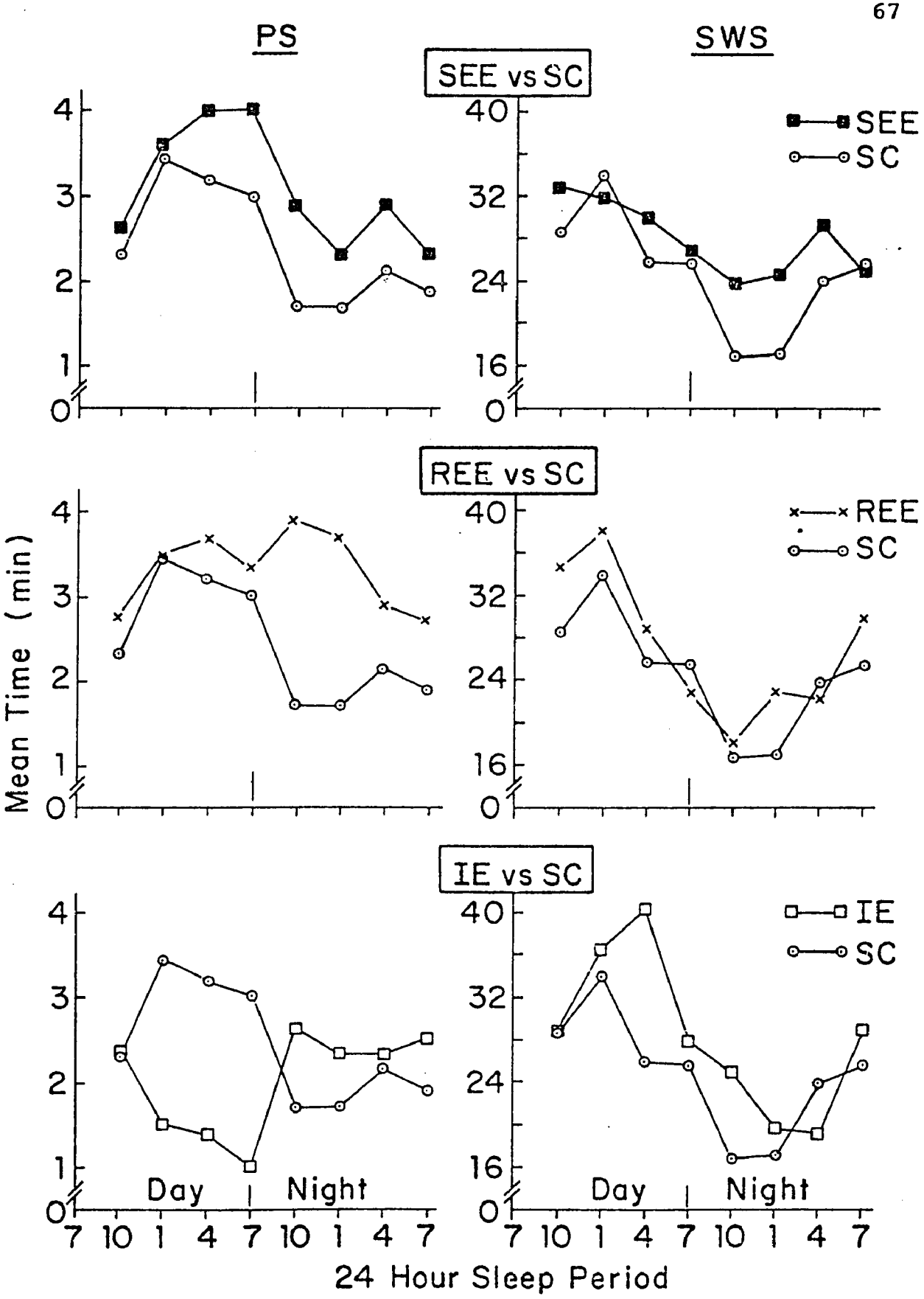
PS
SWS
SEE vs SC
SEE
SC
REE vs SC
REE
SC
IE vs SC
IE
SC
Mean Time (min)
4
3
2
1
0
40
32
24
16
Day
Night
7 10 1 4 7 10 1 4 7
24 Hour Sleep Period

Figure 7

Mean PS and SWS time in environmentally enriched (SEE and REE), social control (SC), and impoverished (IE) reared mice: Animals are differentially reared for 30 days, then (i) implanted with chronic indwelling EEG and EMG electrodes, (ii) adjusted to the recording chamber for 7 days and electrode cable for 3 days, and (iii) 48 hr recording obtained. Note circadian PS and SWS augmentation in SEE and REE mice and PS reduction in IE animals. SEE (N = 10), REE (N = 9), SC (N = 9), IE (N = 5).

Night Cycle

SEE, REE, and IE mice exhibit a significant increase in the number of SWS episodes compared to SCs (SEE vs SC; $F = 4.3$, $df = 1/228$, $p < .03$; REE vs SC; $F = 5.5$, $df = 1/214$, $p < .01$; IE vs SC; $F = 7.2$, $df = 1/166$, $p < .007$). Only SEEs show increased amounts of SWS compared to SCs at this time ($F = 8.2$, $df = 1/228$, $p < .004$). Between-groups values for mean duration of SWS episodes and % SWS/TST are not significantly different.

Measures of PS

Results are summarized in Table VI. Comparisons of values for total amount of PS between differentially reared groups are shown in Figure 7. SC mice spend $8.9 \pm 1.6\%$ of their TST in PS over the 24 hr period; $9.6 \pm 1.4\%$ in the day cycle and $8.2 \pm 1.8\%$ in the night cycle. These findings are also consistent with previously reported data from this laboratory (Fishbein, et al., 1974).

SEE and REE mice exhibit a greater number of PS episodes and longer PS duration compared to SCs (PS episodes; SEE vs SC; $F = 3.5$, $df = 1/438$, $p < .05$, REE vs SC; $F = 14.1$, $df = 1/413$, $p < .0001$; PS duration; SEE vs SC; $F = 15.9$, $df = 1/438$, $p < .0001$) but only REEs exhibit a significant increase in % PS/TST (REE vs SC; $F = 11.3$, $df = 1/413$, $p < .0001$). On the other hand, IE mice demonstrate significantly shorter durations of PS episodes compared to SCs ($F = 6.9$, $df = 1/314$, $p < .009$).

Separate within-groups ANOVAs reveal that REEs and IEs demonstrate significant increases in % PS/TST in the Night cycle compared to the Day cycle (REE; $F = 7.5$, $df = 1/213$, $p < .006$; IE; $F = 11.2$, $df = 1/114$, $p < .001$) whereas SEE and SC rearing induces a uniform distribution of % PS/TST over the 24 hr cycle (SEE; $F = 3.0$, $df = 1/238$, $p < .08$, SC; $F = 1.3$, $df = 1/212$).

Day Cycle

SEEs show significant ihcreases compared to SCs in total amount of PS ($F = 5.4$, $df = 1/222$, $p < .02$) and in PS duration ($F = 5.4$, $df = 1/222$, $p < .02$) whereas REEs only exhibit increased duration of PS episodes (REE vs SC; $F = 5.3$, $df = 1/211$, $p < .02$).

There are no significant differences between either of the enriched groups and SCs on % PS/TST..

IEs are significantly impaired on all PS measures relative to SCs at this time, (PS episodes; $F = 6.6$, $df = 1/160$, $p < .01$; total amount PS; $F = 13.4$, $df = 1/160$, $p < .0003$; PS duration; $F = 19.0$, $df = 1/160$, $p < .0001$; % PS/TST; $F = 14.2$, $df = 1/160$, $p < .0002$).

Night Cycle

REEs show significant increases on all PS measures compared to SCs (number of PS episodes; $F = 15.6$, $df = 1/214$, $p < .0001$; total PS; $F = 24.5$, $df = 1/214$, $p < .001$; PS duration; $F = 22.6$, $df = 1/214$, $p < .0001$;

% PS/TST; $F = 13.1$, $df = 1/214$, $p < .0004$).

SEEs exhibit an augmentation in total PS time (SEE vs SC; $F = 6.8$, $df = 1/228$, $p < .009$).

There are no significant differences between IEs and SCs on any measure of PS during the Night cycle.

Comparisons between SEE and REE Environments

No significant differences exist between SEE and REE mice on either measures of SWS or PS during the Day cycle. In the Night cycle, however, SEEs demonstrate a selective increase on measures of SWS (SWS duration; $F = 5.7$, $df = 1/228$, $p < .01$; % SWS/TST; $F = 5.0$, $df = 1/228$, $p < .02$) whereas REEs show significant increases on measures of PS (PS amount; $F = 5.1$, $df = 1/228$, $p < .02$; % PS/TST; $F = 12.7$, $df = 1/228$, $p < .0004$).

Discussion

The results of Experiment 6 clearly demonstrate that prolonged rearing in qualitatively distinct environments alters the amount of sleep and its circadian rhythmicity. Mice reared in either enriched or impoverished environments exhibit diurnal and nocturnal SWS augmentation but only SEE and REE mice show selective increases on all measures of PS throughout the 24 hr cycle. IE mice exhibit reductions on all PS measures; this effect is highly significant during the Day cycle.

The absence of a significant % PS/TST effect in SEEs

relative to SCs during the Night cycle is due to a concomitant increase in SWS in the SEE group. In addition, SEEs demonstrate a selective increase on measures of SWS compared to REEs during the Night cycle but REEs show an increase on measures of PS compared to SEEs at this time. That this REE induced PS augmentation is not due to the corresponding SEE increase in SWS is evidenced by the finding that both enriched groups show a significant increase in PS compared to SCs in this Night cycle. The selective SWS increase in SEEs may result from the environments' effects on locomotor activity. For example, SEE mice have been observed to be extremely active for several hours following reinsertion into the environment following its change; this group is significantly more active than IEs in the first minute of open-field exposure (Experiment 7a). Hobson (1968) has demonstrated a correlation between increased locomotor activity and augmented SWS time.

PS augmentation after enriched rearing persists for two weeks; this finding confirms and extends previously reported data demonstrating elevated PS levels after aversively motivated training lasting from hours (Fishbein, Kastaniotis, and Chattman, 1974; Leconte, Hennevin, and Bloch, 1974; Lucero, 1970) to days (Experiment 4; Kiyono and Seo, 1978). In addition, enriched rearing in the present study not only increases PS episodic frequency but also its duration, suggesting that the brain possesses considerable

functional plasticity to process the intensive and continuously novel stimuli. Thus, the recent demonstration of an initially delayed but then protracted increase in PS time after light cycle avoidance training (Fishbein, et al., 1974), may have resulted from the moderate intensity and duration of the conditioning stimuli rather than, as these investigators suggested to the existence of a "biological ceiling" effect in PS occurrence.

My results suggest the possibility that enriched and impoverished rearing may alter a neurophysiological sleep mechanism which controls (i) sleep episodic frequency and (ii) sleep duration. First, Hobson and McCarley (1975) have shown that triggering of PS is characterized by an increase in firing rates of cells in the Gigantocellular Tegmental Field (FTG) in the pontine tegmentum that results from a release from inhibition derived from the locus coeruleus (LC) and dorsal raphe cells; inhibitory cells which turn off before and during PS. Injection of carbachol, a cholinergic agonist into the FTG area produces phasic and tonic phenomena which are characteristic of the PS state.

Second, there is now considerable evidence to indicate that oscillation of chronobiological rhythms is influenced by activity of the suprachiasmatic nucleus (SCN) in the hypothalamus. Light-dark environmental stimuli reaches the CNS via a direct retinohypothalamic pathway having its terminus in the SCN. Investigators have shown that SCN

lesions abolishes the circadian rhythmicity of adrenal corticosterone (Moore and Eichler, 1972) pineal serotonin (5-HT) N-acetyltransferase (Moore and Klein, 1974) locomotor activity and drinking behavior (Stephen and Zucker, 1972), heart rate (Saleh and Winget, 1977), and the sleep-wakefulness cycle (Ibuka and Kawamura, 1975). For example, Ibuka and Kawamura (1975) reported that bilateral lesions of the SCN resulted in complete abolition of sleep circadian rhythmicity which persisted for 63 days. Total sleep (SWS + PS) was not significantly decreased. Other studies have shown that SCN neurons contain high 5-HT content and that SCN neuronal firing rates are significantly increased after iontophoretic application of acetyclcholine (ACh) and decreased after either 5-HT, dopamine, or norepinepherine. Neuronal excitation by ACh mimics the excitation produced by optic nerve stimulation or light acting on the eye which increases the activity of 70% of SCN neurons (Nishino, Koizumi, and Brooks, 1976). Further, Zatz and Brownstein (1979) recently reported that intraventricular injections of carbachol near the SCN mimicked the ability of light to reduce nocturnal pineal 5-HT-N-acetyltransferase activity as well as phase shifting this enzyme's circadian rhythm. Finally, Swanson and Cowan (1975) have demonstrated the existence of neuroanatomical connections between the SCN and the pontine tegmentum.

The prominent involvement of the cholinergic system

in all of these findings fit well with recent data demonstrating extensive neurochemical alterations in cortical cholinergic systems following environmentally enriched and impoverished rearing (Rosenzweig and Bennett, 1976). Other studies have demonstrated a correlation between elevated neocortical ACh levels with increased PS (Celesia and Jasper, 1966; Gadea-Ciria, Stadler, Lloyd, and Bartholini, 1973; Jasper and Tessier, 1970) and PS deprivation with a reduction of brain ACh levels (Bowers, Hartmann, and Freedman, 1966). The importance of brainstem cholinergic systems in PS has also been demonstrated by Hobson and McCarley as described above and by Sitaram, et al. (1976), who have reported a significant reduction in latency to onset of the first PS period following physostigmine infusion during SWS in normal volunteers.

The results of Experiment 6 suggest the possibility that differential rearing may affect sleep episodic frequency and duration by influencing the activity of the SCN and the FTG/LC complex. The demonstration of PS augmentation following environmental enrichment and PS reduction after impoverished rearing provides additional support to my contention that PS is a requisite neurobiological process for the maintenance and stability of long-term memory (Fishbein and Gutwein, 1977; Experiments 1-5 this study; Shiromani, Gutwein, and Fishbein, 1979).

CHAPTER V

EFFECTS OF ENRICHED AND IMPOVERISHED ENVIRONMENTAL REARING ON OPEN-FIELD ACTIVITY AND BODY WEIGHT

Experiments 7a and 7b are preliminary investigations into the behavioral and physiological effects of differential rearing upon open-field activity and body weight.

Experiment 7a

Measurement of Open-Field Activity

Apparatus

The open-field is a rectangular wooden box measuring 45.8 x 18.4 x 22.5 cm. A disposable cardboard floor is divided into quadrants measuring 11.0 x 9.5 cm which allows for precise measurement of ambulation. A mirror attached to one side of the apparatus enables the experimenter to observe the animal without being seen.

Procedure

After 30 days of differential rearing, animals are removed from their respective environments, transported to the open-field apparatus in a holding cage, and placed against a retaining wall of the open-field. Locomotor activity, defined as animal crossing into a quadrant with all 4 paws is automatically scored for 5 consecutive minutes at 1

minute intervals. Testing is carried out between 0900 and 1430 hr. SEE (N = 36), REE (N = 37), SC (N = 36), IE (N = 30).

Results

Results are shown in Figure 8. A fixed effects repeated measures ANOVA (Winer, 1971) was used to analyze these data with environment the between-subjects factor and time the within-subjects factor. The analysis shows a significant difference in locomotor activity as a function of differential rearing ($F = 3.9$, $df = 3/528$, $p < .01$), no significant effect of time ($F = 1.02$, $df = 4/528$), and a significant environment X time interaction ($F = 5.2$, $df = 12/528$, $p < .001$).

Pairwise comparisons between environments shows the SC group to be significantly more active than REE animals ($F = 8.4$, $df = 1/132$, $p < .001$) and IE mice ($F = 7.9$, $df = 1/132$, $p < .01$), but IEs are not significantly different from REEs ($F = .004$, $df = 1/132$). There is a small but non-significant difference between the SC and SEE groups ($F = 2.9$, $df = 1/132$, $p < .09$). Trend analysis performed on these data indicate a significant linear component for SEE mice (SEE lin; $F = 10.5$, $df = 1/190$, $p < .001$) and a small but non-significant linear component for REEs (REE lin; $F = 3.2$, $df = 1/180$, $p < .07$). SEE mice habituate to the open-field with increased exposure (Duncan Range Test; min1 vs min5, $p < .01$). SCs show a significant quadratic

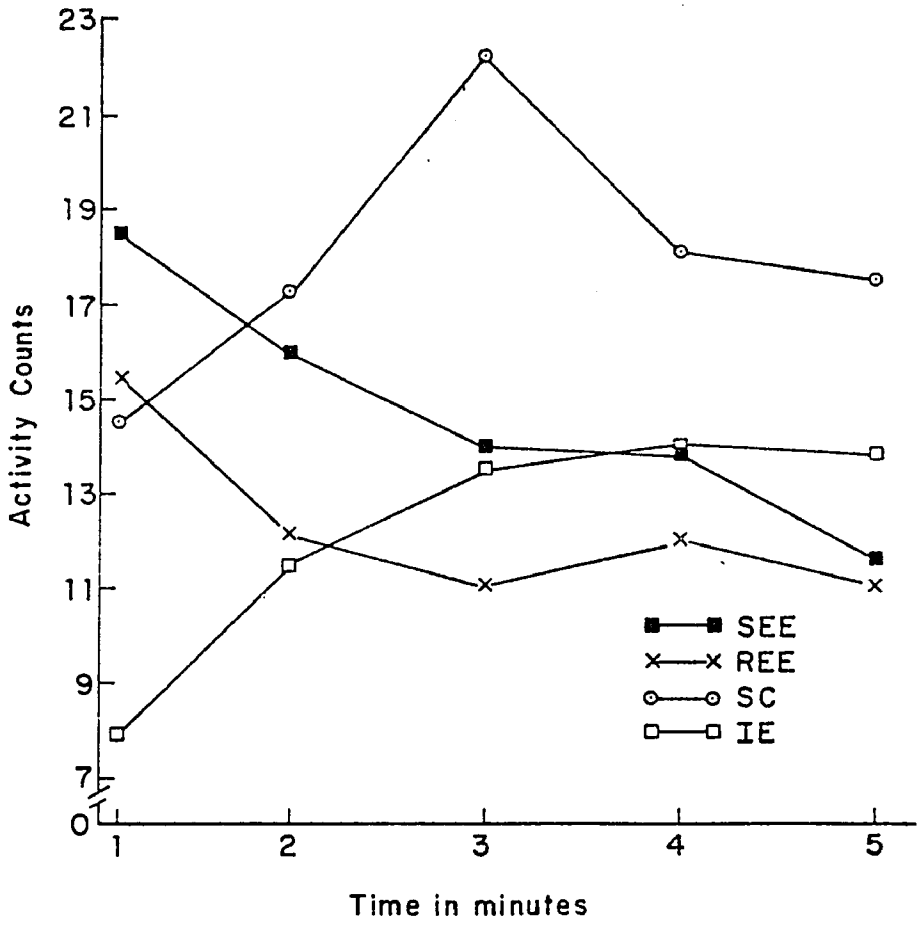
23
21
19
17
15
13
11
9
7
0
Activity Counts
1
2
3
4
5
Time in minutes
SEE
REE
SC
IE

Figure 8

Open-field activity in Environmentally enriched (SEE and REE), Social Control (SC), and Impoverished (IE) reared mice. Animals are differentially reared for 30 days, then observed in the open-field with locomotor activity scored for 5 consecutive min at 1 min intervals. Note significantly higher activity levels of SEE and REE mice compared to IEs in the first minute. SEE (N = 36), REE (N = 37), SC (N = 36), IE (N = 30).

component (SC quad; $F = 4.1$, $df = 1/145$, $p < .04$) with the greatest activity occurring at the third minute. Of particular interest is the finding that SEE and REE activity levels in the first minute are significantly higher compared to IEs (Duncan Range Test; $p < .01$) but are not significantly different from each other or from the SC group.

Discussion

These results are in line with previously reported data showing that enriched rats are more active than SCs and IEs upon initial exposure to a novel environment which is then followed by habituation. SCs and IEs show an initially low level of activity followed by increased activity with continued exposure to the open-field (Ehrlich, 1959; 1961; Forgays and Reid, 1962; Woods, et al., 1961; Stewart, 1961; Denenberg and Morton, 1962a, b; Lore and Levowitz, 1966; Zimbardo and Montgomery, 1957; Rajalakshmi and Jeeves, 1968; Einon, Morgan, and Sahakian, 1975; Beatty and Fessler, 1976). Of interest in the present experiment is the finding of a difference in reactivity to the open-field between SEE and REE mice. Both groups show high levels of activity on min 1 compared to SC and IE groups but SEEs exhibit a significant rate of habituation whereas REEs do not. Walsh and Cummins (1976) have suggested that enriched rearing may increase arousal responses to novel stimuli; perhaps the arousal inducing properties of the more complex SEE environment are greater than that of the REE environment. If so,

this would explain the more rapid habituation to the bare open-field environment in SEEs compared to the REE group. Additional research is required to clarify this issue.

Experiment 7b

Measurement of Body Weight

Method

Mice were weaned at 21 ± 2 days and placed into one of the four environments at random. Initial body weights between groups was comparable. All animals were weighed after 30 days of differential rearing.

Results

Results are shown in Table VII. A one-way ANOVA performed on these data reveals a significant difference in body weight between groups ($F = 3.9$, $df = 1/132$, $p < .01$). A Duncan Range Test for pairwise comparisons shows that SEEs weigh significantly less than REE, SC, and IE groups ($p < .05$), none of which differ from each other.

Discussion

Data on the effects of differential rearing on body weight have heretofore been exclusively reported for the rat (Geller, Yuwiler, and Zolman, 1965; Greenough, Yuwiler, and Dollinger, 1973; Rosenzweig, Bennett, and Diamond, 1972a). These investigators have consistently reported that IE rats weigh significantly more compared to rats reared in enriched environments. In a recent study, Fiala, Snow, and Greenough

TABLE VII

BODY WEIGHTS OF ENVIRONMENTALLY ENRICHED (SEE AND REE), SOCIAL CONTROL (SC), AND IMPOVERISHED (IE) FOLLOWING 30 DAYS OF DIFFERENTIAL REARING

ENVIRONMENT	N	MEAN BODY WEIGHT (gm) ± S.E.M.
SEE	38	22.8 ± 0.5[a]
REE	37	24.2 ± 0.4
SC	30	25.0 ± 0.5
IE	30	24.5 ± 0.5

[a] $p < .05$ (Duncan Range Test) vs REE, SC, and IE groups.

(1977) measured food and water consumption and weight gain in Long-Evans hooded rats reared in either enriched or impoverished environments for a period of 30 days. They found that IEs (individually housed in standard laboratory cages) consumed significantly more food and water and weighed significantly more than enriched littermates. These investigators suggest that increased IE body weight results from restricted activity. Supporting evidence is provided by Mayer (1968) who reported that food intake is greater in rats at extremely low levels of activity than at moderate levels. Furthermore, body weights of these differentially reared Long-Evans hooded rats have approached each other when they were shifted to the corresponding environment (Dr. W. T. Greenough, Personal Communication). Data from the present experiment demonstrating that SEE mice weigh significantly less than IE's cannot be ascribed to a greater level of activity since analysis of open-field behavior (Experiment 7a) shows no overall significant activity differences between SEE and IE groups. Instead, I propose that the low SEE body weight may result from the environments' induction of an increased metabolic rate. We have frequently observed SEEs to be extremely active immediately following re-insertion into the environment. During the latter stages of SEE rearing, when environmental complexity is at its maximum, these animals regularly take between 3-5 hr to find the new location of food and water upon re-

insertion into the environment. Once food and water have been found, the majority of SEE mice do not feed but immediately retrace the environment and continue with their exploratory behavior. This partial food and water deprivation regimen coupled with high activity levels may induce a permanent increase in metabolic rate; a notion supported by our additional observation that SEEs housed individually during the retention interval (Experiment 5) with ad libitum food and water do not increase their weight to SC levels. The present data suggest the necessity for additional experiments to determine the physiological mechanisms that may subserve metabolic alterations induced by SEE rearing.

CHAPTER VI

GENERAL DISCUSSION AND CONCLUSIONS

The results of this study provide considerable support for the hypothesis that PS is essential for the maintenance, stability, and storage of long-term memory (cf. review by Fishbein and Gutwein, 1977).

In Chapter I, the effects of the protein synthesis inhibitor, ANI, on PS and 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 of PS for 9 consecutive hr and ANI 40 mg/kg treated animals for 6 consecutive hr with no PS rebound in all three groups. ANI increased SWS commencing 3 hr post-injection, continuing for 9 consecutive hr, and then returning to SAL control levels. This effect was not dose-dependent. In experiment 2, part a, ANI 120 mg/kg and 210 mg/kg but not 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 only in mice given immediate post-

training injections of ANI 40 mg/kg. ANI 20 mg/kg or ANI 10 mg/kg alone or in combination with immediate ECS was ineffective in extending the lability of the memory trace. These data clearly indicate that PS in the 3 hr period after aversively motivated training is not essential for long-term memory processing and thus does not support the hypothesis of Bloch; Pearlman and Greenberg. Four specific results of these experiments merit review:

First, ANI's specific effects on sleep 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 post-injection, then persists for 9 consecutive hr, and is not dose-dependent. The neurochemical mode of action for ANI's dissociable effects are presently unknown, but this finding merits further investigation.

Second, the finding of ANI-induced SWS augmentation with concomitant amnesia does not support the results of Fowler, et al. (1973), who reported induction of amnesia following SWS but not PS deprivation.

Third, ANI 40, 120, and 210 mg/kg decreases PS duration in addition to PS frequency with no PS rebound.

Fourth, prolonging the duration of protein synthesis inhibition by subamnesic doses of ANI extends the period of memory trace susceptibility to disruption by ECS and shows that PS plays an integral role in this stability and

maintenance of LTM.

Data from Experiment 4 and 5 in Chapter III showed that 30 days of environmental enrichment resulted in a significant and selective increase in PS as well as enhanced recall of a multiple trial, shock, brightness discrimination Y maze 28 days after training. Conversely, impoverished reared mice exhibited a decrease in PS and impaired task performance relative to controls. Of particular interest here is the finding that enriched rearings' elevation of PS persists for at least two weeks; this confirms and extends previously reported data demonstrating elevated PS levels after aversively motivated training lasting from hours (Fishbein, et al., 1974) to days (Experiment 4). In addition, enriched rearing not only increases PS episodic frequency but also PS duration (opposite to ANI's effect) suggesting that the brain possesses considerable functional plasticity to process the intensive and continuously novel stimuli provided by environmental enrichment.

In Experiment 6, the effects of enriched and impoverished environmental rearing on sleep circadian rhythmicity was examined. Enriched mice showed a general increase on measures of SWS in the 24 hr cycle but were comparable to controls during the Day cycle. On the other hand, enriched rearing produced a significant and selective augmentation of PS throughout the 24 hr cycle. Impoverished

reared mice showed a general increase in SWS during the Day cycle but with significant reductions of PS at this time. It was suggested that alterations of chronobiological sleep rhythms after differential rearing may be mediated by the interaction of the Suprachiasmatic Nucleus in the hypothalamus and the Gigantocellular Tegmental Field and Locus Coeruleus in the pontine tegmentum.

The results of the present experiments are of considerable significance with respect to research in the areas of (1) the electrophysiology and pharmacology of human information processing, (2) the effects of aging on sleep, learning, and memory, (3) the relationship between sleep circadian rhythmicity, affective disorders, and memory storage processes, and (4) child and human development.

It is generally agreed that one of the difficulties encountered in studying the basic mechanisms involved in memory storage lies in the fact that we have not yet determined the relationship between the early phases of memory storage and subsequent memory retrieval. A good share of evidence (including the results of Experiments 1-3 in this report) suggests that there exists a short-term storage which initiates long-term storage. This is similar to Weiskrantz's view (1966) that the LTM trace requires some degree of "priming" before it can survive autonomously. Gold and McGaugh (1975), for example, have posited the existence of a single-trace two process view of memory storage.

Their theory is essentially based on the observation that the memory trace may still be activated after interrupting the consolidation phase. Rusinov (1962) and Albert (1966) have shown that cathodal polarization of the cortical surface can be used to interrupt consolidation, and Albert has claimed to be able to reactivate the trace by injecting strychnine, a finding that can be explained by the failure of retrieval hypothesis, i.e., the memory trace is viable but, due to a variety of circumstances, is temporarily unrecoverable.

It appears, therefore, that understanding the mechanism of memory storage is not only a matter of elucidating the fixation process but also a matter of understanding the mechanisms of long-term maintenance of memory storage and of retrieval. Viewed in this way, newly registered information is constantly processed (during the consolidation phase) and perhaps compared with information already in storage. This view fits well with the electrophysiological studies of John (1972) who has found changes in waveforms of evoked potentials when there is a discrepancy between newly presented information and information already stored.

These studies of memory suggest that consolidation exists on a quite prolonged time scale. Moruzzi (1966) proposed that one of the functions of sleep is to permit the passive recovery of synapses involved in learning. It is now generally well accepted that sleep is, of course,

essential for learning and information processing, but the results of this series of experiments clearly indicate that sleep, and in particular, PS, is actively rather than passively involved in memory storage processes.

PS deprivation administered prior to learning may impair subsequent consolidation by interfering with processes involved in the conversion of an unstable STM trace into stable LTM storage. Long-term PS deprivation following learning may disrupt the active maintenance of an established memory already in long-term storage. The retention deficits observed in both cases can result from the same process; alteration of brain neurotransmitter activity, e.g., protein synthesis inhibition and cholinergic neurotransmission. In the first instance, when PS deprivation is administered prior to training, storage into or retrieval from LTM may be impaired because the necessary brain levels of neurotransmitters have been altered. In the second instance, when long-term PS deprivation follows learning, sufficient amounts of brain neurotransmitters are present during the learning experience but are likely to be altered during subsequent memory processing. This manipulation places the memory trace into an unstable (non-retrievable or residual) condition thereby increasing its susceptibility to disruption by amnestic agents long after the learning experience occurs (evidenced by the results of Experiment 3 in this study and other recent reports) Andry

and Luttges, 1972; Andry and Luttges, 1973; Flood et al., 1978; Quinton, 1978). Thus, amnesia may occur as a function of one of two processes, or both. In the case of PS deprivation prior to learning, amnesia may be a case of incomplete conversion; if interference with conversion is total no retrieval occurs. On the other hand, if conversion is incomplete, a residual template may hold the trace for subsequent consolidation by way of the naturally occurring PS mechanism. In the case of prolonged PS deprivation following learning, amnesia may be a function of either the memory trace being held in an unstable state or perhaps an interference with a retrieval process. In other words, when amnesia is only temporary due to PS deprivation, normal retrieval may be restored when normal levels of PS are restored. In this case, the memory template is not disrupted.

Data from Experiments 1-5 in this study suggest a relationship between PS, protein synthesis, cholinergic neurotransmission, and memory processing. There is now good evidence to indicate that PS deprivation induced either by drug or impoverished rearing; and PS augmentation induced either by formal training or enriched rearing affects a broad range of neurotransmitter activity in the central nervous system; an effect that is of particular significance with respect to the modulation of memory storage processes.

In order to more clearly delineate the relationship

between information processing and sleep, future research must focus on the effects of waking experiences upon specific brain structures and on the effects of drugs on PS and memory. For example, it will be necessary to provide correlative EEG data (including dose-response curves) indicating whether administration of modulating agents produces alterations similar to those seen in animals administered PS deprivation via the water-tank technique. Data of this sort will considerably aid in further elucidating the interactions between PS and neurotransmitter systems and their effects on memory.

The results of Experiments 4 and 5 which demonstrated environments' considerable influence on PS, learning, and memory, is of particular relevance and benefit to the study of aging on sleep and information processing. For example, several investigators have shown that human sleep patterns undergo significant alterations with advancing age. Sleep becomes more fragmented and awakenings during the night are longer and more frequent (Feinberg, 1976). There is also a significant reduction in PS (Feinberg, 1976; Marsh, 1977; Prinz, 1977). Further, the nightly amount of PS correlates positively with performance scores on the Wechsler Adult Intelligence Scale (WAIS) in both normal aged subjects and in aged subjects with evidence of organic brain development (Feinberg, et al., 1967; Marsh, 1977; Prinz, 1977). For this reason, PS decrements have been

considered by some investigators to reflect normal age-related as well as pathological changes in the functional integrity of the brain. In addition clinical descriptions of the intellectual impairment found in senescent humans frequently identify memory dysfunction as the central behavioral characteristic. Memory disorders can range in severity from malignant amnesia, i.e., Alzheimer's disease and Korsakoffs syndrome to a benign form of forgetfulness, the major symptom of which is the inability to recall certain pertinent events from a total experience which is otherwise accurately recalled (Kral, 1962; Rozin, 1975).

There is now an expanding amount of evidence which indicates the existence of selective aberrations in cholinergic and catecholaminergic metabolism in senescent animals and humans (Finch and Hayflick, 1977), e.g., decreased levels of AChE, decreased synthetic capacity of catecholamines as evidenced by reduced incorporation of tyrosine into norepinepherine and dopamine, and reduced activity of tyrosine hydroxylase in specific brain regions. There is also a suggestion of reduced uptake of catecholamines (Timiras and Bignami, 1976; Sun, 1976) and an increased sensitivity (perhaps at the receptor level) to exogenous catecholamines. These changes in cholinergic and catecholaminergic systems may have profound electrophysiological and behavioral effects in aging populations. For example, Davis and Yamamura (1978) recently reported the existence

of AChE neuronal depletion and cognitive impairment in aged patients suffering from senile dementia. Finally, a recent review by Birrin and Schaie (1976) suggests that memory dysfunction in aging populations may partially result from "impoverished" environments brought on by the loss of a spouse and/or institutionalization. Data from Experiments 4 and 5 showing PS augmentation and enhanced recall in environmentally enriched animals suggests the design of basic studies to examine the capability of environmental therapeutic regimens to ameliorate the deleterious consequences of memory and sleep dysfunction in aged populations.

Data from Experiment 6 is relevant on two counts: First, in a recent review of the literature on the regulation of both physiological and behavioral processes by neurobiological clocks, Binkley (1979) concluded that time-keeping mechanisms in the brain may either reset, increase or decrease the rate of circadian rhythmicity solely via time cues or light-dark cycles. Experiment 6, however, provides the first clear evidence that circadian rhythmicity of an integral physiological process; sleep, can be considerably altered by environmental rearing.

Second, there is now a good deal of evidence to suggest that alterations of circadian rhythms correlates with affective disorders. One example: Kripke, Mullaney, Atkinson, and Wolf (1978) recently tested the hypothesis that a desynchronization of circadian rhythms may underlie

manic-depressive disorders. In particular, they considered the possibility that manic-depressive cycles may represent a "beat phenomenon" between some body systems exhibiting a free-running circadian rhythm that is different from 24 hr and other body functions that remain synchronized to the 24 hr environmental clock. Volunteering manic-depressive patients measured their own body functions for prolonged time periods. Measures included: (1) mood self-rating on a 5 point scale, (2) oral temperature measurement, (3) eye-hand coordination task, (4) wrist-pulse counting, (5) blood pressure, (6) gross activity measurement, and (7) determination of volume, Na concentration, K concentration, and concentration of free hydrocortisone from urine collections. The results showed a dramatic progressive phase advancing of the peak of oral temperature, pulse rate, and blood pressure indicating the presence of free-running circadian rhythms with periods shorter than 24 hr (21.8-24 hr). The mood rating was lowest when the temperature peak occurred between midnight and 6 a.m. and highest when the peak occurred between 6 p.m. and midnight. These results are consistent with the authors' hypothesis that some circadian rhythms free-run faster than 1 cycle per day in manic-depressive patients, and that the ensuing internal desynchronization may be related to mania and depression. Indeed, it has often been noted that experimentally-induced phase shifts or isolation enhances psychosomatic symptoms,

dysphoric mood changes, irritability, and depression (Aschoff, 1965). These observations indicate that specific abnormal phase relations of circadian rhythms may induce depressive symptoms even in normal subjects. In addition, a series of recent reports has demonstrated altered periods of circadian rhythms in schizophrenics (Mills, Morgan, Minors, and Waterhouse, 1977), the blind (Miles, Raynal, and Wilson, 1977), and in patients with neurotic and psychosomatic complaints (Lund, 1974). Further, Kupfer and colleagues (1977) have shown that sleep, and in particular PS, is a valid predictor of various psychopathologic states. For example, common features of the EEG sleep of depressed psychotic patients are decreased PS time, decreased PS activity, and decreased PS latency to the first PS episode. Although the etiology of several psychoactive complaints have been ascribed to biochemical dysfunction this may be a consequence rather than a cause of the disorder. The results of Experiment 6 in the present study and the experiments reviewed here suggest that environment plays an important role in sleep circadian rhythmicity. Manipulation of environmental factors may be of therapeutic value in the treatment of affective disorders by way of altering the PS mechanism.

Basic studies on the effects of enriched and impoverished rearing on locomotor activity and body weight are presently scarce and therefore Experiments 7a and 7b have

served as preliminary investigations into the effects of differential rearing on these behavioral and physiological measures. Results indicate that the environment exerts significant effects on both open-field activity and body weight. These data may be pertinent to investigations on the influence of severe malnutrition in infancy on the intellectual functioning of school-age children. For example, studies of malnutrition in humans now show that subjects severely malnourished in infancy later perform less well on tests of learning and intelligence (cf. review by Richardson, 1976) and show significantly decreased levels of PS (Drucker-Colin, et al., 1976) compared to siblings not subjected to the severe malnutrition. Investigators in this area have generally agreed that early life malnutrition leads to mental retardation and cognitive impairment (cf. review by Manocha, 1972). On the other hand, Richardson (1976) has questioned whether malnutrition in infancy should not be more accurately viewed as a barometer of a more generally disadvantageous set of environmental conditions, which collectively, are sufficient to account for later observed differences in cognitive abilities between experimental and "control" subjects without invoking the variable of malnutrition. Formulated in this manner, the issue focuses on the qualitative effects of environment as the primary influence on subsequent cognitive development rather than severe malnutrition, which may be a consequence

and/or an adjunct of impoverished rearing. Support for this contention comes from a series of studies reported by Richardson (1976) who demonstrated a significant interaction between malnutrition and the enriching aspects of the human environment. Jamaican schoolchildren hospital-treated for severe malnutrition during the first two years of life (e.g., marasmus, kwashiorkor, or marasmus-kwashiorkor) showed impaired intellectual and general school behavior performance in a follow-up study conducted several years later (ages 6-10 years). Richardson examined the degree to which various aspects of home environment contributed to this dysfunction. His striking finding is that the effect of severe malnutrition upon later school performance depended primarily upon the quality of the home environment. If the environment was enriched, the effects of the malnutrition episode were not observed in tests of cognitive ability and general behavior. Richardson's results provide good support for enriched environmental rearing serving as one therapeutic model for ameliorating the effects of nutritional deprivation and suggests that the concept of severe malnutrition in infancy alone causing CNS damage and subsequent mental retardation is simplistic. On the other hand, the data from experiments 7a and 7b of this study corroborate Richardson's findings by providing a functional profile of environments' effects on motor activity and body weight. The potent effect of environment on behavior in these

experiments is highlighted by my observation that during the latter stages of SEE rearing, when environmental complexity is at its maximum, these animals regularly take between 3-5 hours to find the new location of food and water upon re-insertion into the environment. Once food and water have been found, the majority of SEE mice do not feed but immediately retrace the environment and continue with their exploratory behavior. In view of the preliminary scope of these experiments, additional studies on the basic effects of differential rearing on a variety of performance measures is required. These investigations will enable us to fully increase our understanding of environments' influence on behavior.

In sum, the data I report in this study provides considerable support for the hypothesis that PS occurring over a prolonged time period is a requisite neurobiological mechanism for the processing, maintenance, and storage of long-term memory. Clearly, the continued investigation of the function of PS in information processing and of the relationship between PS, specific brain structures, brain neurotransmitter activity, and the environment will continue to provide valuable information about the characteristics of memory storage processes.

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