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L-Tryptophan, REM sleep, and the behavioral changes occurring from REM sleep deprivation

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L-TRYPTOPHAN, REM SLEEP, AND THE BEHAVIORAL CHANGES OCCURRING FROM REM SLEEP DEPRIVATION

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Abstract

Behavioral changes occurring following REM Sleep Deprivation (RSD) are well documented in the rat and include an increase in open-field activity and a decrease in thresholds to electrical stimulation. The daily administration of L-tryptophan was intended to stabilize the serotonin system and thus prevent the alteration in behavior normally induced solely by RSD. Sixty male Sprague-Dawley rats were randomly assigned to the sleep groups RSD, Wet Control, Dry Control and among triads randomly assigned to either the experimental drug or a placebo condition. Each triad was given pre- and posttreatment tests for open-field activity and thresholds to electrical stimulation Results indicated a RSD by stress confound and thus could be viewed as a complication in discussing the results of this study. Although there appeared to be no alteration in open-field behavior resulting from the different sleep conditions, the electrical threshold data suggest that tryptophan may have attenuated the effects of RSD.

L-Tryptophan, REM Sleep, and the Behavioral Changes Occurring from REM Sleep Deprivation

The behavioral effects of REM Sleep Deprivation (RSD) are well documented in the rat and include, for example, an increase in aggression (Hicks, Moore, Hayes, Phillips & Hawkins, 1979; Kuroda, Gonzales, Gomez, Reyes, & Hicks, 1982; Moore, McRainey & Hicks, 1981); sexual behavior (Canchola, Monroy & Velazquez-Moctezuma, 1986); locomotion (Van Hulzen & Coenen, 1981); open-field activity (Hicks & Moore, 1979); reductions of thresholds to electrical stimulation (Hicks, Moore, Findley, Hirshfield & Humphrey, 1978); the disruption of avoidance learning (Harris, Overstreet & Orbach, 1982); and a decline in the immobility response as well as an increased bolus count during repeated exposure to 5-minute swimming tests (Hawkins, et al., 1980).

Physiological changes that result from REM Sleep Deprivation include, among others, increased basal metabolic rate (Puentes, Bautista, Mistry, Shaw & Hicks, 1989) and a decrement in the P3-N3 amplitude of photically evoked potentials in the visual cortex (Van Hulzen & Coenen, 1984). Complete and prolonged RSD in the rat leads to severe ulcerative and hyperkeratotic skin lesions on the animal's tails and paws as well as increased food intake, energy expenditure, heart rate, protein metabolism, and plasma norepinephrine (Kushida, Bergmann, & Rechtschaffen, 1989). Prolonged RSD has also been associated with decreases in body weight, and surprisingly, decreased body temperature and plasma

thyroxine (Kushida, et al., 1986; Rechtschaffen, Bergmann, Everson, Kushida, & Gilliland, 1989).

The pharmacological changes that result from RSD include increased serotonin (5-HT) synthesis (Hery, Pujol, Lopez, Macon & Glowinski, 1970); norepinephrine turnover (Pujol, Mouret, Jouvet & Glowinski, 1968; Schildkraut & Hartmann, 1972); reductions in beta-adrenoreceptor number and sensitivity (Mogilnicka, Przewlocka, Van Luijtelaar, Klimek & Coenen, 1986); and an increase in dopamine activity (Farber, Miller, Crawford & McMillen, 1983).

To further extend knowledge of the effects of RSD, recent experiments have used designs in which a pharmacological agent was introduced following the deprivation procedure in order to examine RSD-induced alterations in specific systems. The resulting changes in behavior could then be distinguished, and the RSD-induced effects on neural physiology inferred. In their review of the literature, Vogel, Minter and Woolwine (1986) noted that several experiments have investigated the acute effects of a single dose of a particular agent following deprivation, and that collectively, these studies have established a specific methodology for the study of RSD. For example, Velazquez-Moctezuma, Monroy, Beyer and Canchola (1984) investigated the possible effects of RSD on the steroid hormones. The acute administration of the gonadal steroids estradiol benzoate and progesterone following RSD increased the lordosis response in ovarectomized rats when compared to the controls. Likewise, Trotta (1984) investigated the effect of RSD on the dopaminergic system

and noted that the acute administration of the dopamine agonist apomorphine following RSD increased aggression when compared to normal and control animals. This suggested to Trotta that RSD may produce an increase in receptor supersensitivity. Using a similar design, Santos and Carlini (1983) first noted that the development of receptor supersensitivity by manipulating the dynamics of a given neurotransmitter is not restricted to the dopaminergic system; they then investigated the effects of RSD on serotonin receptor sensitivity as measured by the serotonin-syndrome (i.e., behavioral patterns consisting of lateral head weaving, head tremor, forepaw padding, and hindlimb abduction). Animals deprived of REM sleep and then challenged with the serotonin precursors L-tryptophan or L-5-hydroxytryptophan demonstrated a larger incidence of the serotonin-syndrome when compared to the control animals. In addition, both Sallanon, Janin, Buda and Jouvet (1983), and Tobler and Borbely (1982) investigated the effects of RSD and the serotonin synthesis inhibitor p-chlorophenylalanine (PCPA) on subsequent rebound of REM sleep using similar methodology to that described earlier. That is, the acute administration of a pharmacological agent was introduced following deprivation, and subsequent changes in behavior were investigated. These experiments were designed to assess the relationship between RSD and 5-HT in REM-rebound. Results indicated that the administration of PCPA following RSD did not impair the subsequent rebound of REM sleep. Finally, Harris, et al. (1982) sought to clarify the relationship between the cholinergic

and catecholamine systems in their ability to reverse the memory disruption produced by the flower-pot technique. They reported that the acute administration of imipramine could reverse the RSD-induced memory disruption in rats maintained on the large platform, while the acute administration of both imipramine and physostigmine was required to reverse the RSD-induced memory disruption exhibited by the animals maintained on a small platform.

Statement of the problem

The foregoing discussion suggests that RSD may alter normal physiological states and thus it may, on occasion, be possible to bring the physiological system underlying the sleep process back into an equilibrium using a proper pharmacological intervention. However, only a few experiments have been reported which were designed to demonstrate that RSD-induced behavioral changes could be modulated using appropriate pharmacological interventions concomitantly with the deprivation process. In such studies, the predicted consequence of administering such pharmacological agents should be a blocking of the RSD-induced alterations in the target behavior(s). Perhaps a reason for the limited scope of research in this area is due to the fact that the pharmacological mediators of REM sleep remain unclear. It has even been suggested that presently, we know less about these systems than we did 20 years ago (Kandel & Schwartz, 1985).

To explain, initially a serotonergic hypothesis of sleep received strong support from Jouvet (1967) who reported that administration

of PCPA, a serotonin synthesis inhibitor, reduced brain 5-HT levels and REM sleep while subsequent administration of L-tryptophan, a serotonin precursor, raised 5-HT levels and produced a temporary increase in REM sleep. The early enthusiasm for this hypothesis was soon quelled by Dement, Mitler and Henriksen (1972) who observed that REM sleep returned to baseline levels in cats that had been chronically treated with PCPA, in spite of a persistent depletion of 5-HT. These results, along with others, suggested to King (1974) that although 5-HT may be involved in sleep mechanisms, the process is extremely complicated and cannot be explained by examining only one system. The apparent complexity of the pharmacology of sleep has served to limit investigation in this area and, as a result, the specific role of 5-HT in sleep remains unclear.

The purpose of this experiment is to further elucidate the relationship between the neurotransmitter serotonin, REM sleep and the behavioral changes that follow RSD. Although 5-HT may not be the only factor that mediates sleep, it certainly plays an important role (Sallanon et al., 1983). Therefore, L-tryptophan was chosen as a pharmacological agent because it is known to affect the 5-HT system. (For the interested reader, a comprehensive review of the 5-HT system appears in Appendix A, while a review of the 5-HT system across the vigilance states is presented in Appendix B and a review of alterations in the 5-HT system resulting from RSD is presented in Appendix C).

Serotonin and Vigilance

Overall, the literature that is reviewed in Appendix C argues that RSD reduces the availability of 5-HT in the synaptic cleft and thus reduces its activity on both pre- and postsynaptic receptors. This RSD-mediated reduction in availability of 5-HT at pre- and postsynaptic receptor sites would remove vigilance-suppressing functions and thus produce the alterations in behavior commonly reported following RSD. Koella (1988) notes that the inhibition of cell discharge which results when serotonin is applied to single neurons in various areas of the brain (including the motor area, the limbic system, the visual and auditory cortices, and the hippocampus) provides ample evidence to implicate the serotonergic system as a vigilance-controlling apparatus.

To summarize briefly, Koella hypothesizes that selective inhibition of cholinergic, noradrenergic and dopaminergic systems at the appropriate times serves to suppress vigilance. That is, while cholinergic and noradrenergic systems project to higher function vigilance-enhancing networks, the dopaminergic system projects to motor and lower function vigilance-enhancing networks. While the animal is awake, high serotonergic output prevents hyperarousal and may spare those systems not used for a particular behavioral act. Koella suggests that serotonin is therefore capable of producing a proper vigilance profile through integrating serotonin vigilance-supressing functions. Thus, the observed changes in behavior which

are associated with RSD may be due to alterations in the proper vigilance profile.

L-tryptophan

The administration of L-tryptophan during RSD in this study was intended as a means of stabilizing the 5-HT system and thus blocking changes in certain behaviors that have been shown to be sensitive to RSD. The mode of action of L-tryptophan on increased serotonin synthesis allows for an appropriate intervention point for maintaining an equilibrium in the serotonin system across the deprivation process. Gessa and Tagliamonte (1974) note that tryptophan hydroxylase has an unusually high Km for its substrate tryptophan and that changes in neuronal tryptophan are associated with changes in brain 5-HT levels. That is, increasing levels of neuronal tryptophan lead to an increase in brain 5-HT concentration. It should be noted that other neutral amino acids in the diet compete with tryptophan for uptake at the blood brain barrier such that ingested tryptophan in protein may not result in an increase in either neuronal tryptophan levels or brain 5-HT content (Boadle-Biber, 1982; Furnstrom & Wurtman, 1971). However, peripheral administration of L-tryptophan through intraperitoneal injections increases both tryptophan and 5-HT levels in brain areas containing 5-HT cell bodies and nerve terminals. Because 5-HT levels were not measured in this experiment, a summary table which verifies the association between intraperitoneal injections of L-tryptophan and increased 5-HT levels has been included in Table 1.

Table 1

Percent Increase in Brain Tryptophan, 5-HT, and 5-HIAA Following Intraperitoneal Injections of L-tryptophan

Author	Size	Gender	Dose	Brain Tryptophan		Brain 5-HT		Brain 5-HIAA	
Borbely, Neuhaus, & Tobler (1981)[a]	260-338g	male	150mg/kg i.p	60'-	61%	60'-	38%	Not given .	
Bourgoin, Faivre-Bauman, Bende, Glowinski & Hamon (1974)		male	200mg/kg i.p	60'-	869%	60'-	35%	60'-	110%
Curzon, Fernando, & Marsden (1978)	200-250g	male	100mg/kg i.p	30'- 90'- 150'-	500% 340% 66%	30'- 90'- 150'-	20% 32% 16%	30'- 90'- 150'-	53% 82% 52%
Curzon & Marsden (1975)	180-220g	male	50mg/kg i.p	30'- 90'- 150'-	486% 185% 10%	30'- 90'- 150'-	21% 23% 17%	30'- 90'- 150'-	45% 87% 56%
Eccleston, Ashcroft, & Crawford (1965)	160-180g	male	400mg/kg i.p	60'- 120'- 240'-	2700% 2071% 128%	60'- 120'- 240'-	93% 115% 28%	60'- 120'- 240'-	154% 370% 125%
Furnstrom & Wurtman (1971)	150-200g	male	50mg/kg i.p 125mg/kg i.p	60'- 60'-	Not given Not given	60'- 60'-	44% 71%	60'- 60'-	Not given Not given
Grahame-Smith (1971)[b]	180-220g	male	30mg/kg i.p 50mg/kg i.p 100mg/kg i.p 150mg/kg i.p 200mg/kg i.p	60'- 60'- 60'- 60'- 60'-	500% 733% 1233% 1966% 3400%	60'- 60'- 60'- 60'- 60'-	50% 100% 200% 175% 175%	60'- 60'- 60'- 60'- 60'-	Not given Not given Not given Not given Not given
Tagliamonte, Biggio, Vargiu & Gessa (1973)	180-200g	male	50mg/kg i.p	30'- 60'- 120'- 180'-	525% 424% 107% 6%	30'- 60'- 120'- 180'-	18% 17% 10% 0%	30'- 60'- 120'- 180'-	59% 80% 27% 11%
Ternaux, Boireau, Bourgoin, Hamon, Hery, & Glowinski (1976)	250-300g	male	100mg/kg i.p	60'- 120'-	Not given Not given	60'- 120'	Not given 246%	60'- 120'-	Not given Not given
Wojcik, Fomal, & Radulovacki (1980)	400-500g	male	30mg/kg i.p	15'- 45'-	Not given Not given	15'- 45'-	4% 18%	15'- 45'-	38% 46%

Note. [a]Animals were pre-treated with PCPA (300mg/kg i.p.) 24 hours prior to treatment with tryptophan. [b]Animals were pre-treated with the Monoamine oxidase inhibitor tetrabenzine tranylcypromine (20mg/kg i.p.) 30 minutes prior to treatment with various doses of L-tryptophan.

The administration schedule of L-tryptophan was intended to raise neuronal serotonin to levels corresponding with that seen during a normal sleep cycle. Quay (1968) investigated the daily variations of serotonin in various brain regions in the rat, including the frontal cortex, visual cortex, hypothalamus, thalamus, and the lower brain stem. Results indicated circadian rhythmic changes in serotonin, with peak concentrations occurring during the latter part of light phase. Indeed, Hery, Chouvet, Kan, Pujol and Glowinski (1977) reported an increase in 5-HT synthesis in the cerebral cortex during the light period as determined by the accumulation of [^{3}H]5-HT from [^{3}H]5-tryptophan. The circadian variation in brain 5-HT reported by both studies indicate an elevation in 5-HT during the latter two-thirds of the light phase which dropped rapidly during the first two hours of the dark period.

Brain serotonin levels are elevated 15 minutes following the administration of L-tryptophan and persist for two hours (Neckers, Biggio, Moja & Meek, 1977). The administration of L-tryptophan during RSD is intended to increase 5-HT, and thus block changes in certain behaviors which have been shown to be effected by RSD. Therefore, it is important that RSD has a demonstrated association with the alteration in the behaviors being measured. The dependent measures employed, open-field activity and thresholds to electrical stimulation, have been chosen based upon accounts in the literature which provide ample support for changes in these behaviors due to RSD.

Open-field Behavior

The open-field test has been a particularly useful tool in studying motivational behavior (Russell, 1973). Therefore, in light of the motivational theory of REM sleep deprivation which states that RSD increases waking neural excitability (Vogel, 1979), it is not surprising to find a number of research projects which have utilized the open-field test to study the effects of RSD. These experiments are summarized in Table 2. It is interesting to note that although there is a good deal of variability among the experiments listed in Table 2, the results have been fairly robust. That is, there is a demonstrated ability of RSD to increase open-field activity and to reduce defecation and urination regardless of apparatus size, illumination, duration of tests, sex and species. In addition, although there is variability for RSD across age with older animals being less active, Goodrich (1967) found that exploration in mature, non-sleep deprived animals decreased with increasing age, and Hicks, Pettey, Okuda and Thomsen (1979) hypothesized that age and RSD may covary with these behaviors.

The interpretation of the results obtained from open-field experiments is less clear as increases in ambulation have been explained as indications of both increased emotionality (Albert, Cicala & Siegel, 1970) and reduced emotionality (Hicks & Moore, 1979). This discrepancy has been addressed in the literature, and it appears that the interpretation of the results greatly depends upon the criteria used to assess behavior. Specifically, a distinction

Table 2

The Effects of REM Sleep Deprivation on Open Field Behavior

Author	Age	Gender	Light	Open-Field	# of Tests	Duration	Behaviors
Albert, Cicala & Siegel, (1970)	150-175g	male	70 watt	9 x 7.5 x 6 in	3	3 min	# of cage crossings*
Boysner (1970)	Not reported	Not reported	Not reported	(y-maze)	4	3 min	# of entries & rearings*
Hicks, & Adams (1976)	30 day	female	Not reported	55.2 x55.2 x 22.9 cm	1	20 min	# of grid crossings*
Hicks, Thomsen, Pettey, & Okuda (1976)	45 day	female	Not reported	55.2 x 55.2 x 22.9 cm	1	20 min	# of grid crossings
Hicks & Moore (1979)	60 day	male	40 watt bulb102 cm above	81.3 x 81.3 x 38 cm	1	15 min	# of grid crossings*, frequency defecation**, unrination** & freezing**
Hicks, Petty, Okuda, & Thomsen, (1979)	30,60,90 & 120 day	female	Not reported	55.2 x 55.2 x 22.9 cm	1	10 min	# of grid crossings*
Hicks, Gomez, Gonzales, Kuroda,Orme & Reyes (1981)	60 day	female	Not reported	81.3 x 81.3 x 38 cm	2	15 min	frequency of defecation**, and unrination**
Moglinicka, Boissard, Hunn & Delini-Stula (1985)	220-240g	male	75 watt	65 x 65 x 48 cm	1	8 min	# of grid crossings*, and frequency of defecation**,
Ogilvie & Broughton (1976)	30, 90 day	male	Not reported	1.2m x1.2 m	5	3 min	# of grid crossings*, frequency defecation**,
Van Hulzen & Coenen (1981)	75-90 day	male	2-3 lux	40 x 25 x 45 cm	1	45 min	# of grid crossings*, and # of rearings*

* significant increase for RSD compared to controls: $p < .05$

* * significant decrease for RSD compared to controls: $p < .05$

has been made between wall-hugging behavior and activity occurring in the center of the field. It has been noted that activity in the center of the field indicates an increase in exploration and a reduction in emotionality, whereas wall-hugging behavior typifies a reduction in exploration and an increase in emotionality (Aitken, 1974; Corman & Shafer, 1968; Hicks & Moore, 1979). Therefore, in order to distinguish between exploration and emotionality, this experiment measured discrete behavioral patterns consisting of the number of crossings for those grids bordering the walls, and the number of crossings in the central area of the apparatus. Those interested in a detailed treatise on the interpretation of open-field behavior may refer to Hicks and Moore (1979), Walsh and Cummins (1976), Aitken (1974), Russell (1973), and Whimbey and Denenberg (1967).

Thresholds to Electrical Stimulation

As stated, threshold to electrical stimulation was chosen as a dependent measure because the literature provides ample support for its sensitivity to RSD. Hicks et al. (1978) investigated the thresholds to electrical stimulation in rats subjected to RSD. In that study, pre-experimental thresholds were established through an ascending series of 0.5-second stimulations given to the upper part of the tail until the animals exhibited a tail-arch response. Thresholds were measured again following 72-hours of RSD, and it was noted that RSD not only reduced thresholds to electrical stimulation immediately following RSD but also lasting 24-hours

into the RSD recovery period. Hicks, Coleman, Ferrante, Sahatjian, and Hawkins (1979) confirmed these results and reported a RSD-induced reduction in thresholds which persisted for 96-hours.

Hypothesis

The administration of L-tryptophan during RSD is intended to stabilize the 5-HT system and thus prevent the changes in behavior normally induced solely by RSD. The dependent measures employed, open-field activity and thresholds to electrical stimulation, have been chosen based upon accounts in the literature which provide ample support for changes in these behaviors due to RSD. Hypothesis 1 states that there will be a significant difference between RSD animals administered L-tryptophan and RSD animals administered a placebo. That is, the RSD animals administered a placebo will demonstrate an increase in exploration and a reduction in electrical thresholds when compared to the RSD animals treated with L-tryptophan. As an expected corollary, the RSD animals administered L-tryptophan will not exhibit differences, when compared to the combined controls, on any of the behavioral measures tested.

Method

Subjects

The animals were 60 male Sprague-Dawley rats that were 60 days old at the start of the experimental period. Prior to treatment, animals were randomly assigned to the sleep groups (i.e., RSD, Wet Control [WC], Dry Control [DC]) and among sleep groups randomly

assigned to either the experimental drug or the placebo conditions. Therefore, the 60 animals were randomly assigned 10 each to one of the six sleep by drug groups.

REM Deprivation Technique

REM sleep deprivation was achieved using the water-tank procedure described in detail by Hicks and Moore (1979). Suggestions that this technique induces undue stress have been answered by findings which indicate that the water-tank procedure produces little or no stress when properly employed (Fishbein & Gutwein, 1977; Hicks, Okuda & Thomsen, 1977). Of particular relevance to this study are findings that increased serotonin turnover in rats deprived of REM sleep is not due to non-specific stress related to the deprivation procedure (Borbely, Steigrad & Tobler, 1980; Cramer, Tagliamonte, Tagliamonte, Perez-Cruet & Gessa, 1973).

During the four-day treatment period, all of the animals were housed in 18.9 liter buckets that had been modified such that food and water was available ad lib from a feeder on the side of the cage. In addition, the top of each bucket was covered with wire mesh. During treatment, each animal in the RSD condition was placed in the bucket on a 6.5-cm platform which was surrounded by water to within 1-cm of the platform. The WC group was identical in every regard except that the animals spent the treatment period on a 16.5-cm platform. These platforms are large enough to minimize these animals' contact with the water at REM

onset. Thus, this group served as a non REM-sleep deprived control for exposure to the wet environment. The DC group spent the treatment period in the apparatus with wood shavings covering the floor and a large platform in place, and therefore served as a non-deprived control against which exposure to the wet environment could be evaluated.

Open-Field Test

Open-field activity was measured in an 81.3 x 81.3 x 38-cm open-field apparatus with 10.2 x 10.2-cm grids under an illuminance of 8 lux. With the exception of the frequency of defecation, which was scored at the time of testing, the number of crossings for the grids bordering the walls, the number of crossings in the central area of the apparatus and the incidence of freezing (i.e., remaining motionless for at least a 5-second period) were recorded on video tape during each 15-minute test period. Later, the video tapes were scored as described by Hicks and Moore (1979) by three observers that were blind to the animals' treatment conditions. The open-field container was washed with an industrial strength cleanser following each test to eliminate odor cues.

Thresholds for Electrical Stimulation

Pre-experimental thresholds to electrical stimulation were established for each animal. The animals were restrained using a 10 x 5 x 5-cm plastic Harvard Universal Rat Restrainer. Electrodes were placed onto the upper portion of the tail. Intensity of shocks was controlled by dial settings on a Grass (Model S88) Stimulus Generator

connected with a Grass (CCU1-A) Constant Current Unit. Each test consisted of an ascending series of 0.5-second stimulations, with a frequency of electrical square waves of 125 pulses/second and a pulse width of 1.6-milliseconds. The animals' threshold for electrical stimulation (in milliamps) was considered to be the point at which the animal exhibited tail withdrawal and movement of the hind quarters. At this amperage, the motor response is considered a spinal reflex and it does not elicit a vocalization (Carroll & Lim, 1960; Paalzow & Paalzow, 1975).

Injections

The drug solution was prepared before use and was administered by intraperitoneal injection. Experimental animals received 80 mg/kg of L-tryptophan, (25 mg/mL of tryptophan in saline). This concentration is not soluble at room temperature, and thus the solution was heated to 75°C and then cooled to 45°C prior to injection. Control animals were administered an isovolumetric injection of 45°C saline according to the same schedule as the experimental group.

Procedure

During the 10-day adaptation period, all animals were housed individually in the dry REM deprivation apparatus with a large platform in place on a 12-hour light/dark cycle (lights on at 0700 hours; lights off at 1900 hours) with food and water available ad lib throughout the experiment. Ambient room temperature was kept at 25 °C. All animals were handled for 7-minutes daily.

Following adaptation, and before treatment, the animals were placed into the open-field apparatus and video taped for 15-minutes. Upon completion of the open-field test, the animals were allowed to rest for a 5-minute period pursuant to measuring the thresholds to electrical stimulation. Immediately following this test, the animals were placed into treatment conditions. During the four-day treatment period the animals received intraperitoneal injections at 1100, 1300 and 1600 hours of either L-tryptophan (80 mg/kg) or isovolumetric saline. The injection schedule was chosen in order to elevate 5-HT levels to the corresponding peak levels seen in non-deprived animals (Quay, 1968). Upon completion of the four-day treatment period, animals were towel dried and allowed to rest for 5-minutes prior to the open-field test. All tests were conducted between 0730 and 0930 hours on all testing days. Upon completion of the open-field test, the animals were allowed to rest for a 5-minute period following which thresholds to electrical stimulation were measured. The animals were then placed back into their home cages with wood shavings covering the floors. The animals then remained in their home cage condition until termination of the experiment.

Results

The means and standard deviations computed for the pre- and posttest body weights for each sleep by drug treatment group are presented in Table 3. As a check on the relative stressfulness of the treatment conditions, a 2 (Tests) x 2 (Drug) x 3 (Sleep) factorial

ANOVA with repeated measures over the first factor was computed to assess pre-posttreatment differences in body weight. Results of this analysis revealed a significant main effect for Sleep, (F [2,54] = 5.41, $p < .01$); and for the Sleep x Tests interaction (F [2,54] = 44.12, $p < .001$). However, the main effects for Drug and Tests, the Sleep x Drug interaction, the Drug x Tests interaction, and the Sleep x Drug x Tests interaction were not significant (i.e., F [1,54] = 0.00; F [1,54] = 0.96; F [2,54] = 2.40; F [1,54] =1 .15; and F [2,54] = 1.08, respectively).

Table 3

The Means and Standard Deviations for the Pre- and Posttest Body Weights for Each Sleep by Drug Treatment Group

	L-tryptophan		Placebo	
	Pretest	Posttest	Pretest	Posttest
	Mean SD	Mean SD	Mean SD	Mean SD
RSD	275.3 ± 15.23	263.0 ± 21.18	267.5 ± 11.44	253.1 ± 4.51
WC	270.0 ± 10.87	270.8 ± 8.02	276.5 ± 19.81	283.3 ± 19.75
DC	272.7 ± 9.91	283.7 ± 9.13	270.0 ± 13.18	284.3 ± 14.37

As can be seen from the means and standard deviations presented in Table 3, both RSD groups lost weight throughout the experiment and thus it may be that RSD groups were confounded by stress. Therefore, an analysis of covariance was considered in order

to provide a statistical correction for these differences. The covariate was computed by subtracting pretreatment from posttreatment weights and converting this difference into a T-score. According to Keppel (1982), the magnitude of any statistical correction afforded by an ANCOVA depends in large measure on the linear correlation between the covariate and the response variable. Thus, separate Pearson Product Moment Correlation Coefficients were computed between the covariate (the weight change index) and each response measure (i.e., the Exploration Index and the electrical threshold: with the result that $\underline{r}$ = .015 and $\underline{r}$ = .24, respectively). Therefore, because of the low correlation between the covariate and both these response measures, only the analyses of variance and the appropriate t-tests have been reported.

Open-Field Results

Although three measures are commonly used to evaluate open-field behavior two of these (the frequency of defecation and freezing) proved to be unreliable. That is, of the 120 tests administered, boli were observed on only 2 occasions and freezing only sporadically. Therefore, frequency of defecation and incidence of freezing were not included as measures of open-field activity in testing the formal hypothesis of this study. In addition, it should be noted that during recording of the open-field data, malfunctions of the video equipment resulted in the loss of data for four posttreatment trials, and thus those data could not be included in the analyses.

Separate Pearson Product Moment Correlation Coefficients were calculated between scorers for the Exploration Index. The mean inter-rater reliability was assessed as .95.

A distinction has been made between wall-hugging behavior and activity occurring in the center of the field. Activity in the center of the field is often taken to indicate an increase in exploration and a reduction in emotionality, whereas wall-hugging behavior typifies a reduction in exploration and an increase in emotionality (Aitken, 1974; Corman & Shafer, 1968; Hicks & Moore, 1979). A single Exploration Index was computed as follows: Exploration Index is equal to the number of central crossings divided by the total crossings, multiplied by 100.

Confirmation of the hypothesis required that only one of the six sleep by drug groups exhibit variation on the response measure. Specifically, of the six groups, it was predicted that only the RSD animals given a placebo would show an increase in exploration. With this in mind, it seemed unlikely that results from the omnibus ANOVA would provide an adequate test of the hypothesis. Therefore, separate one-tailed t-tests were conducted as formal tests of the hypothesis that the RSD animals administered a placebo would demonstrate an increase in exploration when compared to the RSD animals challenged with L-tryptophan, and that the RSD animals administered L-tryptophan would not differ from the combined controls.

Table 4

Means and Standard Deviations for the Open-Field Exploration Index

	L-tryptophan		Placebo	
	Pretest	Posttest	Pretest	Posttest
	Mean SD	Mean SD	Mean SD	Mean SD
RSD	10.53 ± 2.80	11.62 ± 1.70	8.34 ± 5.04	10.91 ± 3.31
WC	9.20 ± 4.70	11.04 ± 5.79	11.79 ± 2.11	14.90 ± 5.48
DC	8.23 ± 4.22	8.69 ± 7.02	10.95 ± 4.27	11.71 ± 4.66

The means and standard deviations for the Exploration Index are presented in Table 4. Using the data summarized in Table 4, one-tailed t-tests were conducted on the posttreatment Exploration Index. No significant differences were found between the RSD animals given L-tryptophan and the RSD animals given a Placebo, t (18) = 0.70; p = .49. In addition, the RSD L-tryptophan animals did not differ from the combined controls, t (45) = 0.48; p = .63. An analysis was performed to examine the difference for the posttest open-field index between the RSD animals administered a placebo and the combined controls. Results of this analysis revealed that the RSD animals given a placebo did not differ from the combined controls t (45) = 0.07; p = .94.

As expected, results from the 2 (Tests) x 2 (Drug) x 3 (Sleep) factorial ANOVA revealed that with the exception of Tests F (1,48) =

4.04; p = 0.05 neither main effects nor their interactions were significant (i.e., for Sleep F [2,54] = 1.42; p > .05; Drug F [1,54] = 1.79; p > .05; Sleep x Drug F [2,54] = 1.71; p > .05; Sleep x Tests F [2,48] = 0.40; p > .05; Drug x Tests F [1,48] = 0.41; p > .05; and for the Sleep x Drug x Tests interaction F [2,48] = 0.04; p > .05).

Electrical Threshold Results

As has been noted, thresholds to electrical stimulation were measured as a second means of testing the hypothesis of this study. Once again, confirmation of the hypothesis required that only one of the six sleep by drug groups exhibit variation on the response measure (i.e., only the Placebo-RSD animals were expected to demonstrate a reduction in electrical thresholds). As such, the omnibus ANOVA was not expected to provide an adequate test of the hypothesis, and therefore, separate one-tailed t-tests were conducted as formal tests of the hypothesis.

The means and standard deviations computed for the electical thresholds for each of the six sleep by drug groups are presented in Table 5. The data summarized in Table 5 were first analyzed utilizing separate one-tailed t-tests which were conducted on the posttest electrical thresholds. The RSD animals administered a placebo did not differ from the RSD animals challenged with L-tryptophan t (18) = 1.51; p = .07. Results of the analysis comparing the RSD L-tryptophan animals with the combined controls failed to reach significance as hypothesized, t (48)= -0.75; p = .45. In addition,

Table 5

Means and Standard Deviations for Thresholds to Electrical Stimulation

	L-tryptophan		Placebo	
	Pretest	Posttest	Pretest	Posttest
	Mean SD	Mean SD	Mean SD	Mean SD
RSD	1.34 ± 0.67	0.83 + 0.31	1.32 + 0.91	0.64 + 0.23
WC	1.30 + 0.94	0.93 + 0.59	1.21 + 0.71	0.99 + 0.47
DC	0.99 + 0.47	1.13 + 0.79	1.20 + 0.88	1.01 + 0.40

an analysis was performed to examine the difference in posttest electical thresholds between the RSD animals administered a placebo and the combined controls. Results of this one-tailed t-test revealed that the RSD animals given a placebo experienced a significant reduction in thresholds to electrical stimulation when compared to the combined controls t (48) = -1.84; p = 0.04.

Once again, as expected, results from the 2 (Tests) x 2 (Drug) x 3 (Sleep) factorial ANOVA revealed that with the exception of Tests $F(1,54) = 9.40$; $p < .005$ neither the main effects nor their interactions were significant (i.e., for Sleep F [2,54] = 0.05; Drug F [1,54] = 0.21; Sleep x Drug F [2,54] = 0.16; Sleep x Tests F [2,54] = 2.28; Drug x Tests F [1,54] = 0.69; Sleep x Drug x Tests F [2,54] = 0.13).

Discussion

A differential pattern for body weight was observed between pre- and posttreatment tests for the sleep groups. Specifically, both RSD groups lost weight throughout the treatment while the control animals either gained weight or remained stable. This finding allows for the possibility of a RSD by stress confound and thus could be viewed as a complication in discussing the results of this study. However, as was pointed out, since the correlation coefficients which were computed between the covariate (weight) and both response measures were negligible, the observed reductions in body weight in the RSD groups probably had little, if any, effect on these variables. Therefore, the confounding of these data by stress seems only a remote possibility.

Open-field

As mentioned, the hypothesis was tested using separate one-tailed t-tests which examined the relationship between the two RSD groups, and between the L-tryptophan-RSD animals and all the combined controls. The ability of RSD to increase open-field activity, and to reduce freezing and defecation is well documented (Albert et al., 1970; Hicks et al., 1976; Hicks et al., 1981; Ogilvie & Broughton, 1976). Surprisingly, boli were observed in only 2 of the 120 open-field tests administered. The sparsity of boli observed throughout testing may have resulted from repeated handling which the animals experienced during adaptation and while receiving injections throughout treatment. To explain, Russell (1973) and Aitken (1974)

reported that handled rats defecate less and are more active in the open-field, presumably due to reduced fear levels. Further, as can be seen from Table 4, high levels of activity were observed in the open-field prior to testing and increased only slightly during the posttreatment test. In addition, the incidence of freezing, which has been utilized as an index of fear in the open-field (Mogilnicka et al., 1985; Moore, Hayes, & Hicks, 1979; Russell, 1973) was rarely observed during all of the tests, and seemed unrelated to the animals' treatment.

Thus, the lack of defecation, the infrequent incidence of freezing and the high level of activity suggest that exposure to the open-field did not induce fear in any of the animals during either the pre- or posttreatment tests. As a result, the planned comparisons computed for the open-field index revealed no significant differences between the untreated RSD group, and both the L-tryptophan-RSD animals and the combined control group. Here it should be noted that in view of these data, it is doubtful that the weight loss of the RSD groups was indicative of non-specific stress. To explain, had the RSD animals been stressed by their sleep treatment, the open-field results should have been in the opposite direction to those that were observed.

To summarize, the pattern of these data indicate that the open-field data may not have provided an adequate test of the hypothesis. As stated, the excessive handling of all the animals was an inescapable aspect of the injections and may have contributed to the

lack of difference in fear-related behaviors observed between groups.

Electrical Thresholds

Thresholds to electrical stimulation were measured as a separate test of the hypothesis that the RSD animals given a placebo would demonstrate a reduction in electrical thresholds compared to the RSD animals receiving L-tryptophan. As an expected corollary it was hypothesized that the RSD animals given L-tryptophan would not experience a reduction in electrical thresholds when compared to the combined controls.

Inspection of the data reveal that with the exception of the L-tryptophan animals placed in the DC condition, all other groups regardless of sleep condition or drug treatment demonstrated a reduction in electrical thresholds between the pre- and posttreatment tests. The significant main effect calculated for Tests was anticipated and is not relevant to the hypothesis as electrical thresholds are reduced upon repeated exposure (Hicks et al., 1978; Hicks, Coleman, Ferrante, Sahatjian & Hawkins, 1979).

Of particular relevance to the hypothesis, however, are the analyses of the posttreatment electrical thresholds. Recall that the RSD animals administered a placebo demonstrated a reduction in thresholds when compared to the RSD animals receiving L-tryptophan. Although these results failed to reach significance at $p < .05$, given the statistical power of the RSD groups test, the level could be treated as significant at $p < .10$. This result, when viewed in

conjunction with the other threshold data, provide tentative support for the hypothesis. To explain, while the L-tryptophan-RSD animals did not differ from the controls, the Placebo-RSD animals did exhibit a significant reduction in posttreatment electrical thresholds when compared to the combined controls.

The observed patterns suggest that L-tryptophan may have attenuated, but not eliminated the effects of RSD, and as such they provide support for the hypothesis. This result, in which some but not all of the effects of RSD are reduced is compatible with reports in the literature which indicate that gradations in a behavior are produced following different lengths of exposure to RSD. Hicks, Moore, Hayes, Phillips, and Hawkins (1979) reported that animals deprived of REM sleep for 48 and 96 hours showed an increased incidence of interspecies aggression when compared to the controls. The animals deprived of REM sleep for 96 hours showed a greater incidence of aggression than did the animals deprived of REM sleep for 48 hours. They concluded that RSD substantially increased interspecies aggression in a dose-related fashion. In addition, Mogilnicka et al. (1985) reported that animals deprived of REM sleep for 24 and 72 hours demonstrated a shorter latency to approach a novel object and spent a significantly longer time in exploration than did the non-deprived controls. Mogilnicka et al. (1985) also reported that a 72-hour deprivation period produced a larger effect than their 24-hour deprivation period, and, like Hicks, Moore, Hayes, Phillips, & Hawkins (1979), suggested that RSD influenced behavior in a dose-

related fashion. The ability of RSD to produce gradations in a response measure are not limited to behavioral effects. Animals continuously deprived of REM sleep show increasing physiological debilitation (i.e., severe ulcerative and hyperkeratotic skin lesions, an increase in food intake, energy expenditure, heart rate, protein metabolism, and Norepinephrine) as deprivation progresses. (Kushida et al., 1986; Kushida, et al., 1989; Rechtschaffen, et al., 1989). In addition, animals which were deprived of REM sleep for three five and seven days showed increased turnover of 5-HT corresponding to the length of deprivation (Cramer et al. 1973).

The foregoing discussion suggests that different durations of RSD lead to gradations in the level of certain behavioral, physiological, and pharmacological measures. Although RSD associated reductions in electrical thesholds were not eliminated following the administration of L-tryptophan, the posttreatment variation which was observed may have resulted through the attenuation of RSD in a dose-related fashion. If this were the case, the ability of L-tryptophan to mediate changes in RSD-induced behavior would depend not only upon the the dose of L-tryptophan but also upon the duration of the RSD treatment. In this study only one level of RSD and L-tryptophan were utilized and perhaps the dose of L-tryptophan that was employed here would have completely eliminated the RSD-induced behavioral changes in some groups, had the amount of RSD been reduced.

Conclusions

Taken together these results provide only mixed support for the hypothesis. As mentioned, the open-field data may not have tested the hypothesis due to inescapable difficulties inherent in the administration of injections. However, the electrical threshold data were consistent with the hypothesis, and although the least sensitive test (L-tryptophan-RSD animals vs RSD-placebo animals) approached significance at $p = .07$, the more powerful test (placebo-RSD vs combined controls) was significant at $p = .04$. This pattern of the electrical threshold data suggest that the effects of RSD were attenuated but not eliminated following injection of L-tryptophan. However, as was discussed, the ability of L-tryptophan to completely eliminate changes in a behavior may depend upon the doses of L-tryptophan and/or the durations of RSD utilized. This possibility is interesting and warrants futher examination.

The use of complex behavioral measures may not, due to the intricacies of their underlying mechanisms and sensitivity to the test environment, be best suited to test this hypothesis. For example, open-field activity is a complex voluntary behavior which is sensitive to the test conditions and the test environment. Walsh and Cummins (1976) discussed the complexity of the open-field test and commented on the myriad of variables in the test situation which effect behavior: "In the open-field test, the whole situation (rather than any one specific stimulus component) is the independent variable." With this in mind, future research should employ

behaviors which are relatively involuntary and less complex when investigating the relationship between L-tryptophan, RSD, and the behavioral changes occuring from RSD. Examples of behaviors in this class include thresholds to electrical stimulation, metabolic rate and evoked potential responses. In addition, researchers should be sensitive to inescapable difficulties presented by the administration of injections during treatment. Some of these potential difficulties include the modification of behavior resulting through repeated handling, and the introduction of stress through the administration of injections. Furthermore, although the literature indicates that RSD effects 5-HT in a dose-related fashion, neuronal 5-HT was not measured in this experiment. The possibility that L-tryptophan brought the physiological system underlying the sleep process back into an equilibrium through interaction with the serotonergic system remains speculative. Therefore, future research should measure neuronal 5-HT as a means of verifying the validity of these injections, and elucidating the pharmacology of REM sleep and the behavioral changes occuring from RSD.

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APPENDICES

Appendix A

The Serotonergic System

A description of the serotonergic system is presented below and a graphical representation of this system is presented in Figure 1. The letters in parentheses refer to appropriate aspects of the figure.

Tryptophan is the only essential amino acid which is partially bound to albumin in the plasma; and therefore, only tryptophan which is free in plasma can cross the blood brain barrier (Hamon & Glowinski, 1974) (A). Although tryptophan is taken up at neuronal membranes, it is believed that the rate limiting step for tryptophan uptake is at the blood brain barrier (Pardridge, 1979). Gessa and Tagliamonte (1974) argue that the enzyme tryptophan hydroxylase has an unusually high Km for its substrate tryptophan, and that changes in neuronal tryptophan are associated with changes in brain 5-HT levels (B-1). That is, increasing levels of neuronal tryptophan lead to an increase in brain 5-HT concentration. Indeed, Neckers et al. (1977) measured tryptophan and tryptophan hydroxylase activity using high pressure liquid chromatography coupled with fluorescence detection in various brain structures. Results indicated that whenever tryptophan content increased in a brain region or nuclei, the tryptophan hydroxylase activity was also increased. More important were the findings which suggested that lowered tryptophan levels in vivo increased the Vmax for tryptophan hydroxylase. Neckers and his colleagues suggest that this increase in

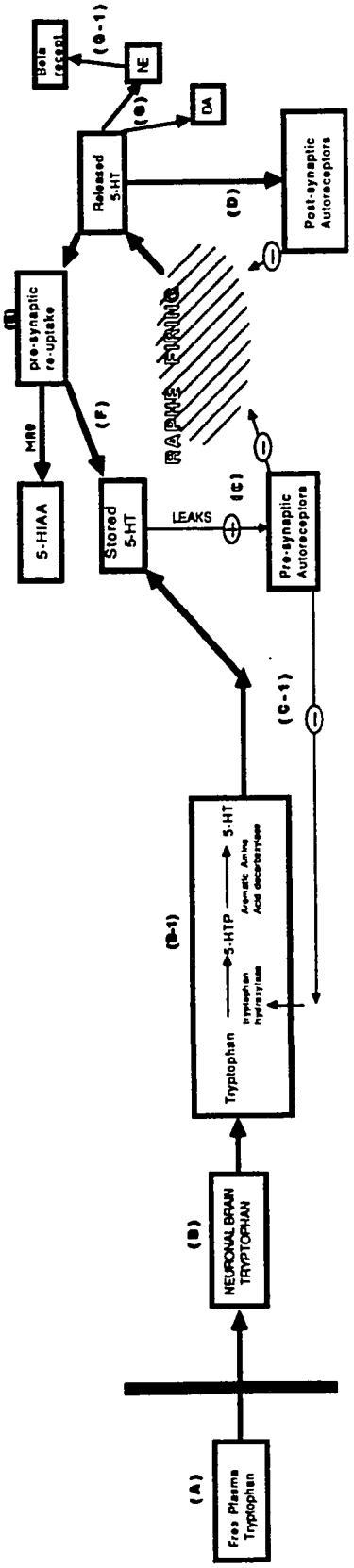

Figure 1
The serotonergic system

tryptophan hydroxylase activity may buffer the effects of a severe depletion of tryptophan on brain 5-HT concentration.

The ability of 5-HT to depress its own release, as measured by single cell recordings from the raphe nucleus, has been demonstrated and is believed to function through the presence of both pre- and postsynaptic auto-receptors (Aghajanian, 1972; Cerrito & Raiteri, 1979). Pharmacological evidence indicates that these receptors have distinct physical and structural properties (Luki, Nobler & Frazer, 1984). Aghajanian (1972) noted that the administration of 5-HT precursors such as L-tryptophan or 5-Hydroxytryptophan led to an increase in brain serotonin content and raphe cell fluorescence as well as a subsequent decrease in raphe cell firing. Gallager and Aghajanian (1976) have hypothesized that local changes in serotonin may affect raphe firing through the leakage of serotonin from the raphe soma onto presynaptic autoreceptors (C). Hamon and Glowinski (1974) suggest that activation of these presynaptic autoreceptors activates a negative feedback mechanism which then reduces the synthesis of serotonin through inhibition of tryptophan hydroxylase and neuronal tryptophan transport (C-1). In addition, Aghajanian, Graham and Sheard (1970) reported that both Monoamine oxidase inhibitors, regardless of structural category, and the tricyclics, depressed raphe cell firing. Although these drugs have different modes of action, they are believed to reduce raphe firing by increasing the availability of serotonin in the synaptic cleft, and thus activating the negative feedback mechanism (D).

Reinhard and Wurtman (1977) have demonstrated that presynaptic re-uptake of serotonin from brain synapses is required before it can be metabolized into 5-Hydroxy Indoleacetic acid (5-HIAA) (E), therefore, measuring 5-HIAA provides a good index of serotonin release. Curzon, Fernando and Marsden (1978) measured serotonin and 5-HIAA levels in both normal animals, and animals whose serotonin was partially depleted by administration of PCPA. They noted that when serotonin levels were reduced, although serotonin could be increased by the administration of L-tryptophan, its subsequent breakdown into 5-HIAA remained unchanged. This was in contrast to normal animals which showed an increase in accumulation of 5-HIAA compared to serotonin following treatment with L-tryptophan. It was concluded that in systems partially depleted of serotonin, the transmitter either has little availability for release or is more readily taken up and re-used. (F).

As discussed in the introduction, the serotonergic system may function as a vigilance controlling apparatus mediating the vigilance-enhancing activity of the catecholamines (Koella, 1988). Indeed, the relationship between serotonin, the catecholamines and vigilance has received a great deal of attention (Frankhuyzen & Mulder, 1980; Janowsky et al., 1982; Pujol et al., 1968; Schildkraut & Hartmann, 1972; Wojcik et al., 1980). Therefore, studies investigating these relationships may provide indirect lines of inquiry for elucidating the relationship between serotonin, REM sleep and the behavioral changes occurring from REM sleep deprivation.

Serotonin has an inverse relationship with the catecholamines (Janowsky et al., 1982; Pujol et al., 1968; and Schildkraut & Hartmann, 1972), such that an increase in release of serotonin is associated with a decrease in release of both Norepinephrine (NE) and Dopamine (DA). Conversely, a decrease in serotonin is associated with an increase in NE and DA. (G).

Appendix B

Vigilance States and the Serotonergic System

Much of the knowledge on REM sleep has been derived from experiments examining the behavioral and pharmacological manifestations occurring from its deprivation. However, before examining such data, it may be appropriate to review the serotonin system in relation to the sleep cycle in non-deprived animals.

The daily variations of tryptophan, 5-HT, and 5-HIAA concentration have been thoroughly examined in the rat brain (Hery, Rauer, & Glowinski 1972; Hery et al., 1977; Ogasahara, Taguchi & Wada, 1980). Hery et al. (1977) subjected animals to a 12:12 light dark cycle for three weeks. Following this adaptation period, the animals were tritiated with [^{3}H]L-tryptophan and then sacrificed at various times of the day. Tissue was taken from the fronto-parietal cerebral cortex and homogenized. [^{3}H]Tryptophan, [^{3}H]5-HT, and [^{3}H]5-HIAA levels were separated by ion exchange chromatography and the eluates were then examined for spectrofluorimetric estimations of the [^{3}H]Amine. Results indicated that 5-HT synthesis (i.e., the accumulation of [^{3}H]5-HT formed from [^{3}H]Tryptophan) was increased during the light phase. However, [^{3}H]5-HIAA was reduced during this period and reached maximal levels during the dark phase. The [^{3}H]5-HT/[^{3}H]5-HIAA ratio peaked during the light phase and was significantly reduced during the dark period. Hery et al. (1977) suggest that an increase in [^{3}H]5-HIAA or the reduction of the

[^{3}H]5-HT/[^{3}H]5-HIAA ratio is related to enhanced release and utilization of serotonin.

Hery et al. (1972) measured [^{3}H]Tryptophan, [^{3}H]5-HT, and [^{3}H]5-HIAA levels using identical methods, however tissue from the cortex, hypothalamus and the brain stem were analyzed. Results in the various brain structures for [^{3}H]Tryptophan, [^{3}H]5-HT, and [^{3}H]5-HIAA followed the same pattern described earlier: [^{3}H]5-HT levels were generally higher than [^{3}H]5-HIAA during the light phase while the opposite was true for the dark period. Hery, et al., (1972) suggests that the accelerated formation of [^{3}H]5-HIAA from newly synthesized [^{3}H]5-HT is mainly the result of active transmitter release from presynaptic terminals.

Surprisingly, Hery et al. (1977) noted that free plasma tryptophan levels were maximized during the dark phase. These findings have been confirmed by Semba, Toru and Mataga (1984) who, in addition to finding the same circadian variations in serotonin and 5-HIAA during a normal light cycle, also found the same rhythm in animals maintained in constant dark conditions.

A depiction of the raphe system across the vigilance states is presented in Figure 2. During the light phase in which the animals spend a disproportionate amount of time in sleep, an increase in serotonin synthesis as determined by the accumulation of [^{3}H]5-HT from [^{3}H]Tryptophan (A) is accompanied with a reduction in its release and utilization as measured by both the serotonin metabolite [^{3}H]5-HIAA and the [^{3}H]5-HT/[^{3}H]5-HIAA ratio (B, C & D

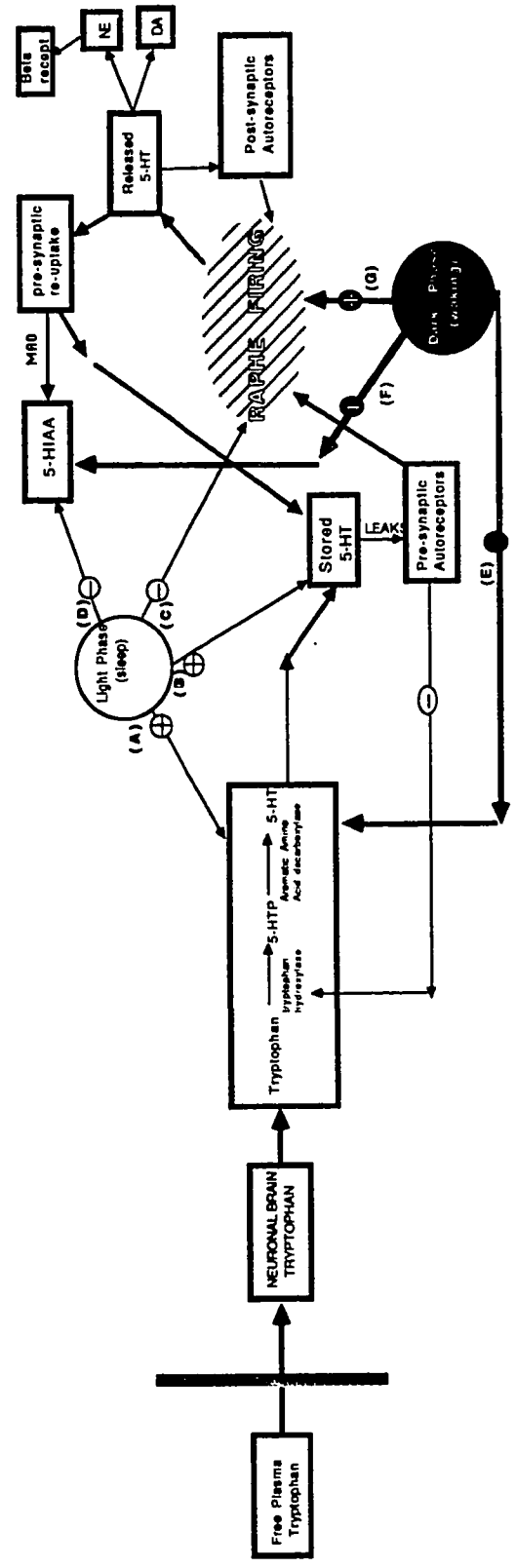

Figure 2
Vigilance states and the serotoninergic system

respectively). During the dark phase, in which the animals engage predominantly in waking behavior, a decrease in serotonin synthesis (E) is accompanied with an increase in its utilization and release as measured by the formation of [^{3}H]5-HIAA from newly synthesized [^{3}H]5-HT (F & G). These results are consistent with the evidence presented in Appendix A. That is, the pattern in which local changes in serotonin concentration affect raphe firing through interactions with presynaptic autoreceptors is characteristic of the serotonergic system.

The firing rate of the dorsal raphe is proportional to the amount of serotonin discharged (Aghajanian et al., 1970). Therefore, based on the evidence presented so far, one would predict a decrease in firing of the dorsal raphe nucleus during sleep and an increase in firing during the waking phase. Indeed, these predictions are supported by the data reported by McGinty and Harper (1976) and Ogasahara et al., (1980). McGinty and Harper monitored the activity of the dorsal raphe neurons across the sleep-waking cycle in freely moving cats. The results indicated a reduction in dorsal raphe unit firing during slow wave sleep and marked reduction or cessation in firing during REM sleep (C). During waking, the dorsal raphe exhibited a characteristic pattern of firing (G). These patterns of raphe firing were confirmed in the rat using the microwave fixation technique (Ogasahara et al., 1980). The data support the hypothesis that REM sleep provides an interruption in neurally-mediated 5-HT release.

The neurotransmitters known as the monoamines include Serotonin (5-HT), Dopamine (DA) and Norepinephrine (NE). The literature which has been cited demonstrates that serotonin is at least correlated to the sleep cycle, exhibiting definite circadian fluctuations across the different vigilance states. Do the other monoamines exhibit the same type of pattern? The answer for the DA system is probably no. Mogilnicka (1981) found no change of DA firing across any of the sleep cycles. NE on the other hand, may be involved in sleep states (Aston-Jones & Bloom, 1981; Chu & Bloom, 1973; Chu & Bloom, 1974). Additionally, during REM rebound, at time which is characterized by an increase in 5-HT synthesis and a decrease in 5-HT release, there is an increase in activity of noradrenergic neurons (Pujol et al., 1968).

Appendix C

Effects of REM Sleep Deprivation on the Serotonin System

RSD has been reported to increase neuronal brain tryptophan transport, tryptophan hydroxylase activity, and brain 5-HT synthesis (Hery et al., 1970). RSD may also induce receptor supersensitivity of serotonergic presynaptic auto-receptors. Santos and Carlini (1983) noted an exaggerated incidence of both the serotonin syndrome and the number of head shakes in animals deprived of REM sleep and challenged with L-tryptophan. They suggest that these behaviors result from activation of central serotonin receptors and indicate receptor supersensitivity of serotonergic presynaptic auto-receptors.

The observation that those Monoamine oxidase inhibitors which alter catecholamine systems also disrupt normal sleep patterns led to the investigation of the effects of RSD on both norepinephrine and dopamine (Pujol et al., 1968; Schildkraut & Hartmann, 1972). The ability of RSD to increase NE turnover and cause a decrease in Beta-adrenoreceptor number and sensitivity has been reported (Mogilnicka et al., 1985.). The effect of RSD on the Dopaminergic system is less clear as both decreases in receptor sensitivity (Tufik, Lindsey & Carlini, 1978; Zwicker & Calil, 1986) and failures to find alterations in receptor sensitivity (Farber et al., 1983) have been reported. These results are accompanied by reports of increases in DA transmission (Ghosh, Hrdina, & Ling 1976).

Borbely et al. (1980) and Hery et al. (1970) have hypothesized that RSD may enhance the release of 5-HT from terminals. This

would then decrease intraneuronal levels of 5-HT and thus trigger a negative feedback mechanism which would increase tryptophan transport across the neuronal membrane, and thus increase 5-HT synthesis. It has been noted that in neurons partially depleted of 5-HT, the transmitter has little availability for release or is taken-up and re-used more readily (Cerrito & Raiteri, 1979). It has also been noted that activation of the presynaptic autoreceptors by increased accumulation of the amine reduces 5-HT synthesis by way of a negative feedback mechanism (Hamon & Glowinski, 1974).

Taken together, these data provide a framework by which the effects of RSD may be explained. This framework is presented graphically in Figure 3.

During RSD, continued release of the amine results in a decrease in intraneuronal levels of 5-HT (A); these partially depleted 5-HT levels may then either reduce the availability for release, or increase the re-uptake from the synaptic cleft (B). This would result in reduced availability of 5-HT at presynaptic autoreceptors, and thus prevent inhibition of tryptophan transport, and tryptophan hydroxylase activity (D). This, in turn, would result in an increase in 5-HT synthesis (E). As described earlier, serotonin has a distinct relationship with the catecholamines, such that a decrease in availability of 5-HT would also be associated with an increase in both NE and DA activity (F).

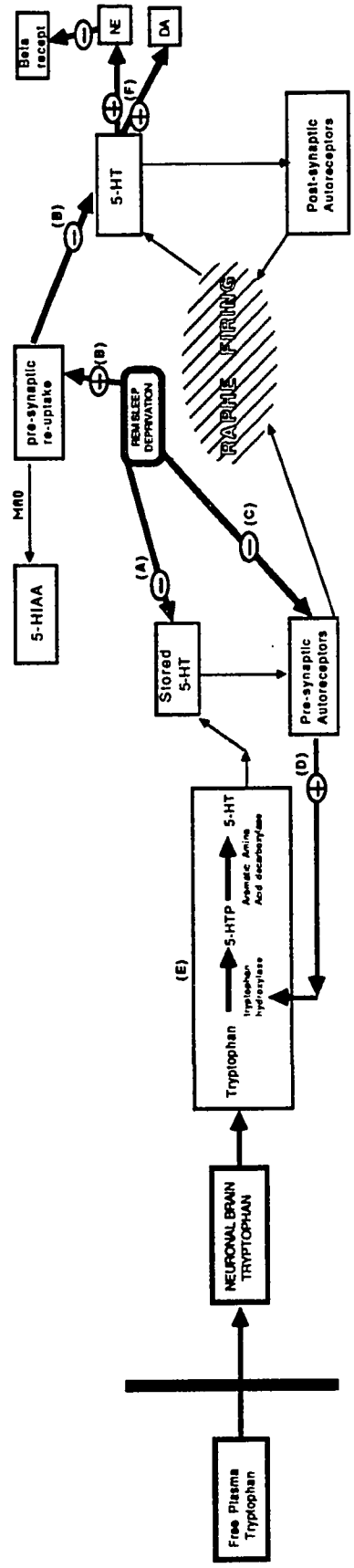

Figure 3
Effects of REM sleep deprivation on the serotonin system

It is hypothesized here that RSD reduces the availability of 5-HT in the synaptic cleft, thus reducing its activity at both pre- and postsynaptic receptors. Therefore, it may be possible to prevent the reported induced behavioral changes occurring from RSD through the administration of a 5-HT agonist concomitantly with the deprivation process. After reviewing the proposed paradigm, it becomes apparent that not all of the putative 5-HT agonists are appropriate. For example, RSD has been of particular interest to researchers due to its similarities with the anti-depressants in the therapeutic ability to improve depressive symptoms (Vogel et al., 1986). The class of Mono amine oxidase inhibitors which prevent class A Mono amine oxidase, and the tertiary tricyclics primarily affect the 5-HT system (Fuller, 1982; Savage, Mendels & Frazer, 1980). Although the mode of action for the therapeutic effect of these drugs remains unclear, they are classified as 5-HT agonists. Montigny and Aghajanian (1978) reported a supersensitivity of 5-HT receptors following chronic administration of tricyclic anti-depressants, a result also reported following RSD (Santos & Carlini, 1983). Vogel et al. (1986) hypothesized that the therapeutic effects of the anti-depressants result from the deprivation of REM sleep. Therefore, the combined effects of RSD and administration of antidepressants may be complementary and would be inappropriate for this study.

Intraperitoneal injections of L-tryptophan raise both tryptophan and serotonin in brain areas containing 5-HT cell bodies and nerve terminals. Wojcik et al. (1980) hypothesize the increase of 5-HT in

serotonergic terminals either 'spills over' into the synaptic cleft and interacts with receptors, or increases the amount of stored 5-HT which then provides excess 5-HT at the times when the 5-HT neurons normally fire. Therefore, the mode of action of intraperitoneal injections of L-tryptophan on increased serotonin activity at pre- and postsynaptic receptor sites allows for an appropriate intervention point for maintaining an equilibrium in the serotonin system across the deprivation process.