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The effects of rapid-eye-movement sleep deprivation on synaptic plasticity in the rat hippocampus

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THE EFFECTS OF RAPID-EYE-MOVEMENT SLEEP DEPRIVATION ON SYNAPTIC PLASTICITY IN THE RAT HIPPOCAMPUS

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ABSTRACT

THE EFFECTS OF RAPID-EYE-MOVEMENT SLEEP DEPRIVATION ON SYNAPTIC PLASTICITY IN THE RAT HIPPOCAMPUS

by Roger N. Morrissette

Rapid-Eye-Movement sleep (RS) consolidation theory postulates that one function of RS is to modulate plastic processes, particularly learning and memory. Proponents argue that RS serves to transfer perceptual data from short-term to long-term memory storage. With the discovery of long-term potentiation (LTP), an empirical method of testing consolidation theory becomes available. LTP is a neural response to a brief, high-frequency train of stimuli, resulting in a long-lasting potentiation in the neuron's response to a fixed stimulus. Preliminary research indicates that LTP normally occurs when animals learn. Additionally, results indicate that this synaptic plasticity is significantly affected during the sleep-wakefulness cycle. RSd was initiated using the platform technique and LTP was elicited and monitored in hippocampal slices of control and 4-day RSd rats. Results show a significant increase in population spike amplitudes for RSd rats as compared to controls.

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The Effects of Rapid-Eye Movement Sleep Deprivation on Synaptic Plasticity in the Rat Hippocampus

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Footnotes

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The Effects of Rapid-Eye Movement Sleep Deprivation on Synaptic Plasticity in the Rat Hippocampus

Rapid-Eye-Movement sleep (RS) consolidation theory postulates that one function of RS is to modulate plastic processes, particularly learning and memory (Ellman, Spielman, Luck, Steiner & Halperin, 1991; Fishbein & Gutwein, 1977; Horne & McGrath, 1984). It is hypothesized that information perceived during the day is appraised, sorted and stored during RS. Much work has been done with human and non-human subjects to determine the effect of RS on learning and memory (Ellman et al., 1991; Fishbein & Gutwein, 1977; Horne & McGrath, 1984; McGrath & Cohen, 1978; Pearlman, 1978), and in so doing, a variety of learning measures have been used in conjunction with various RS deprivation (RSd) methodologies. In developing the rationale for this study, I hypothesized that if an animal is exposed to a learning acquisition trial and then allowed to sleep, then that animal's RS will serve to consolidate retention of the task at hand. Thus, deprivation of RS following a learning trial should create a deficit in learning. Therefore, the following section of my review is focused on post-acquisition learning and methodologies of RSd.

Posttrial RSd and Learning

Work done by Pearlman and his colleagues examined the effects of learning when RSd follows various learning tasks. They found that RSd blocks the appearance of the latent learning effect (Pearlman, 1971). Latent learning occurs when an animal is introduced to a learning task, like a maze, and is allowed to run about in it without being tested or rewarded. Although there is no evidence of learning from the initial exposure, the animal will later learn a conditioned task in the maze much faster than an unexposed animal. In Pearlman's (1971) latent learning set-up, 32 male albino rats were given one trial per day in a six-unit multiple T-maze with no reward given at

completion of the learning task. All rats were equally habituated to the maze. On the fourth day of training, food was placed in the goal box for the first time. Sixteen of the 32 animals were then RS deprived. On the fifth day, both groups of animals ran the maze to an empty goalbox. RSd rats were significantly less successful in running the maze than controls. The results suggest that RSd interferes with the integration between the unrewarded acquisition and the rewarded acquisition.

RSd immediately following a shuttlebox avoidance learning trial has been shown to decrease retention of previous learning (Leconte & Bloch, 1970; Leconte & Hennevin, 1973; Leconte, Hennevin, & Bloch, 1974; Pearlman & Greenberg, 1973). In Pearlman and Greenberg's (1973) procedure, five groups of three month old female Wister rats underwent four consecutive series of 10 training trials in a Lehigh Valley Electronics shuttlebox. Of the three deprivation groups, one was RS deprived by the inverted flower pot technique (Vimont-Vicary, Jouvet-Mounier, & Delorme, 1966), one by intra-peritoneal (IP) injections of imipramine (5mg/kg), and one by IP injections of pentobarbital (35 mg/kg). Two groups served as controls. After the 24-hour training period, all animals were given 10 retention trials that were identical to the learning tasks. Results indicate that two hours of RSd immediately following shuttlebox avoidance training causes a significant retention deficit. No significant differences across the three deprivation techniques were found. Pearlman and Greenberg (1973) concluded that the deficit of retention in the shuttlebox avoidance task following training may have been due to a disruption in the memory consolidating function of RS. Additionally, immediate posttrial RSd has also been associated with learning deficits for bar-press acquisition (Pearlman, 1973; Pearlman & Becker, 1974a), spatial reversal learning (Pearlman & Becker, 1974b), spatial probability maximizing (Pearlman & Becker, 1974b), brightness discrimination (Pearlman &

Becker, 1973), and two-way avoidance (Leconte & Bloch, 1970).

Contradictory evidence suggests that retention is less effected when RSd follows simple or instinctual learning tasks (Albert, Cicala, & Siegal, 1970; Danguir & Nicolaidis, 1976; Joy & Prinz, 1969; Miller, Drew, & Schwartz, 1971; Pearlman, 1971; Pearlman & Becker, 1973). Pearlman (1971), in his second experiment, used exploratory feeding trials and showed no difference between RSd and control rats. These exploratory or 'appetitive' trials involve monitoring the animal's natural food exploration behavior. Danguir and Nicolaidis (1976) used one-trial taste aversion , Pearlman and Becker (1973) used Y-maze position habit trials, and one-way active avoidance trials were used by both Joy and Prinz (1969) and Albert et al. (1970). Most of these learning acquisition trials involve innate or simple responses that may not be linked to the RS system. Contradictory results have also been found with more complex learning measures. Van Hulzen and Coenen (1979) demonstrated no differences in shuttle-box avoidance frequency for RSd and control groups. Animals in these trials were deprived of RS for only one hour following a training session. The fact that it had been previously shown that the first three hours following a shuttlebox learning trial was a critical period for RS, and subsequent retention (Leconte & Hennevin, 1973; Leconte et al., 1974) may explain the limited results. Van Hulzen and Coenen note that a stable memory trace may not have had time to develop, and thus may not have been affected by the RS deprivation. Although individual results are dependent on the experimenter's choice of learning measure, the consensus suggests that RSd, following a learning task, impairs complex learning (Fishbein, 1970; Horne & McGrath, 1984; Leconte & Bloch, 1970, Pearlman, 1971, 1978; Pearlman & Becker, 1974a, 1974b; Pearlman & Greenberg, 1973).

Several studies have shown that RS duration increases in the sleep period

immediately following learning acquisition (Hennevin, Leconte, & Bloch 1974; Leconte & Hennevin, 1971; Leconte, Hennevin, & Bloch, 1973; Lucero, 1970; Pearlman, 1978; Smith, Lowe, & Smith, 1977). Leconte et al. (1974) found that a critical period of free sleep immediately following training sessions was sufficient for learning to occur. A positive relationship was observed between the degree or level of learning and the amount of subsequent RS attained during this critical period. Once learning is complete or the task is mastered, RS returns to normal. If the animal is exposed to another learning task, RS again increases. Based on these results, a newly formed memory trace is believed to be integrated during RS following acquisition. It is hypothesized that RS's function involves the integration of new information into existing structures.

Measuring Memory, Neurophysiologically

Currently, the idea of measuring a memory trace, neurophysiologically, seems insuperable. In this regard, a popular theory for memory involving neurophysiological function between presynaptic and postsynaptic cells was introduced by the psychologist Donald O. Hebb in 1949. Hebb suggested that there may be a structurally consolidating neural property of memory. He stated:

> When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased (p. 62).

In 1973, Bliss and Lomo observed a long-lasting potentiation in a neuron's response to a fixed stimulus following a brief, high-frequency train (HFT) of electrical

stimuli. This long-term potentiation (LTP) phenomena reignited interest in Hebb's neurophysiologic postulate. Basically, LTP is a form of neural plasticity. As a result of an electrical tetanus, and the influence of both presynaptic and postsynaptic fibers, a change in synaptic efficacy results. LTP has been found to occur in several areas of the brain but predominates in the hippocampus (Shepherd, 1988). The role of the hippocampus in memory has been assessed by correlating unilateral hippocampus removal with performance on various memory tests. Unilateral hippocampal removal of areas in both the right and left hippocampus show significant impairment for several learning acquisitions in humans (Corkin, 1965; Milner, 1965, 1968; Petrides & Milner, 1982; Smith & Milner, 1981).

In the dentate granule cells of the hippocampal-dentate complex, LTP can last for several hours, days or even weeks (Racine, Milgram, & Hafner, 1983). Additionally, the CA1 pyramidal cell region of the hippocampus has been shown to be an equally resourceful area for LTP elicitation (Bliss & Lynch, 1988). In fact, the Schaffer collateral-commissural pathway which ends in the CA1 region of the hippocampus, has been the major pathway studied for investigating LTP mechanics. The large monosynaptic excitatory projections from the CA3 to the CA1 pyramidal cells in the hippocampus provides an ideal structure for investigating a hebbian synapse. Figure 1 shows a schematic representation of a rat hippocampal slice with the Schaffer collateral-commissural pathway, CA1 and CA3 cell body layers specified. Typical placement of recording and stimulating electrodes are also displayed. An example of a typical population action potential (derived from a composite of action potentials) before and after a high-frequency train (HFT), demonstrating LTP of both the excitatory postsynaptic potential (EPSP) and population spike, is shown in Figure 2.

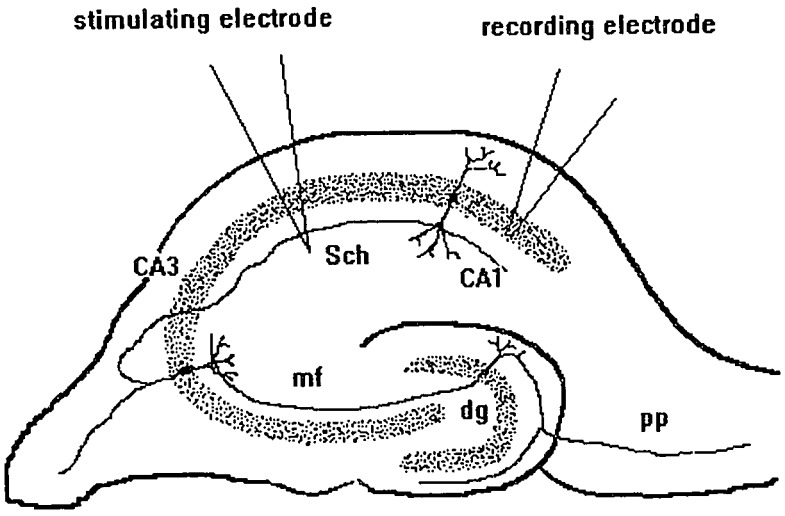

Figure 1. A schematic representation of the rat hippocampal slice displaying typical electrode positioning for recording synaptic transmission in the Schaffer collateral-commissural pathway. The trisynaptic pathway goes from the perforant path (pp) of the entorhinal cortex, to the dentate gyrus (dg) granule cells, along the mossy fibers (mf) to the CA3 pyramidal cells and then along the Schaffer collaterals (Sch), to the CA1 pyramidal cells.

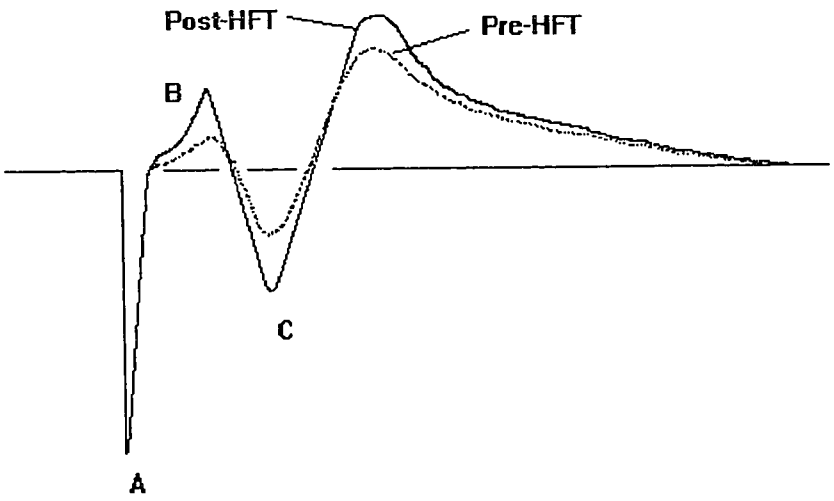

Figure 2. An example of a typical population action potential before and after a high-frequency train (HFT) of stimulation. The amplified response following the HFT demonstrates LTP in both the EPSP and the population spike. The first downward spike (A) is the stimulus artifact, the next rising phase (B) is the field EPSP and the next downward and upward spike (C) make up the population spike.

Although the evidence that links LTP to learning and memory is, at present, only suggestive, several studies have supported this notion (Berger, 1984; McNaughton, Barnes, Rao, Baldwin, & Rasmussen, 1986; Morris, Anderson, Lynch, & Baudry, 1986). For example, it has been demonstrated that the rate at which rabbits learn a conditioning task increases if LTP is elicited in the hippocampus prior to presentation of the learning task (Berger, 1984). Also, McNaughton et al. (1986) reported that learning can be influenced by the use of procedures that induce LTP in the hippocampus. Additionally, by associating N-methyl-D-aspartate receptors (NMDA), a subclass of glutamate receptors, with LTP (Collingridge & Bliss, 1987; Malenka, Kauer, Perkel, & Nicoll, 1989; Smith, 1987), and using the drug, aminophosphonovaleric acid (AP5), an NMDA channel blocker, Morris et al. (1986) has shown that NMDA receptors are involved in spatial learning. Morris et al. (1986) showed that AP5 selectively impairs place learning and also prevents the induction of LTP following high-frequency stimulation. Collectively, these results offer support for the notion that LTP is correlated with the process of learning.

Sleep and LTP

In the hippocampus, it has been shown that spontaneous single-neuron activity (Olmstead, Best, & Mays, 1973; Suzuki & Smith, 1985) and synaptic field potentials (Buzsaki, Grastyan, Czopf, Kellenyi, & Prohaska, 1981; Leung, 1980; Winson, 1980; Winson & Abzug, 1978) change with the animals behavioral state. Using the trisynaptic pathway from the perforant pathway of the entorhinal cortex to the CA1 pyramidal cell body region (perforant path to dentate gyrus granule cells to CA3 pyramidal cells along the Schaffer collaterals to the CA1 region), Winson and Abzug (1978) recorded CA1 population spikes and EPSP's from chronically prepared, freely moving rats during RS, slow-wave-sleep (SWS), and waking. When stimulating with

low current (between 155 - 205 μamps) in the perforant path, responses in the CA1 region were similar in all three arousal states, but increased stimulation (from 310 - 625 μamps) produced a marked increase in population spike and EPSP amplitude during SWS as compared to RS and waking.

Contradictory excitable effects were found more recently when Bramham and Srebo (1989) stimulated the perforant path and recorded monosynaptic LTP responses from dentate gyrus granule cells in freely moving rats during RS, SWS, and waking states. Stimulation during RS and waking reliably produced LTP of population spikes, EPSP's, and EPSP slope, while stimulations during SWS rarely elicited LTP phenomena. Bramham and Srebo (1989) postulate that since there is a behavioral state-dependent modulation in the same neural region that fosters LTP, it logically follows that LTP may also be manipulated by the behavioral state.

Little is known about the mechanisms underlying the behavioral regulation of neural activity in the hippocampus. Because brainstem monoaminergic projections are implicated in learning and memory functions (Altman, 1985; Roberts, 1981), and because their firing rates tonically vary with the degree of behavioral arousal (Aston-Jones & Bloom, 1981; Jacobs, 1986; McGinty & Harper, 1976; Trulson & Jacobs, 1979), emphasis has been placed on these projections. Preliminary evidence suggested a connection between noradrenergic (Bliss, Goddard, & Riives, 1983; Dahl, Bailey, & Winson, 1983; Stanton & Sarvey, 1985) and serotonergic (Bliss et al., 1983; Srebo, Azmitia, & Winson, 1982) inputs from the locus coeruleus and median raphe nucleus respectively to behavioral arousal and LTP, but contradicting evidence has developed in both cases (Krug, Chepkova, Geyer, & Ott, 1983; Robinson & Racine, 1985; Stanton & Sarvey, 1985).

If the function of RS is to consolidate memory, then the deprivation of RS

should create a need for consolidation. If this consolidation is manifested in LTP, then RSd could create an increase in LTP variables. A simplified alternative to Bramham and Srebo's (1989) in vivo method was used. That is, an in vitro LTP recording technique was used to identify baseline differences between RSd and control rats. I chose to record in the most commonly used monosynaptic pathway for eliciting LTP, the Schaffer collateral to CA1 cell body region in the hippocampus. Additionally, since LTP scoring paradigms are still being argued, I chose multiple facets of the most common scoring measures. For example, in field potential recordings, population spike amplitude is most commonly measured (Andersen, 1987; Collingridge & Bliss, 1987; Teyler & DiScenna, 1987). This variable was broken down into three facets: the first peak-to-peak amplitude (Amp1), the second peak-to-peak amplitude (Amp2), and their average amplitude (AVEa). These three variables in addition to the EPSP slope variable make up the LTP 'scoring' variables. Each of these will be discussed in greater detail in the methods section. Additional common measures used were frequency of occurrence and duration of LTP evaluated via the AVEa. I hypothesized that all six variables, the EPSP slope, the three amplitude values (Amp1, Amp2, and AVEa), and both the frequency and duration of LTP values would all be greater for hippocampal slices of RSd rats than for the control animals.

Methods

Subjects

The experimental animals were 12, 45 day old, male Sprague-Dawley rats weighing approximately 150 gms at the start of the experiment. Controlled light conditions were maintained with lights on from 7:00 a.m. to 7:00 p.m. in a temperature controlled (22°C) holding room. Animals were kept on an ad libitum diet of rat chow and water.

RSd Apparatus and Procedure

RSd was achieved by using an adaptation of the platform technique introduced by Jouvet and his colleagues (Vimont-Vicary et al., 1966). The Jouvet apparatus was modified such that it is similar to that of Hicks and Moore (1979). The RSd platforms were 6.5 cms (small platforms) in diameter while control platform diameters were 16.5 cms (large platforms). Water (19°C) filled the buckets to within 1 cm of the surface of the platform for all conditions.

Twelve animals were housed in 18.9 liter buckets, one rat per bucket. First, animals were exposed to a minimum of 5 days adaptation in a dry bucket (no water) containing the large platform. Subjects in each condition were matched by weight and treatment and counterbalanced with control animals. All rats were then exposed to the large platform wet condition. The control animals stayed on the large platforms surrounded by water for six days, while the matched treatment groups had two days on the large platforms but spent their last four days and nights on the small, RSd platforms. Water in the buckets was changed daily and ambient room temperature was maintained at a constant 22 °C.

Surgical/Slice Preparation and Procedure

At the conclusion of the treatment period, the animals were anesthetized using pentobarbital (35mg/kg), sacrificed by decapitation, and then their brains rapidly removed. Brains were chilled, split into two halves, and both the right and left hippocampus removed. Tissue slices, 500 microns thick, were cut at an angle parallel to the axons of the alveus using a McIlwain tissue chopper. The slices were quickly transferred to the experimental (incubation) chamber where they were set on lens paper saturated with artificial cerebrospinal fluid (aCSF). The composition of the aCSF consisted of: 124 mM NaCl, 2.4 mM $CaCl_2$, 5 mM KCl, 1.24 mM KH_2PO_4,

1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, and, 10 mM dextrose (Oliver, Hoffer, & Wyatt, 1977; Yamamoto, 1972), the pH was between 7.4 and 7.5, and the temperature was maintained between 34 and 35 °C. The level of the aCSF was modulated to keep the slices covered via capillary action while a mixture of 95% O_2 and 5% CO_2 was directed through warm distilled water toward the slices, keeping the chamber both oxygenated and humidified (Haas, Schaerer, & Vosmansky, 1979; Schwartskroin, 1975). Figure 3 displays a sketch of the incubating chamber used.

Recording Apparatus and Procedure

An etched tungsten microelectrode (tip diameter 5-10 μm, length 10-50 μm) served as the stimulating electrode, and a Grass SD9 Stimulator was used to elicit the stimulus. Recordings were made with a glass micropipette (2-15 MΩ) filled with 4 M NaCl . The stimulating electrodes were positioned with Brinkman and Narishige mechanical microdrives and the recording electrodes were positioned by Kopf and Narishige hydraulic microdrives to allow for accurate placement of each electrode. Recording electrodes were connected to a Mentor D. C. intracellular amplifier. Signals were displayed on a Tectronix storage oscilloscope, and then recorded on an IBM 386 hard disk. Run Technologies Inc., DataPac II software, was used to record and score data.

The time from sacrifice to the placement of tissues in the chamber took approximately 8 minutes. Slices were allowed to equilibrate for one hour before the onset of testing. All tests were conducted during the same time of the day to control for possible circadian effects between animals. After equilibration, extracellular potentials were recorded from the CA1 pyramidal cell body layer. The criteria for a healthy slice were that it exhibited a minimum of 5mV population spike using a stimulus strength of less than 10 volts and that its response was stable for 15 minutes.

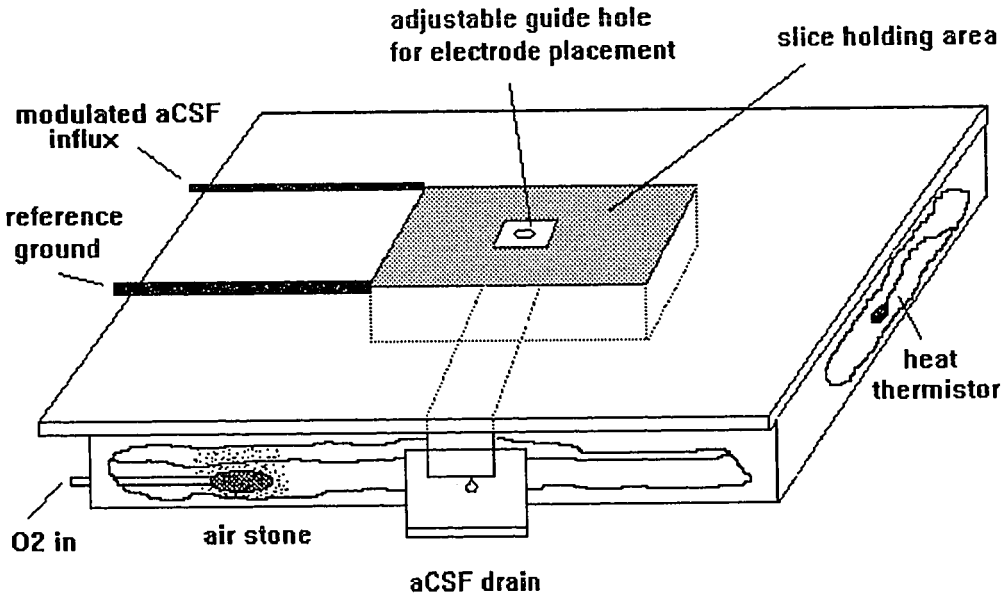

Figure 3. Sketch of the incubating chamber used to house the hippocampal slices. aCSF: artificial cerebrospinal fluid.

Any slice in which the field potential response did not deviate by more than 10% from baseline during the 15 minute trial period was considered stable and used. The stimulus voltage was then reduced to maintain a peak-to-peak population spike amplitude, which was 80% of maximal. Stability of the 80% maximal response was also checked for 15 minutes under the same criteria as mentioned above. Stable slices received a high-frequency train (HFT) of electrical current delivered to the Schaffer collaterals. The HFT (15 Hz) was delivered in three sets of 10 second intervals with 10 seconds separating each interval. The voltage of the HFT was the same as the 80% maximal stimulating voltage. At 5 minutes past the last train of pulses, the frequency values were reduced back to the pre-HFT baseline levels, the tissue was stimulated at the same 80% maximal level and continued to be stimulated every minute for 360 minutes or until there was no response. The field potential was recorded during every stimulation bout. A visual description of the stimulus paradigm is shown in Figure 4.

The Variables and Scoring

Six variables were used in the analysis. Four of the six variables were direct measures of the field potentials. Figure 5 shows a typical field potential response with the three divisions used to isolate the first four 'scoring' variables. Division 1 isolates the population EPSP. DatapacII software was programmed to subtract the lowest amplitude of the field EPSP from the highest and then divide that product by the distance between the same points. In this way a slope, or rate of rise measure was calculated for each EPSP (EPSPs). This EPSPs was the first dependent variable measured. Division 2 accounted for the first population spike amplitude variable (Amp1). The distance between the lowest and highest amplitudes within division 2 were calculated to achieve Amp1. In the same manner, the second population spike amplitude measure (Amp2) was calculated using information from division 3. Just as

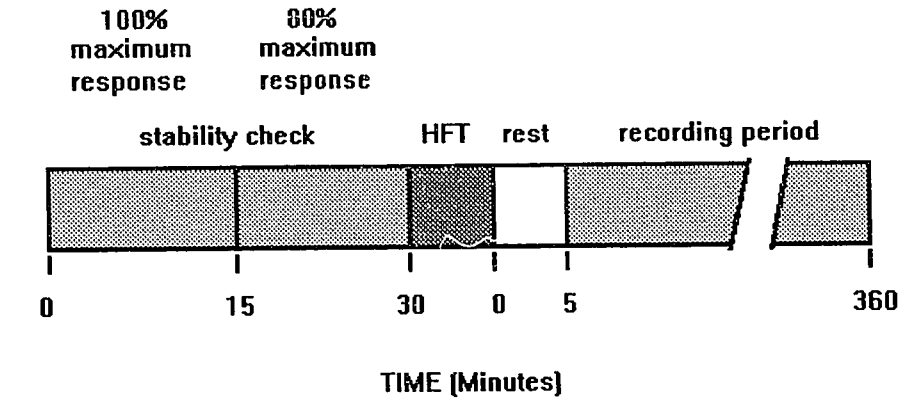

Figure 4. Time line representation of the stimulus paradigm used. Note that the recordings began at 5 minutes past the high-frequency train (HFT) and were taken at one minute intervals until 360 minutes had passed or until there was no response.

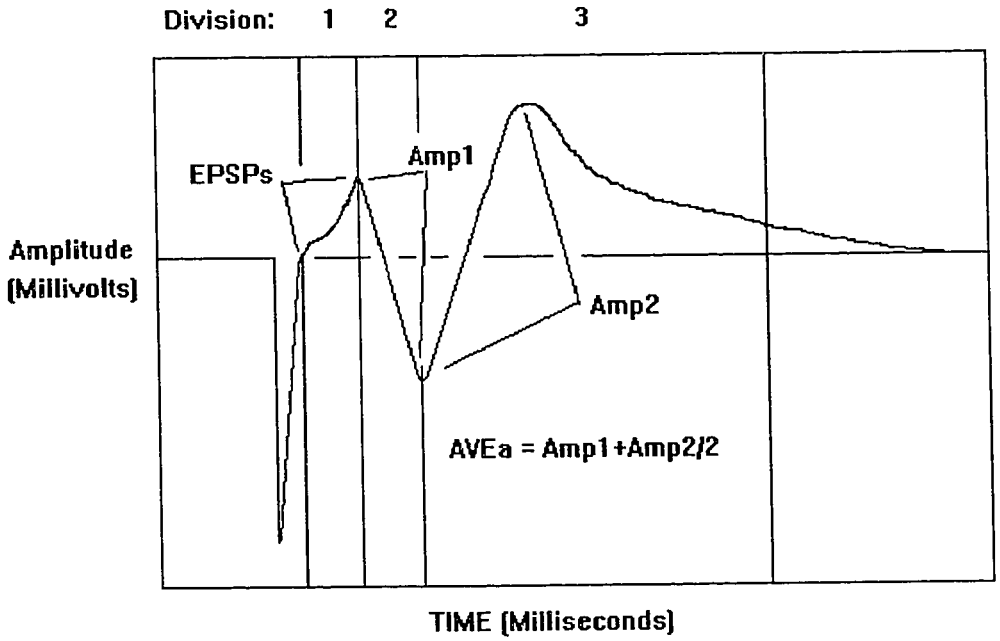

Figure 5. Schematic of a typical field potential, three subdivisions, and variables associated with each division. Division 1 isolates the EPSP. The lowest amplitude in division 1 was subtracted from the highest and then the product was divided by the distance between the two points to determine the EPSP slope. In division 2, the lowest peak was subtracted from the highest peak to determine Amp1. Similarly, in division 3, the lowest peak was subtracted from the highest peak to determine Amp2. AVEa was calculated by simply taking the average of Amp1 and Amp2.

in calculating Amp1, the distance between the lowest and highest amplitudes within division 3 were calculated to achieve Amp2. The fourth dependent variable was simply the average amplitude of Amp1 and Amp2 (AVEa). These four dependent measures were calculated for each one minute stimulation across all twelve subjects. Responses were plotted at fifteen minute time intervals (to 360 minutes) following the HFT. Once all responses were tabulated, an average of the five responses closest to the 15 minute interval was calculated. In this way, a five minute average was taken at each fifteen minute interval. These data were then entered into an EXCEL spreadsheet.

Using the spreadsheet and the pre-HFT baseline values, a value for the percentage change from baseline was calculated for each fifteen minute interval, for each subject, and for all four scoring variables (EPSPs, Amp1, Amp2, and AVEa). According to these measuring criteria, since stable values should not deviate by more than 10%, any increase in the percentage change from baseline scores for these four variables by more than 20% was considered to be LTP. The two remaining variables, the frequency and duration of LTP, were calculated from the average population spike variable (AVEa).

Results

As the data were collected, it became clear that the planned six hours (360 minutes) of recording from each tissue slice was not possible because many of the slices showed considerably less longevity than the rest. Therefore, a truncated timeline was used. In one case the first 15 minute sample was missing, so in order to maximize the database, scores from 30 minutes to 165 minutes were used as an adapted set of measurement points for the analysis. This yielded 135 minutes of data for each subject including all six of the RSd subjects and four of six control subjects. This time period

was used since it eliminated the least amount of samples, while containing the greatest amount of data. In the Appendix, the complete set of raw data for each variable is listed, as well as, the results of five data truncation calculations. The number of both control and RSd cases not rejected during a separate two factor Analysis of Variance (ANOVA) with repeated measures in the second factor, are also listed. This information, along with the individual ANOVAs, illustrates the necessity of using the third truncation between 30 minutes and 165 minutes. Truncations 3, 4, and 5 carry the most cases (control n = 4, RSd n = 6) and are similar enough in their statistical analysis that truncation 3 (the truncation carrying the most data) became the logical choice.

Table 1 lists the means and standard errors for the change from baseline scores for EPSPs, Amp1, Amp2, and AVEa. These values were calculated for each 15 minute interval between 30 minutes and 165 minutes. Graphic representations of the table 1 data for each variable are shown in Figures 6, 7, 8, and 9, respectively.

Using the data summarized in Table 1, a separate two factor ANOVA with repeated measures in the second factor (measurement periods) was computed for the EPSPs, Amp1, Amp2 and AVEa variables. The results of these analyses are as follows. For EPSPs, there was no significant treatment effect ($\underline{F}(8) = 0.015$, $\underline{p} = .90$), or repeated measure effect ($\underline{F}(9) = 1.882$, $\underline{p} = .07$), while a significant interaction ($\underline{F}(17) = 2.129$, $\underline{p} = .04$) was found. The three population spike amplitude values did show significant treatment effects. Amp1 showed a significant treatment effect ($\underline{F}(8) = 7.19$, $\underline{p} = .03$), and non-significant repeated measures ($\underline{F}(9) = 0.220$, $\underline{p} = .99$), and interaction ($\underline{F}(17) = 1.332$, $\underline{p} = .25$). The treatment effect of Amp2 ($\underline{F}(8) = 5.93$, $\underline{p} = .04$), as well as the interaction ($\underline{F}(17) = 2.194$, $\underline{p} = .03$), were significant, while the repeated measures effect was not ($\underline{F}(9) = 0.960$, $p = .42$). And expectedly similar to

Table 1

Means (M) and standard errors (SE) for the change from baseline scores for EPSPs, Amp1, Amp2, and AVEa for each 15 minute measurement between 30 minutes and 165 minutes. Cell totals for control = 4 and RSd = 6.

Time (Minutes):		30	45	60	75	90	105	120	135	150	165
EPSPs											
Control	M	-21.94	53.25	25.60	7.48	-22.57	-20.49	-37.53	-42.58	-51.58	-50.73
	SE	11.28	165.03	83.13	75.22	37.07	40.12	26.53	28.71	24.76	23.28
RSd	M	-15.63	-19.11	-15.33	-12.34	-19.26	-18.28	-18.29	-19.83	-19.65	-27.38
	SE	9.93	6.02	15.07	27.41	25.15	25.06	27.98	24.32	18.33	22.10
Amp1											
Control	M	-29.19	-36.89	-33.77	-37.06	-41.45	-44.73	-44.04	-46.19	-49.92	-53.99
	SE	26.88	33.49	37.53	38.45	34.38	31.53	30.17	29.60	34.30	35.53
RSd	M	-7.30	-9.53	-7.07	-8.99	-10.68	-6.69	-1.92	-1.35	-0.37	-1.33
	SE	11.75	14.60	14.65	14.45	18.56	18.31	21.83	21.25	20.12	22.57
Amp2											
Control	M	-23.28	-1.21	-20.70	-21.73	-27.70	-32.46	-32.75	-21.93	-23.12	-23.71
	SE	21.76	40.90	27.08	29.87	25.51	23.20	24.03	25.01	27.73	27.31
RSd	M	2.76	1.20	3.48	4.19	2.26	7.00	12.85	14.42	15.67	15.10
	SE	10.89	13.21	14.95	14.62	20.23	21.49	25.34	25.56	22.13	22.24
AVEa											
Control	M	-25.79	-16.92	-26.61	-28.66	-34.00	-38.06	-38.39	-40.55	-44.37	-48.35
	SE	23.90	33.66	31.34	32.83	29.01	26.52	27.65	25.55	30.69	33.59
RSd	M	-2.10	-3.94	-1.68	-1.70	-6.44	0.71	6.04	6.81	8.21	7.55
	SE	11.13	13.60	14.47	14.50	21.97	19.75	23.17	23.12	20.66	21.85

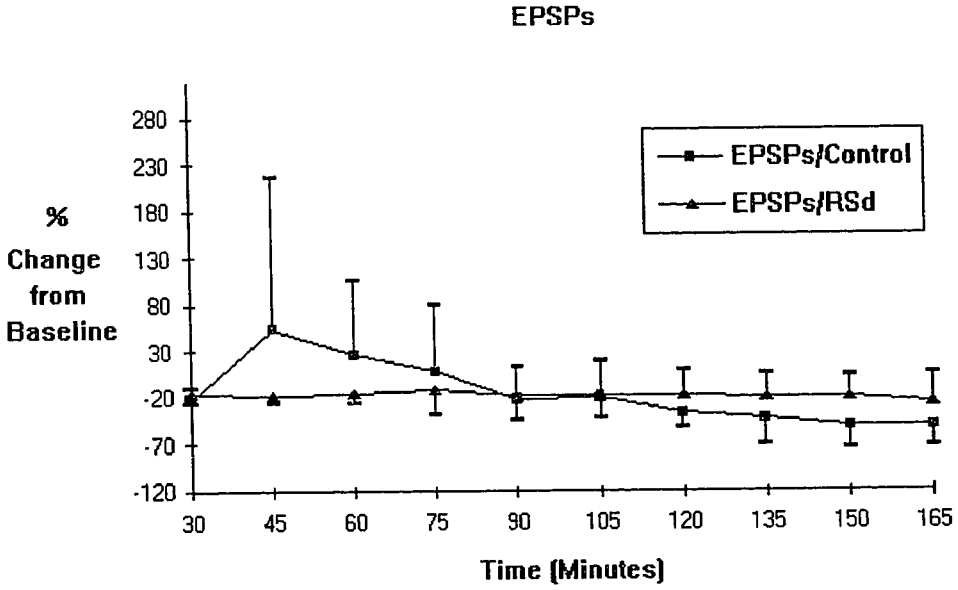

Figure 6. Graphic representation of EPSPs means and standard errors for control and RSd hippocampal slices at 15 minute intervals from 30 to 165 minutes past the high-frequency train.

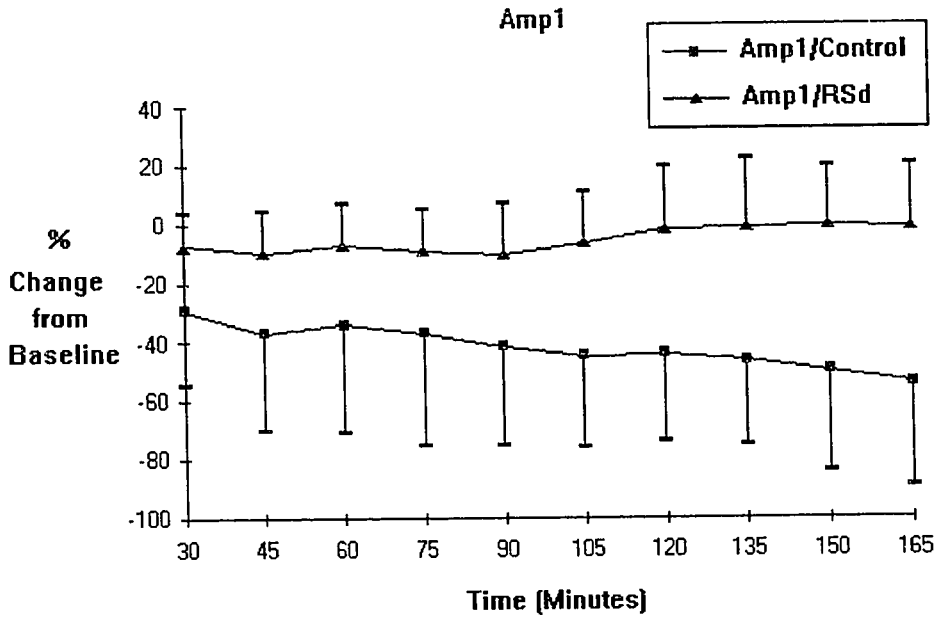

Figure 7. Graphic representation of Amp1 means and standard errors for control and RSd hippocampal slices at 15 minute intervals from 30 to 165 minutes past the high-frequency train.

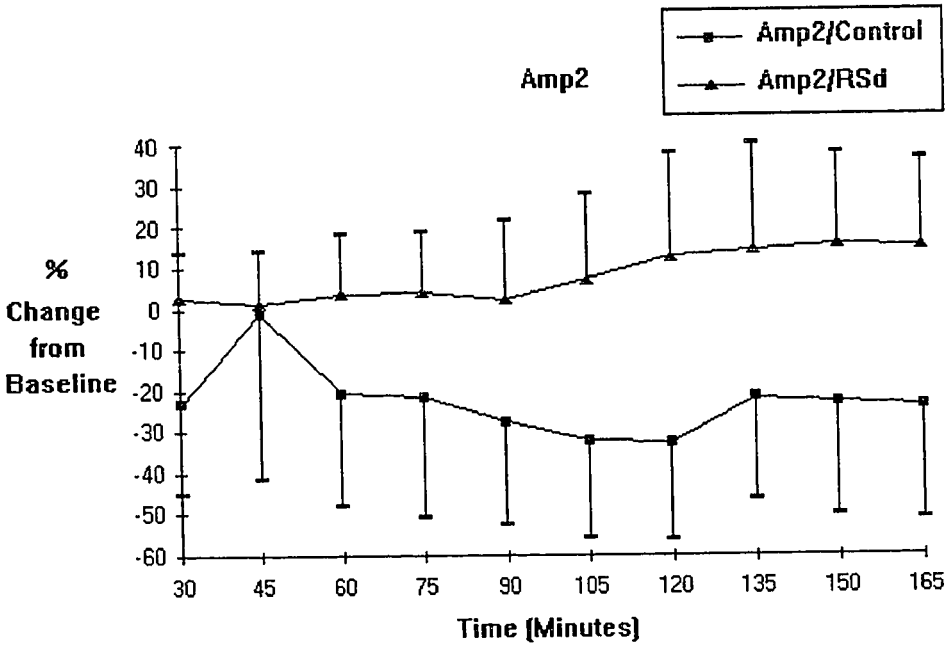

Figure 8. Graphic representation of Amp2 means and standard errors for control and RSd hippocampal slices at 15 minute intervals from 30 to 165 minutes past the high-frequency train.

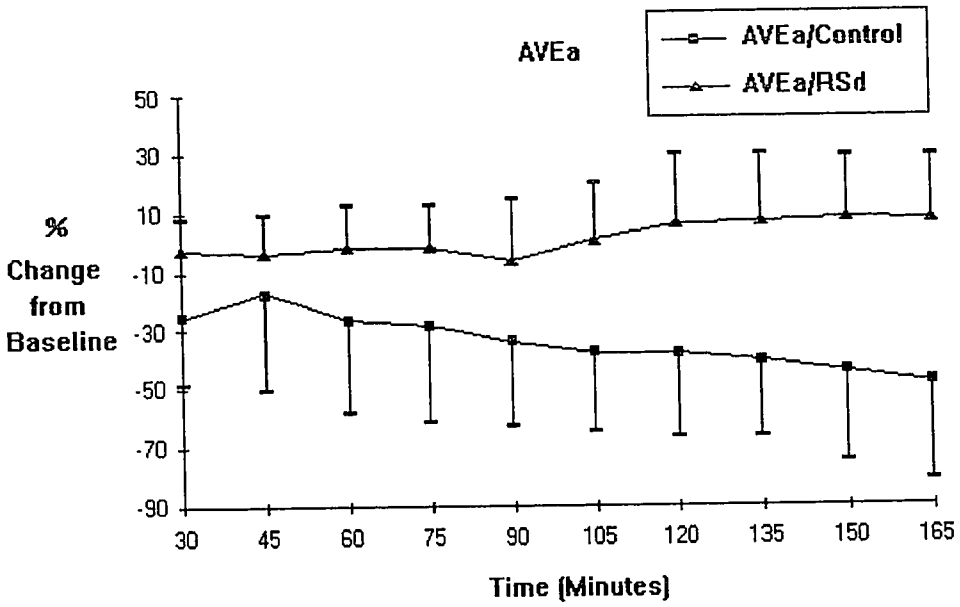

Figure 9. Graphic representation of AVEa means and standard errors for control and RSd hippocampal slices at 15 minute intervals from 30 to 165 minutes past the high-frequency train.

Amp1 and Amp2, the AVEa variable had a significant treatment effect ($F(8) = 7.844$, $p = .02$) and interaction ($F(17) = 2.627$, $p = .01$), while the repeated measure effect was nonsignificant ($F(9) = 0.285$, $p = .98$).

To determine the frequency of occurrence and duration of LTP, the data from AVEa was recoded into nominal form. Table 2 shows the recoded data for all slices in the time period. For control slices, there was only 1 LTP occurrence in 40, 15 minute trials (2.5%), while the RSd slices showed 14 LTP occurrences in 60 possible 15 minute trials (23.3%). Obviously the single LTP occurrence for the control slices lasted less than 15 minutes, while the duration of LTP occurrences for the RSd slices lasted approximately 30 minutes, 75 minutes, and 120 minutes respectively. Using the data in Table 2, a Chi-square statistic was computed to test the significance of frequency of LTP across groups. The results of this analysis were not significant ($X^2(1, n = 10) = .625$, $p > .05$). Although the three RSd duration values exceeded the single LTP duration value, the limited sample leaves any inference about LTP duration values questionable. Based on this nominal recoding of data, it is clear that for frequency of occurrence and duration of LTP, RSd slices and control slices did not differ significantly.

Discussion

Consolidation theory postulates that learning occurs during RS. Images and perceptions taken in during the day are consolidated into memories during this latter stage of sleep. Long-term potentiation, a form of neural plasticity in the hippocampus, has also been linked to learning and may indeed be a measure of memory trace consolidation. This research was designed to explore the relationship between LTP and RS. Using hippocampal slices from both control and RSd rats I measured the differences in the ability of these slices to elicit LTP. I hypothesized that the six

Table 2

Nominal recoded data for AVEa.

	Subject	Time (minutes): 30	45	60	75	90	105	120	135	150	165
Control	1	1	2	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1
	7	1	1	1	1	1	1	1	1	1	1
	9	1	1	1	1	1	1	1	1	1	1
RSd	2	1	2	2	2	2	2	2	2	2	1
	4	1	1	1	1	1	1	1	1	2	2
	6	1	1	1	1	1	2	2	2	2	1
	8	1	1	1	1	1	1	1	1	1	1
	10	1	1	1	1	1	1	1	1	1	1
	12	1	1	1	1	1	1	1	1	1	1

1 = % change less than 20% above baseline 2 = 20% LTP

measures of LTP (EPSP slope, three facets of the population spike, and both frequency of occurrence and duration of LTP) would all be greater for RSd rats than for controls.

All three facets of the population spike (Amp1, Amp2, and AVEa) showed significant increases from baseline values in the RSd condition as compared to controls. That is, significant treatment main effects were observed in all of the population spike amplitudes which are the most commonly used means of establishing LTP occurrence (Andersen, 1987; Collingridge & Bliss, 1987; Teyler & DiScenna, 1987). Since there is no current literature involving RSd and LTP, it is difficult to generalize these results to a common reference. However, these results agree with the results reported by Bramham and Srebo (1989) in a general way, in that they showed that LTP is promoted as easily during RS as it is during waking and that it is not promoted during SWS. Thus, if it can be assumed that RS naturally uses LTP to consolidate information, then RSd may make the tissues more susceptible to LTP induction, if indeed LTP is the tool used to consolidate a memory trace. These amplitude data support the hypothesis of this study that field potential values increase following RSd. However, the EPSP slope variable results failed to support this hypothesis. That is, the means did not show any consistent changes for the RSd condition, nor was the main effect for treatments significant. In this regard, Bramham and Srebo (1989) did show a change in dentate gyrus granule cell EPSP slope following HFT in the perforant path of the hippocampus when the HFT was elicited during RS or waking as compared to SWS. These differences in results may be attributed to the different methodologies used. Scoring procedures similar to Bramham and Srebo's were used although a flaw was detected in both protocols. Scoring parameters for EPSP slope began at the end of the stimulus artifact and ended

at the peak of the EPSP and used minimum and maximum EPSP amplitudes (see Figure 5). As can be seen in Figure 5, the slope represented is curvilinear and both our calculations were based on a linear slope. Therefore, it may be that this scoring procedure is not sensitive enough to pick up the subtle differences in EPSP slope for the two treatment groups.

The values for the frequency of occurrence and duration of LTP do not offer additional support for my hypothesis. Although increases in frequency of occurrence and duration of LTP were seen in the RSd slices, these differences were either non-significant (frequency) or were confounded by the limited sample (duration).

Limitations on the Interpretation of the Results

As has been mentioned, of the six LTP variables used, only the three facets of the population spike offered support for the hypothesis of this study. Results of the EPSP slope changes, a measure of LTP which is derived from these variables, failed to confirm the hypothesis. This was also the case for frequency of LTP occurrence. In the following discussion, I will explore some of the possible reasons for these discrepant results by pointing out that the difference in population spike amplitudes for the RSd slices may have been influenced by several extraneous variables.

First, the RSd slices were more viable than controls. This effect was unanticipated and it was the main reason for truncating the data for the data analytic procedures. One interpretation of these results is that RSd promoted the longevity in the experimental slices. A popular RS theory which postulates that RSd increases neural excitability (Cohen & Dement, 1965; Ellman et al., 1991; Handwerker & Fishbein, 1975; Owen & Bliss, 1970) seems to be consistent with this interpretation. However, it has been reported (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973) that undamaged healthy hippocampal slices can be consistently kept alive for six to

eight hours. Thus, an alternative explanation of these data is that the control procedures in someway shortened the true life span of the slices removed from animals in this treatment. Since both groups of slices were exposed to the same stimulation protocol, this would suggest that RSd allowed the RSd slices to overcome the premature death seen in the control slices. In addition to the problem with longevity, control slices exhibited an atypical depression in their post-HFT population spike values. Alterations in the stimulus intensity and/or duration protocol may result in closer to normal responses.

It should also be noted that long-term depression (LTD), as was seen in the control slices, is not a rare occurrence. It has been shown that HFT electrical stimulation similar to that which normally evokes LTP, can produce generalized depression of the post-synaptic cell (Abraham, Bliss, & Goddard, 1985; Andersen, Sundberg, Sveen, Swann, & Wigstrom, 1980; Bliss & Lomo, 1973; Lynch, Dunwidde, & Gribkoff, 1977). Dudai (1989) suggests that this LTD may be a depression manifested on the converging pathways to the post-synaptic cell which remains during the HFT to the main potentiated pathways. Unfortunately, HFT stimulations to these converging pathways have also acted associatively to create LTP (Barrionuevo & Brown, 1983; Lee, 1983; Levy & Steward, 1979). In fact, Lee (1983) showed that activation of these adjacent, converging pathways promote an enhanced LTP response. At present the cellular basis of LTD is much less understood than that of LTP and unfortunately, any clear understanding of this depression effect is forth-coming.

As a final note, RSd and control slices were alternated in the protocol to avoid any technique improvement effect. The experimenter was not blind to the treatment conditions and therefore was potentially biased towards a RSd effect, but since the

slice stability protocol was maintained for both groups it is difficult to blame experimental bias. Although there are discrepancies in the data, results are still encouraging toward support of the hypothesis that RSd promotes an augmented plastic response. However, there are still many questions remaining before data such as these, could be interpreted as unambiguous support for the RS consolidation hypothesis. Some of these questions might be addressed by the follow-up projects that are briefly described in the following paragraphs.

Suggestions for Future Research

Several adaptations to the protocol used in this study come to mind. First, and obviously, an increase in the number of tissue samples, adjustments to aCSF concentrations, and adjustments to the stimulus intensity and duration protocol seem important changes that may serve to stabilize the slice responses. Second, it would be of interest to add a treatment(s) in which subjects are exposed to some type of complex learning trial prior to collecting tissue samples. These in vitro protocols could offer further insight into the relationships and inconsistencies that emerged from this study.

Following these in vitro studies, in vivo experiments similar to those done by Bramham and Srebo (1989) could be conducted to record the susceptibility of LTP induction during naturally occurring sleep states rather than from deprived conditions. In these experiments, animals could be exposed to a particular learning task and then during the first onset of RS, stimulations of the Schaffer collateral-commissural pathway could be given in an attempt to elicit LTP. Sleep state measures, as well as hippocampal stimulation and recording electrodes, could be chronically implanted so that recordings could be made from freely moving rats or cats.

Conclusion

RS most likely has several functions. The host of behavioral data seems to support memory consolidation as one of those functions. Cellular investigations into the mechanics of learning and memory have only recently been pursued. These cellular protocols, including LTP and LTD paradigms may be a viable means of investigating the function of RS. The data presented here show cellular support for the consolidating function of RS. However, it will only be after continued investigation into the relationship between RS and learning, as per the follow-up protocols mentioned, that any real understanding of this relationship becomes evident. If we fall asleep and enter RS so that we can try to remember all that we've learned in a given day, then the experiments, like the one conducted and those suggested, are good attempts at confirming or condemning this hypothesis.

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Appendix

EPSPs percentage change from baseline for control and RSd groups from 15 to 360 minutes.

Control

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
1	-2.27	-11.36	297.73	120.45	109.09	0.00	25.00	-43.18	-52.27	-59.09	-68.18	
3	27.27	18.18	27.27	27.27	27.27							
5	-37.50	-37.50	-62.50	-62.50	-62.50	-62.50	-62.50	-62.50	-62.50	-75.00	-62.50	-75.00
7	-11.11	-22.22	-22.22	-22.22	-33.33	-44.44	-44.44	-44.44	-55.56	-55.56	-55.56	-66.67
9		-16.67	0.00	66.67	16.67	16.67	0.00	0.00	0.00	-16.67	-16.67	-16.67
11	-17.65	-17.65	-17.65	-29.41								

Time (Mins.):	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
1												
3												
5												
7	-66.67	-66.67	-66.67	-55.56	-66.67	-66.67	-77.78	-55.56	-55.56	-55.56	-44.44	-22.22
9	0.00	-16.67										
11												

RSd

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
2	-36.84	-26.32	-26.32	-26.32	-26.32	-31.58	-31.58	-36.84	-36.84	-36.84	-42.11	-42.11
4	0.00	0.00	-14.29	14.29	42.86	28.57	28.57	28.57	28.57	14.29	14.29	14.29
6	-11.76	-17.65	-11.76	-17.65	-23.53	-23.53	-17.65	0.00	-23.53	-23.53	-29.11	-35.29
8	0.00	-7.69	-15.38	-23.08	-15.38	-30.77	-30.77	-30.77	-30.77	-15.38	-23.08	-23.08
10	-30.77	-23.08	-23.08	-15.38	-23.08	-15.38	-15.38	-23.08	-23.08	-23.08	-46.15	-38.46
12	-9.52	-19.05	-23.81	-23.81	-28.57	-42.86	-42.86	-47.62	-33.33	-33.33	-38.10	-42.86

Time (Mins.)	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
2	-47.37	-47.37	-47.37	-47.37	-42.11	-47.37	-47.37	-42.11	-47.37	-52.63	-57.89	-52.63
4	14.29	0.00	0.00	14.29	14.29	14.29	28.57	28.57	57.14	71.43	14.29	0.00
6	-35.29	-35.29	-35.29	-41.18	-76.47							
8	-30.77	-30.77	-30.77	-30.77	-30.77	-38.46	-38.46	-30.77	-30.77	-38.46	-46.15	-46.15
10	-38.46	-46.15	-46.15	-53.85	-53.85	-61.54	-53.85	-61.54	-53.85	-61.54	-69.23	-69.23
12	-38.10	-52.38	-61.90	-66.67	-80.95	-9.52						

Amp1 percentage change from baseline for control and RSd groups from 15 to 360 minutes.

Control

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
1	-9.51	-6.13	-3.37	-53.07	-51.84	-56.13	-57.36	-61.96	-64.42	-73.93	-86.81	
3	5.66	-7.55	-10.38	-14.45	-16.98							
5	-60.50	-62.18	-71.43	-74.79	-81.51	-80.67	-82.65	-75.63	-76.47	-83.19	-82.35	-74.79
7	-1.69	-8.43	-13.48	-16.85	-23.03	-27.53	-28.09	-28.65	-32.02	-31.46	-27.53	-31.46
9		-40.00	-59.26	9.63	8.15	-1.48	-11.11	-9.63	-11.85	-11.11	-19.26	-22.22
11	0.00	11.64	8.47	4.23								

Time (Mins.):	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
1												
3												
5												
7	-32.02	-28.09	-28.65	-29.21	-29.21	-32.58	-31.46	-32.58	-36.52	-34.27	-34.83	-23.60
9	-25.93	-38.52										
11												

RSd

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
2	-0.67	16.00	17.33	18.00	16.00	20.00	17.33	16.67	16.67	14.00	10.67	12.00
4	-26.39	-15.28	-23.61	-25.00	-25.00	-22.22	-8.33	0.00	5.56	18.06	31.94	43.06
6	-10.81	-7.21	-5.41	0.00	-1.80	3.60	14.41	30.63	27.93	17.12	0.00	-1.80
8	-6.49	-14.29	-19.48	-12.99	-16.88	-28.57	-24.68	-15.58	-15.58	-5.19	-6.49	-10.39
10	-30.68	-11.36	-14.77	-12.50	-15.91	-17.05	-18.18	-23.86	-25.00	-30.68	-36.36	-32.95
12	-12.93	-11.64	-11.21	-9.91	-10.34	-19.83	-20.69	-19.40	-17.67	-15.52	-7.76	-6.47

Time (Mins.)	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
2	10.00	10.00	10.00	9.33	8.67	5.33	4.00	4.67	2.00	-4.00	-8.00	-7.33
4	50.00	62.50	68.06	75.00	73.61	83.33	90.28	93.06	97.22	101.39	101.39	87.50
6	8.11	19.82	10.81	3.60	-94.59							
8	-14.29	-15.58	-9.09	-16.88	-10.39	-12.99	-22.08	-18.18	-20.78	-24.68	-35.06	-37.66
10	-34.09	-36.36	-39.77	-40.91	-44.32	-47.73	-50.00	-61.36	-32.95	-39.77	-62.50	-68.18
12	-10.34	-21.55	-39.66	-36.21	-65.95	-8.62						

Amp2 percentage change from baseline for control and RSd groups from 15 to 360 minutes.

Control

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
1	-7.69	-5.77	43.13	-23.08	-18.13	-30.49	-35.71	-48.63	1.65	1.22	0.74	
3	10.00	-3.85	-6.15	-10.00	-10.77							
5	-48.84	-48.84	-53.49	-56.40	-62.79	-61.63	-63.95	-57.56	-56.98	-62.79	-62.79	-56.98
7	-0.92	-4.61	-9.22	-11.52	-14.75	-17.05	-17.05	-16.59	-19.82	-18.89	-14.75	-17.05
9		-33.88	14.75	8.20	8.74	-1.64	-13.11	-8.20	-12.57	-12.02	-18.03	-21.31
11	0.00	11.64	8.47	4.23								

Time (Mins.):	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
1												
3												
5												
7	-15.21	-12.44	-13.82	-13.36	-13.82	-16.59	-14.75	-17.51	-17.97	-17.97	-17.97	-5.99
9	-24.59	-34.97										
11												

RSd

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
2	0.60	21.69	23.49	25.30	25.90	28.92	27.71	27.71	28.91	26.51	24.70	26.51
4	-20.24	-7.14	-11.90	-10.71	-9.52	-5.95	7.14	16.67	25.00	36.90	51.19	63.10
6	0.00	5.08	8.47	16.95	17.80	23.73	36.44	54.24	53.39	42.37	22.88	18.64
8	4.44	-3.33	-8.89	-11.11	-8.89	-21.11	-15.56	-10.00	-11.11	-1.11	0.00	-5.56
10	-19.44	6.48	1.85	3.70	2.78	1.85	0.93	-0.93	-0.93	-3.70	-9.36	-6.48
12	-7.66	-6.20	-5.84	-3.28	-2.92	-13.87	-14.60	-10.58	-8.76	-6.93	1.09	2.92

Time (Mins.)	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
2	24.70	24.70	25.90	26.51	25.30	23.49	21.69	23.49	19.88	15.06	10.24	12.65
4	70.24	90.48	98.81	102.38	102.38	111.90	117.86	122.62	128.57	133.33	135.71	120.24
6	30.51	44.07	37.29	30.51	-90.68							
8	-10.00	-11.11	-4.44	-12.22	-8.89	-11.11	-21.11	-17.78	-20.00	-22.22	-31.11	-34.44
10	-8.33	-8.33	-12.04	-13.89	-17.59	-22.22	-23.15	-31.48	-0.93	-12.04	-32.41	-38.89
12	-3.65	-14.60	-30.29	-27.01	-55.11	12.14						

AVEa percentage change from baseline for control and RSd groups from 15 to 360 minutes.

Control

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
1	-8.70	-6.09	21.16	-37.39	-34.20	-42.61	-46.09	-55.07	-59.42	-70.14	-83.19	
3	7.63	-5.93	-8.47	-11.86	-13.56							
5	-53.79	-54.48	-60.69	-64.14	-70.34	-69.66	-71.72	-67.83	-64.83	-71.03	-71.03	-64.14
7	-1.02	-6.09	-11.17	-13.71	-18.27	-21.83	-21.83	-21.83	-25.38	-24.37	-20.30	-23.35
9		-36.48	-16.98	8.81	8.18	-1.89	-12.58	-8.81	-12.58	-11.95	-18.87	-22.01
11	1.98	15.35	11.88	9.41								

Time (Mins.):	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
1												
3												
5												
7	-22.84	-19.29	-20.30	-20.30	-20.81	-23.86	-22.34	-24.37	-26.40	-25.38	-25.38	-13.71
9	-25.16	-36.48										
11												

RSd

Time (Mins.):	15	30	45	60	75	90	105	120	135	150	165	180
Subject												
2	0.00	18.99	20.25	21.52	22.15	24.68	22.78	22.15	22.78	20.25	17.72	19.62
4	-23.08	-11.54	-17.95	-17.95	-16.67	-14.10	0.00	8.97	15.38	28.21	42.31	53.85
6	-5.26	-0.88	1.75	8.77	8.77	14.04	26.32	42.98	41.23	30.61	12.28	8.77
8	0.00	-8.43	-13.25	-12.05	-12.05	-24.10	-19.28	-12.05	-13.25	-2.41	-2.41	-7.23
10	-24.49	-2.04	-6.12	-4.08	-6.12	-7.14	-8.16	-11.22	-12.24	-16.33	-21.43	-18.37
12	-10.28	-8.70	-8.30	-6.30	-6.30	-32.02	-17.39	-14.62	-13.04	-11.07	-3.16	-1.58

Time (Mins.)	195	210	225	240	255	270	285	300	315	330	345	360
Subject												
2	17.72	17.72	18.35	18.35	17.09	14.56	13.29	14.56	11.39	5.70	1.27	3.16
4	60.26	76.92	84.62	89.74	89.74	98.72	105.13	108.97	114.10	117.95	119.23	105.13
6	20.18	32.46	24.56	17.54	-92.98							
8	-12.05	-13.25	-6.02	-14.46	-9.64	-12.05	-21.69	-18.07	-20.48	-22.89	-32.53	-36.14
10	-20.41	-21.43	-24.49	-26.53	-29.59	-33.67	-35.71	-44.90	-15.31	-24.49	-45.92	-52.04
12	-6.72	-17.79	-34.78	-31.23	-60.08	2.77						

Repeated measures ANOVA totals for EPSPs, Amp1, Amp2, and AVEa in five truncation conditions.

Truncation	Control n/RSd n	Treatment (A) Main Effect (F/p-value)	Repeated Measure (B) Main Effect (F/p-value)	Interaction (AB) (F/p-value)
EPSPs				
0.) 15 - 360 min. (full timeline)	1/4	0.703/.4633	2.270/.0048**	1.285/.2111
1.) 15 - 180 min.	2/6	0.047/.8350	1.670/.1054	2.555/.0108*
2.) 30 - 180 min.	3/6	0.682/.4362	2.899/.0062**	1.624/.1277
3.) 30 - 165 min.	4/6	0.015/.9042	1.882/.0684	2.129/.0377*
4.) 30 - 150 min.	4/6	0.064/.8064	1.640/.1312	2.136/.0449*
5.) 30 - 135 min.	4/6	0.196/.6699	1.426/.2134	1.944/.0849
Amp1				
0.) 15 - 360 min. (full timeline)	1/4	0.500/.5305	0.155/.9999	0.224/.9999
1.) 15 - 180 min.	2/6	7.026/.0380*	0.439/.9214	1.753/.0958
2.) 30 - 180 min.	3/6	4.983/.0608	0.384/.9386	0.771/.6430
3.) 30 - 165 min.	4/6	7.190/.0279*	0.220/.9862	1.332/.2490
4.) 30 - 150 min.	4/6	6.356/.0357*	0.236/.9745	1.109/.3705
5.) 30 - 135 min.	4/6	5.583/.0457*	0.281/.9432	0.729/.6288
Amp2				
0.) 15 - 360 min. (full timeline)	1/4	0.583/.5009	0.362/.9959	0.159/.9999
1.) 15 - 180 min.	2/6	7.991/.0301*	1.162/.3300	1.346/.2198
2.) 30 - 180 min.	3/6	6.416/.0391*	0.592/.8149	1.535/.1454
3.) 30 - 165 min.	4/6	5.930/.0409*	0.960/.4802	2.194/.0322*
4.) 30 - 150 min.	4/6	5.472/.0475*	1.037/.4181	2.562/.0172*
5.) 30 - 135 min.	4/6	4.132/.0533	0.931/.4900	2.767/.0153*
AVEa				
0.) 15 - 360 min. (full timeline)	1/4	0.559/.5091	0.254/.9997	0.184/.9999
1.) 15 - 180 min.	2/6	7.529/.0336*	0.826/.6146	1.462/.1673
2.) 30 - 180 min.	3/6	5.641/.0492*	0.525/.8668	1.114/.3640
3.) 30 - 165 min.	4/6	7.844/.0232*	0.285/.9769	2.627/.0110*
4.) 30 - 150 min.	4/6	6.763/.0316*	0.339/.9474	2.569/.0170*
5.) 30 - 135 min.	4/6	5.828/.0422*	0.409/.8925	2.152/.0527

* - $p < .05$ ** - $p < .01$