Stress
The International Journal on the Biology of Stress

ISSN: 1025-3890 (Print) 1607-8888 (Online) Journal homepage: informahealthcare.com/journals/ists20

The Enhancement of Hippocampal Primed Burst Potentiation by Dehydroepiandrosterone Sulfate (DHEAS) is Blocked by Psychological Stress

David M. Diamond, Monika Fleshner & Gregory M. Rose

To cite this article: David M. Diamond, Monika Fleshner & Gregory M. Rose (1999) The Enhancement of Hippocampal Primed Burst Potentiation by Dehydroepiandrosterone Sulfate (DHEAS) is Blocked by Psychological Stress, Stress, 3:2, 107-121, DOI: 10.3109/10253899909001116

To link to this article: https://doi.org/10.3109/10253899909001116

Published online: 07 Jul 2009.

Stress, 1999, Vol. 3(2), pp. 107-121
Reprints available directly from the publisher
Photocopying permitted by license only

The Enhancement of Hippocampal Primed Burst Potentiation by Dehydroepiandrosterone Sulfate (DHEAS) is Blocked by Psychological Stress

DAVID M. DIAMOND[a*], MONIKA FLESHNER[b] and GREGORY M. ROSE[c]

[a]*Department of Psychology and Neuroscience Program, University of South Florida, and Medical Research Service, Veterans Affairs Medical Center, Tampa, Florida, USA,* [b]*Department of Kinesiology and Applied Physiology, University of Colorado, Boulder, Colorado, USA and* [c]*Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado*

(Received December 01, 1998; Revised April 02, 1999; In final form April 08, 1999)

This series of studies investigated the effects of psychological stress and the neurosteroid dehydroepiandrosterone sulfate (DHEAS) on hippocampal primed burst (PB) and long-term (LTP) potentiation, two electrophysiological models of memory. The DHEAS and stress manipulations were performed on awake rats, and then PB and LTP were recorded while the rats were anesthetized. DHEAS enhanced PB potentiation when administered to rats under non-stress conditions, but had no effect when given to stressed rats. Further study showed that DHEAS enhanced PB potentiation only when it was administered before, but not after, the rats were stressed. The DHEAS and stress manipulations had no effect on LTP.

This study provides three major findings regarding stress, neurosteroids and hippocampal plasticity. First, DHEAS enhanced a threshold form of plasticity (PB potentiation), but had no effect on a supra-threshold form of plasticity (LTP). Second, stress blocked the DHEAS-induced enhancement of PB potentiation. Third, stress and DHEAS effects on the hippocampus were so durable they could be performed on awake animals and then be studied while the animals were anesthetized. That DHEAS enhanced a subset of forms of hippocampal plasticity under restricted behavioral conditions may help to resolve conflicting observations of DHEAS effects on cognition and mood in people.

Keywords: LTP, memory, neural plasticity, neurosteroids, psychological stress, rat

INTRODUCTION

For almost three decades, research into the neurobiology of learning and memory has focused on long-term potentiation (LTP) as the primary physiological model of memory (Izquierdo, 1994; Maren and Baudry, 1995). LTP is an enhancement of synaptic transmission produced by high frequency electrical stimulation of an afferent pathway. Although LTP can be generated in numerous brain regions, it has been studied most extensively in the hippocampus, a limbic system structure which is critically involved in memory formation (Jarrard, 1995; Zola-Morgan and Squire, 1990). LTP shares many features in common

* Corresponding author: David Diamond, Ph.D., e-mail: ddiamond@chuma1.cas.usf.edu Department of Psychology, BEH 339, 4202 E. Fowler Avenue, University of South Florida, Tampa, FL 33620 USA, Phone: (813) 974–0480, FAX: (813) 974–4617.

with memory, including rapid onset, enhanced magnitude and duration with repeated stimulation, and in many instances, drugs that impair memory also impair LTP (Davis *et al.*, 1992; Hargreaves *et al.*, 1997; Morris, 1989; Tsien *et al.*, 1996). The connection between LTP and memory has been strengthened in recent years by the finding that LTP, as with memory, can be impaired by stress or exogenous administration of stress hormones (Bodnoff *et al.*, 1995; Diamond *et al.*, 1992; Diamond *et al.*, 1994; Foy *et al.*, 1987; Gold *et al.*, 1984; Shors *et al.*, 1989). Although this area of research is not without its controversies (Caramanos and Shapiro, 1994; Eichenbaum, 1995; Holscher, 1997; Saucier and Cain, 1995), LTP remains the primary focus of studies on the neurobiology of memory.

In the last decade, investigators have shown that the threshold for inducing LTP is reduced when the pattern of electrical stimulation mimics patterns of rhythmic electrophysiological activity that occur in the hippocampus of behaving rats (Larson and Lynch, 1986; Rose and Dunwiddie, 1986). Whereas conventional LTP is normally induced by multiple trains of 100–200 pulses, we have shown that a low threshold form of LTP, referred to as primed burst (PB) potentiation, can be induced by as few as 5 physiologically patterned pulses in the behaving rat (Diamond *et al.*, 1988). Studies have also shown that the induction of PB potentiation is more sensitive than conventional LTP to modulation by behaviorally relevant influences, such as aging (Moore *et al.*, 1993), stress (Mesches *et al.*, 1998) and neurotransmitters (Corradetti *et al.*, 1992).

Recently, we found that administration of the neurosteroid dehydroepiandrosterone sulfate (DHEAS) increased the magnitude of PB potentiation (Diamond *et al.*, 1996b). Neurosteroids are a newly described category of hormones that are produced in the brain *de novo,* as well as in the periphery (Baulieu and Robel, 1990). DHEAS is the most abundant adrenal steroid produced in humans. However, levels of DHEAS decline dramatically with physical stress (Lephart *et al.*, 1987) and advanced age (Orentreich *et al.*, 1984). Whereas the stress and age-related decline in DHEAS levels correlate with the increased incidence of physical and mental health disorders, including cancer, atherosclerosis and dementia (Regelson and Kalimi, 1994a; Regelson *et al.*, 1994b; Yanase *et al.*, 1996), supplementation with DHEAS enhances cell survival (Roberts *et al.*, 1987), stimulates immune function (Araneo *et al.*, 1995b), affects neural excitability (Ffrench-Mullen and Spence, 1991; Majewska, 1992) and improves learning and memory (Diamond *et al.*, 1996a; Flood *et al.*, 1992; Flood and Roberts, 1988a; Flood *et al.*, 1988b; Frye and Sturgis, 1995). Thus, the finding of an enhancement of PB potentiation by DHEAS further supported the idea that this hormone can produce beneficial effects on brain function.

Although DHEAS has generated much enthusiasm as a possible "fountain of youth" hormone (Baulieu, 1996), under some conditions DHEAS supplementation has had no effect, or even an impairing effect, on mental functions. For example, while Yen and his co-workers have shown that DHEAS increased a sense of well-being in elderly people (Morales *et al.*, 1994; Yen *et al.*, 1995), others found that DHEAS had no effect on well-being or memory in young and elderly people (Wolf *et al.*, 1997; Wolf *et al.*, 1997). One possible explanation for the contradictory findings is that there is a complex interaction between DHEAS and stress. DHEAS is commonly seen as an anti-stress or anti-glucocorticoid hormone because it can block many of the physiological effects of glucocorticoids (Araneo and Daynes, 1995a; Shafagoj *et al.*, 1992). However, it has also been observed that glucocorticoids can interfere with DHEAS-induced changes in brain physiology (Singh *et al.*, 1994). Moreover, while several studies have found that DHEAS can enhance learning (Flood and Roberts, 1988a; Flood *et al.*, 1988b), Fleshner and co-workers (1997) reported that DHEAS impaired hippocampal-dependent fear conditioning. Thus, at this stage there is a lack of a satisfactory understanding of how DHEAS affects cognition and nervous system function in people and animals.

In this work we investigated the effects of stress and DHEAS on hippocampal PB potentiation and LTP. Specifically, we tested the hypothesis that psychological stress, i.e., exposing rats to an unfamiliar

environment, would impair the capacity for DHEAS to enhance PB potentiation and LTP. Preliminary findings from this study have been presented previously (Diamond *et al.*, 1995).

METHODS

Animals and Housing

Subjects were 53 male Sprague-Dawley rats obtained from Charles Rivers Laboratories weighing approximately 275–325 g upon arrival. The rats were housed 2–3/cage and were given one week to acclimate to the vivarium (12:12 hr light/dark cycle, lights on at 6 AM) before experimental manipulations began.

DHEAS/Saline Injection Protocols

In previous work we reported that the optimal doses of DHEAS for enhancing PB potentiation were 24 and 48 mg/kg under non-stress conditions. Lower (6 mg/kg) and higher (96 mg/kg) doses were ineffective (Diamond *et al.*, 1996b). In the work described here we focused on the capacity for stress to block the DHEAS-induced enhancement of PB potentiation. The 24 mg/kg (12 mg/cc, s.c.) dose of DHEAS was used in all groups because it has reliably produced the largest increase in PB potentiation under non-stress conditions.

There were three basic injection/recording procedures followed in these experiments. The sequence of events in each of the procedures is described in the following paragraphs and is illustrated in Figure 1.

In Protocol 1 (Figure 1) a subject was brought to the laboratory 2–5 days before the injections and recordings were initiated. DHEAS or saline (2 cc/kg, s.c.) was injected at approximately 9:20 AM on the day of the acute recording. In Protocols 2 and 3 the procedures were modified to explicitly study the effects of psychological stress on the DHEAS-induced enhancement of hippocampal plasticity. In protocol 2 (Figure 1) a subject was removed from its home cage in the vivarium, injected with DHEAS, and then placed back in its home cage. The injection was performed in the vivarium so as to minimize disturbing the subject. Ten minutes after the DHEAS injection the subject was brought to the laboratory where it was placed in an electrophysiological recording chamber (22 × 22 × 32 cm). The subject remained in the recording chamber for 20 minutes and then was injected with saline (2 cc/kg, s.c.). Ten minutes later it was anesthetized and prepared for electrophysiological recordings.

In Protocol 3, physiological saline (2 cc/kg, s.c.) was injected when the subject was in the vivarium. Ten minutes after the saline injection the subject was brought to the laboratory and placed in the recording chamber. Twenty minutes later DHEAS was injected and then ten minutes later the subject was anesthetized and prepared for electrophysiological recordings. The critical difference between Protocols 2 and 3 was that in Protocol 2 DHEAS was administered to the subjects ***before*** they were stressed (when they were in the vivarium) and in Protocol 3 DHEAS was administered ***after*** the subjects were stressed (after they had been transported to the laboratory). A control group of subjects was injected with saline in both the vivarium and in the laboratory.

Electrophysiology

The electrophysiological recording procedures employed in this study followed the same methodology we have used previously (Diamond *et al.*, 1992; Diamond *et al.*, 1996b). Subjects were given atropine sulfate (0.2 mg/kg, ip) and urethane (1.25 g/kg, ip), and if necessary, a supplement of urethane (0.2 g/kg, ip) to induce surgical depth of anesthesia. The skull was cleared of connective tissue and holes were drilled in the skull for recording and stimulating electrodes. The stimulating electrode (125 μm diameter stainless steel Teflon coated wire, uninsulated at the tip) was placed in the left side of the ventral hippocampal commissure and the recording electrode (50 μm diameter stainless steel Teflon coated wire, uninsulated at the tip) was placed in the CA1 cell layer of the right hippocampus.

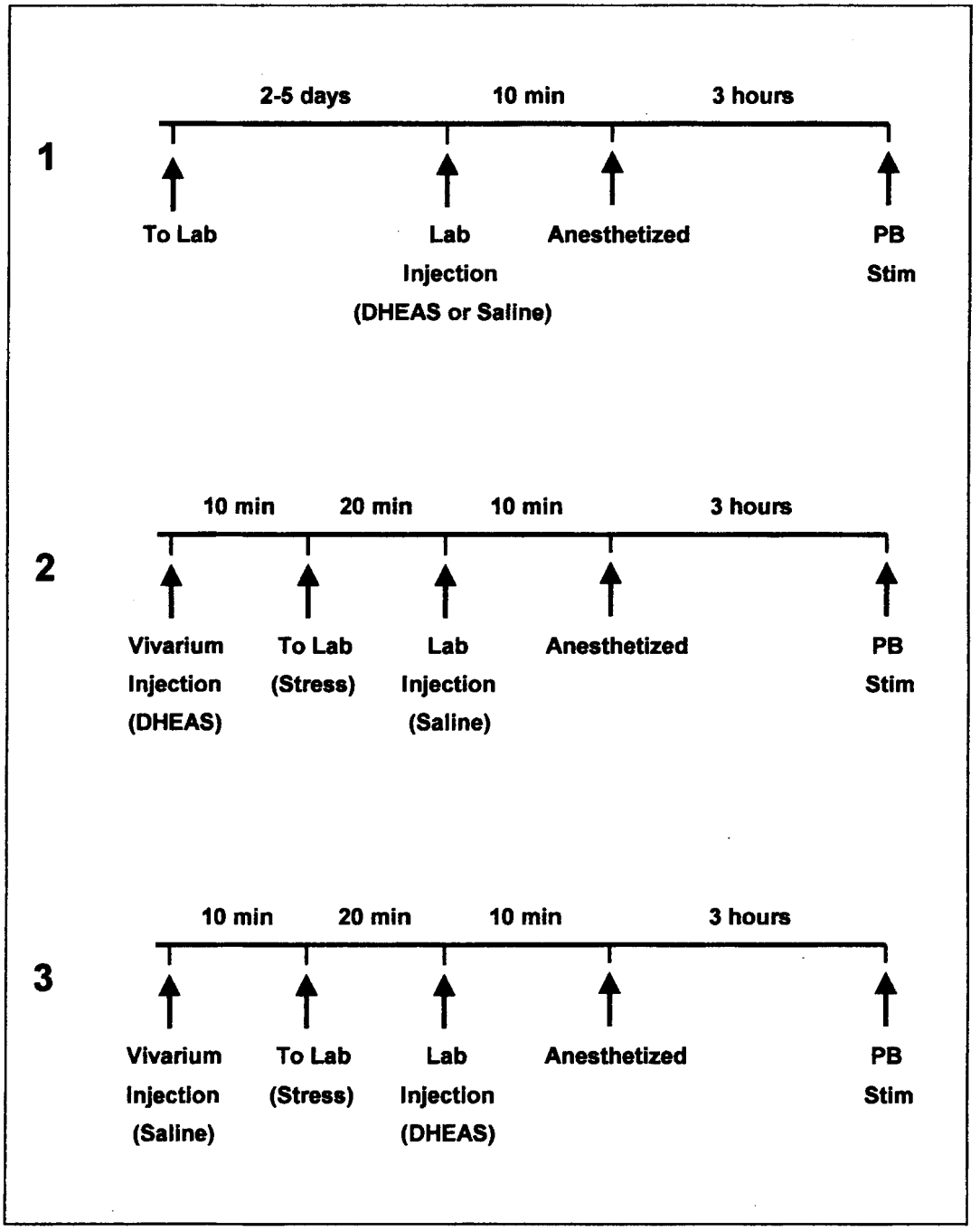

FIGURE 1 Sequence of events in the experimental protocols used in this series of studies. In Protocol 1 subjects were brought to the laboratory 2–5 days before the acute recording. In Protocol 2 subjects were injected with DHEAS in their home environment and in Protocol 3 they were injected with DHEAS after they were transported to the laboratory

Test pulse stimulation consisted of a single pulse (150μsec duration) delivered every 30 sec for 10 min prior to and 30 min following high frequency (PB or LTP) stimulation. The duration (150 μs) and current levels of all test pulses were constant within a recording session. The magnitude of the baseline current was approximately 20–25% of the current used to evoke the maximum amplitude population spike. Two forms of high frequency stimulation were used in each recording session: Primed burst (PB) stimulation, which was composed of a single pulse followed 170 msec later by a high frequency (200 Hz) burst of 4 pulses; and LTP stimulation, which was composed of a single 125 msec train of 25 pulses at 200 Hz. We used only 25 pulses in an attempt to avoid a potential "ceiling effect", i.e., an increase in response so large that it would not be possible to observe any further increases with DHEAS administration. The current level was increased by 25% during the delivery of PB and LTP stimulation, as described previously (Diamond *et al.*, 1996b). This level of stimulation consistently produces a small magnitude of PB potentiation and a large magnitude of LTP in control subjects. Thirty minutes after the PB stimulation was delivered the current was reduced, if necessary, to reduce the size of the population spike to its original baseline. Following 10 minutes of a stable baseline period, LTP stimulation was delivered and test pulses were given for the next 30 minutes to monitor any effects the LTP stimulation may have had on the magnitude of the population spike. Population spikes were digitized and stored in a computer for on- and off-line analyses.

The occurrence of PB potentiation or LTP in individual recording sessions was defined as a statistically significant increase in the magnitude of the population spike 21–30 min after PB or LTP stimulation compared to the respective 10 min pre-stimulation baseline period (t-test). Group effects were evaluated for statistical significance by ANOVA with post-hoc Student Newman Keuls Test and Fisher Exact Test for group incidence effects. Statistical effects were considered significant if $P \leq 0.05$. Data are presented as the mean (± SEM).

Blood Sampling Procedures and Plasma Corticosterone Measurement

At the conclusion of the recordings, which was approximately 4–5 hours after the DHEAS injections, 1.5 cc of blood was obtained by cardiac puncture. The blood was centrifuged and the plasma was analyzed for corticosterone levels by radioimmunoassay (RIA). The brains of the subjects were removed and stored at −70° C. Additional blood samples were obtained from two other groups of rats. In one group, rats were decapitated 20 minutes after they were brought to the laboratory. In a second group, rats were brought to the laboratory and decapitated 2–5 days later. The trunk blood samples from all rats were obtained between 9:30–10AM. Blood sampling was accomplished within 15 seconds of removing rats from their cages.

RESULTS

In the first protocol (Protocol 1; Laboratory Acclimation), two groups of subjects (n=8 per group) were removed from the vivarium and kept in a quiet area of the laboratory for 2–5 days before the injections and acute recordings took place. In the group given DHEAS, all 8 rats developed PB potentiation, with an overall group mean increase in response of 144.9% (± 28.4). By contrast, in the group given saline, only 3/8 rats developed PB potentiation, with an overall group mean increase in response of 19.9% (± 13.7) (Figure 2, Left). The differences in the incidence (Fisher Exact test, $P < .01$) and magnitude ($t = 3.96$, 14 df, $P < 0.001$) of PB potentiation between the DHEAS and Saline groups acclimated to the laboratory were significant.

LTP was unaffected by the DHEAS manipulations in the laboratory acclimated rats. Specifically, the magnitudes and incidence of LTP in the DHEAS-injected (259.3% (± 31.8) increase in 8/8 rats) and Saline-injected groups (229.3% (± 38.6) increase in 8/8 rats) were not significantly different (Figure 2, Right). Thus, acclimating rats to the laboratory environment and insulating them from environmental stress on the day of the recording allowed a DHEAS-induced enhancement of hippocampal PB potentiation, but not LTP, to be expressed.

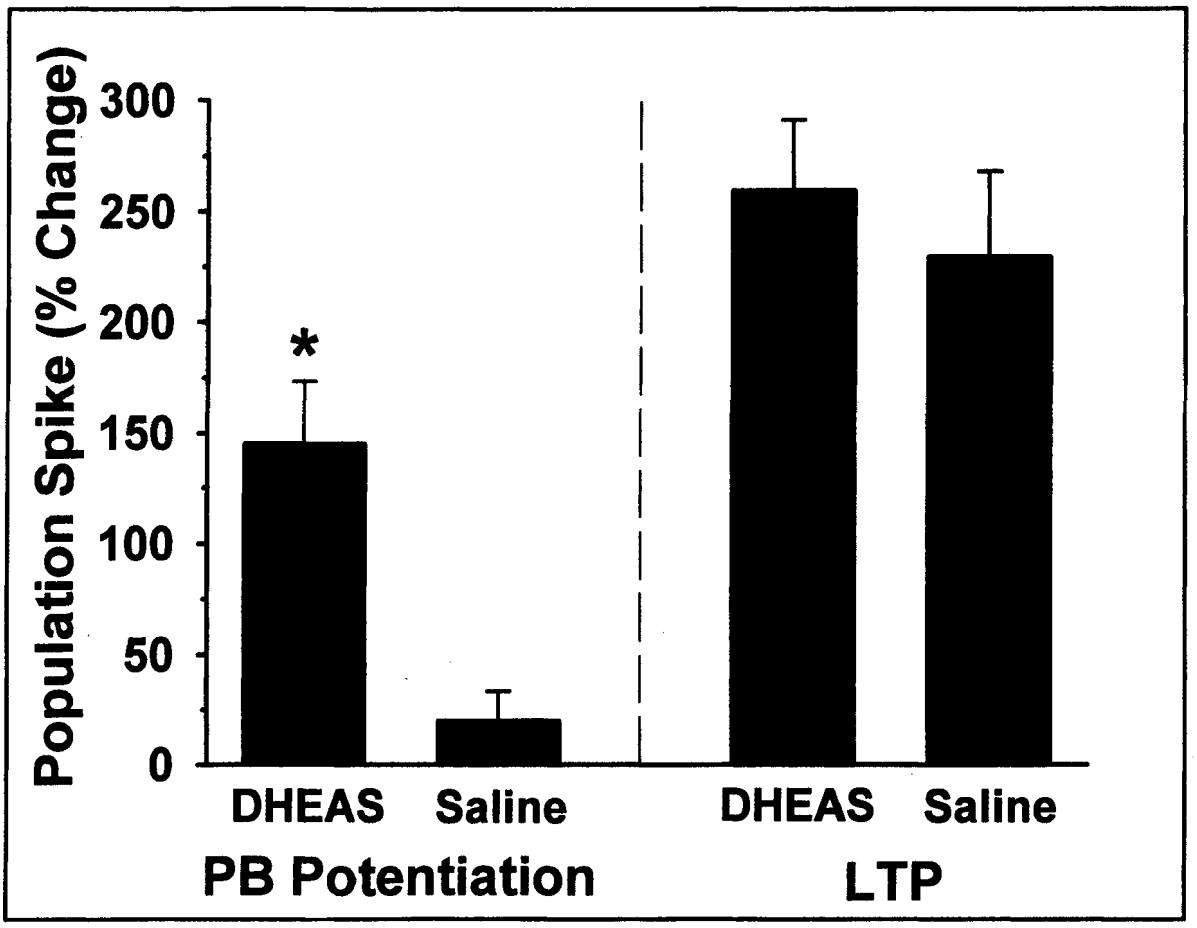

FIGURE 2 DHEAS enhanced PB potentiation, but not LTP, in rats (n=8/group) housed under "stress free" environmental conditions. The left side shows that the magnitude of PB potentiation increased with DHEAS administration, and the right side shows that the magnitude of LTP did not increase further with DHEAS administration. * = $P < 0.05$ vs. saline control group. All data in this and in subsequent graphs represent the mean (± SEM) percent change in the magnitude of response 21–30 min following the PB or LTP stimulation

In the next component of the study, rats were given DHEAS while they were in their home environment (Protocol 2) or 20 minutes after they were transported to the laboratory (Protocol 3). In the group injected with DHEAS in the home environment, PB potentiation occurred in all 8 rats and the overall magnitude of PB potentiation was a 142.7% (± 15.4) increase in response (Figure 3). For the group that was first brought to the laboratory and then injected with DHEAS, PB potentiation occurred in only 4/8 rats and the overall magnitude of PB potentiation was only a 38.9% (± 28.5) increase in response (Figure 3). In control rats injected with saline in both the vivarium and in the laboratory, 4/6 developed PB potentiation, and the overall magnitude of PB potentiation was a 60.3% (± 18.7) increase in response. The difference in the incidence of PB potentiation between each of the two DHEAS groups and saline was not significant, but the difference in magnitudes of PB potentiation among the three groups was significant ($F_{(2,19)}= 6.47$, $P < 0.01$). The group receiving DHEAS before being transported to the laboratory had significantly greater PB potentiation than either the group injected only with saline or the group injected with DHEAS

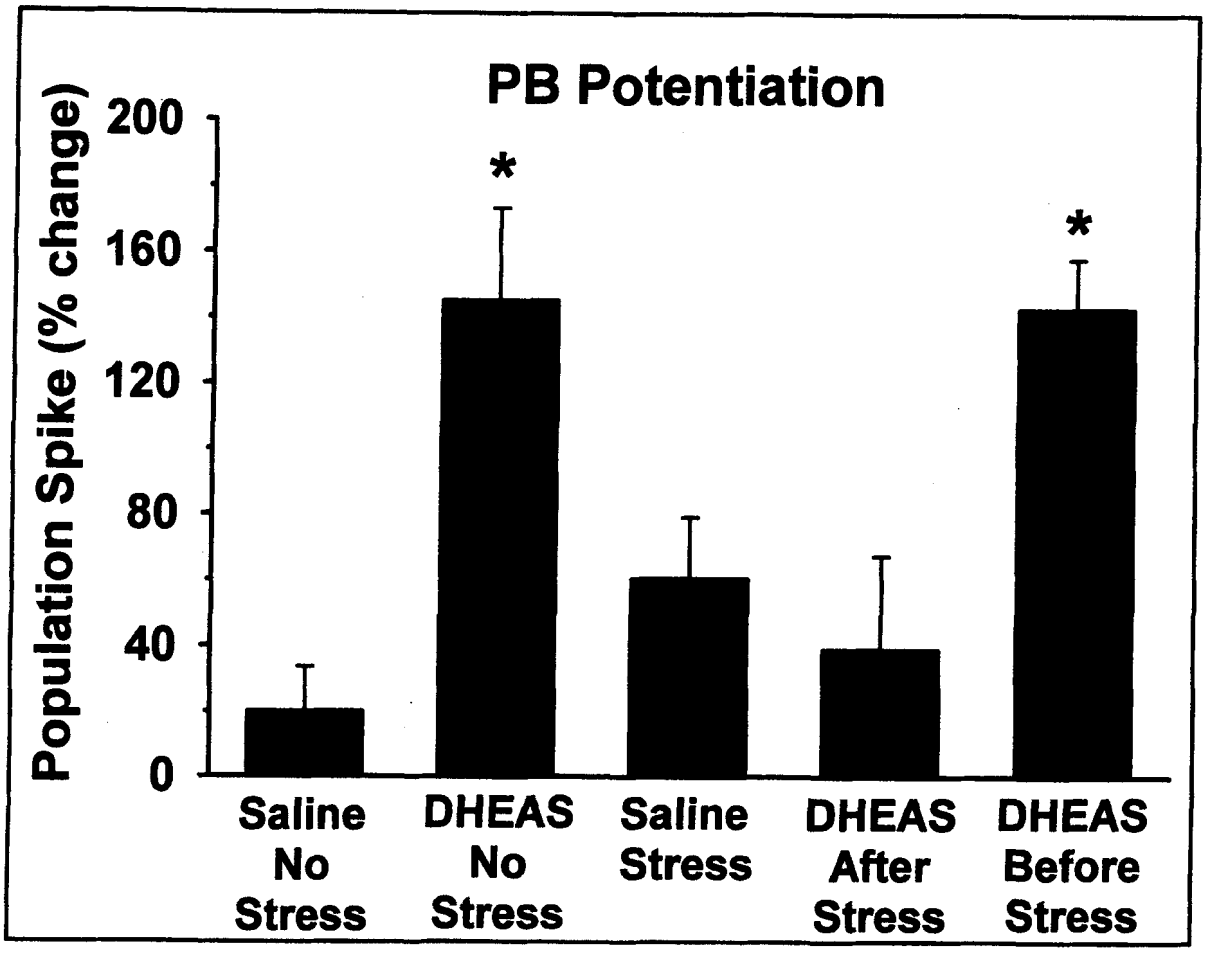

FIGURE 3 DHEAS enhanced PB potentiation only if administered in the absence of stress or before stress occurred. Data from Figure 2 are shown for comparison purposes on the left side of this figure (Saline/No Stress and DHEAS/No Stress). When DHEAS was administered after the subjects were stressed (DHEAS after Stress; n=8) there was no enhancement of PB potentiation. When DHEAS was administered before the subjects were stressed (DHEAS before Stress; n=7) there was an enhancement of PB potentiation. Saline injections in stressed rats (n=6) had no significant effect. * = $P < 0.05$ vs. saline control group

after laboratory transport (Student Newman Keuls Test, $P < 0.05$). These findings indicate that injection of DHEAS before, but not after, stress increased the magnitude of PB potentiation (Figure 3).

The timing of the injection of DHEAS either before or after laboratory transport had no influence on the incidence or magnitude of LTP (Figure 4). LTP occurred in every rat regardless of the DHEAS/Saline injection protocol. The magnitudes of LTP across groups did not differ significantly (Saline-injected: 161.5% increase (± 27.6), DHEAS before stress: 226.6% increase (± 24.8) and DHEAS after stress: 220.5% increase (± 28.8). Although there was a reduction in the mean magnitude of LTP in the Saline-Stress group, the effect was not statistically significant ($P = 0.21$).

Examples of typical population spike recordings which illustrate the effects of DHEAS administration under stress and non-stress conditions on evoked responses are presented in Figure 5. In one session

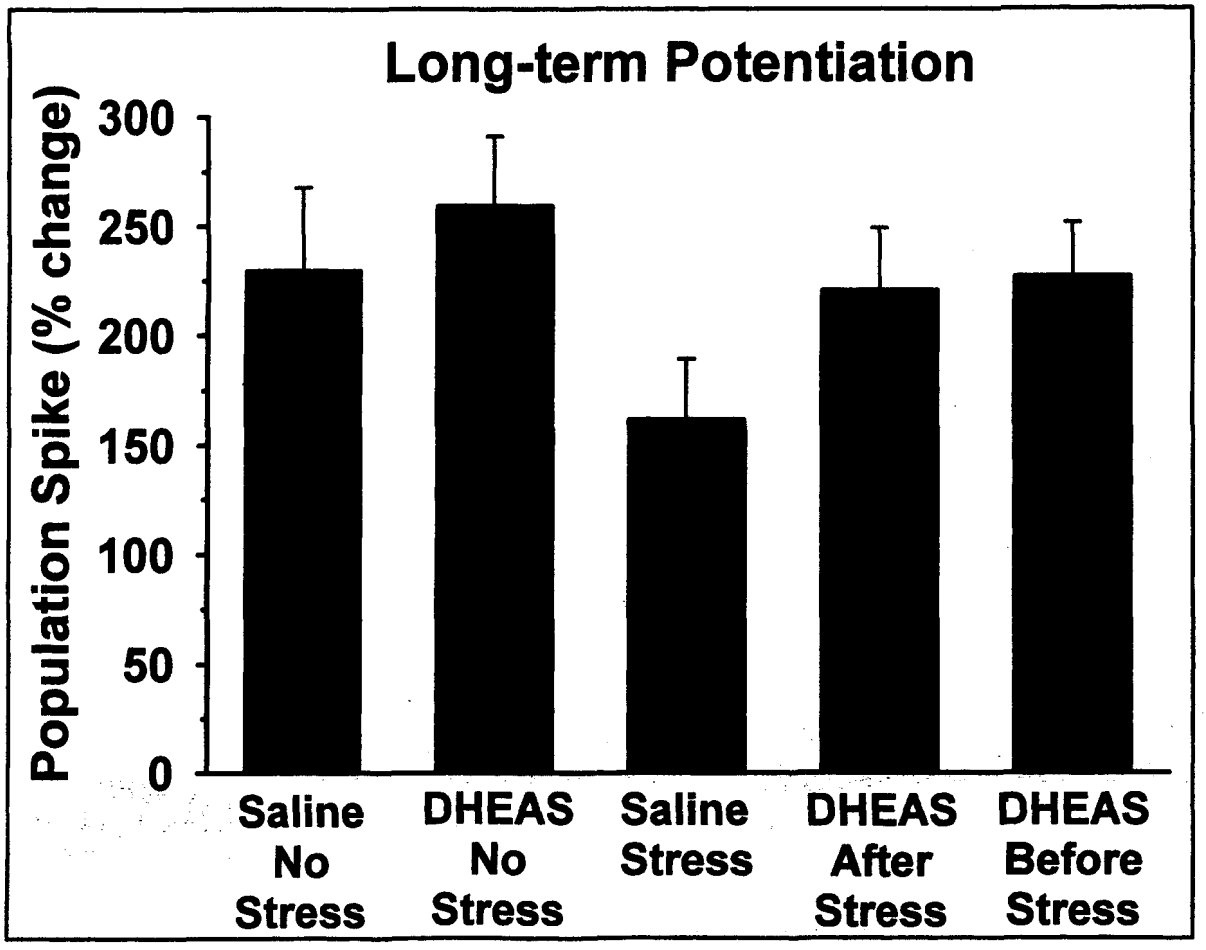

FIGURE 4 LTP was unaffected by the stress and DHEAS manipulations. Data from Figure 2 are shown for comparison purposes on the left side of this figure (Saline/No Stress and DHEAS/No Stress). DHEAS was administered either after (DHEAS after Stress; n=8) or before (DHEAS before Stress; n=7) the stress manipulation. Neither stress nor DHEAS administration had a significant effect on the magnitude of LTP. Saline injections in stressed rats (n=6) had no significant effect

(top four waveforms), the animal was given DHEAS under non-stress conditions (laboratory acclimated group; Protocol 1), and in another session (bottom four waveforms), another animal was given DHEAS under stress conditions (non-acclimated group; Protocol 3). In the first example, the magnitude of the population spike increased in response to PB stimulation (upper left two waveforms) and also in response to LTP stimulation (upper right two waveforms). In the second example, PB stimulation had no effect on the magnitude of the population spike (lower left two waveforms). In contrast, LTP stimulation, given to the same animal later in that session, produced a significant increase in response (lower right two waveforms).

Stress or DHEAS could have affected PB potentiation by altering the general excitability of the hippocampus. We tested this possibility by comparing two baseline measures of excitability across groups: 1) the amount of current used to evoke the baseline test responses; and 2) the magnitude of the baseline (Pre-high frequency) responses. The range of mean

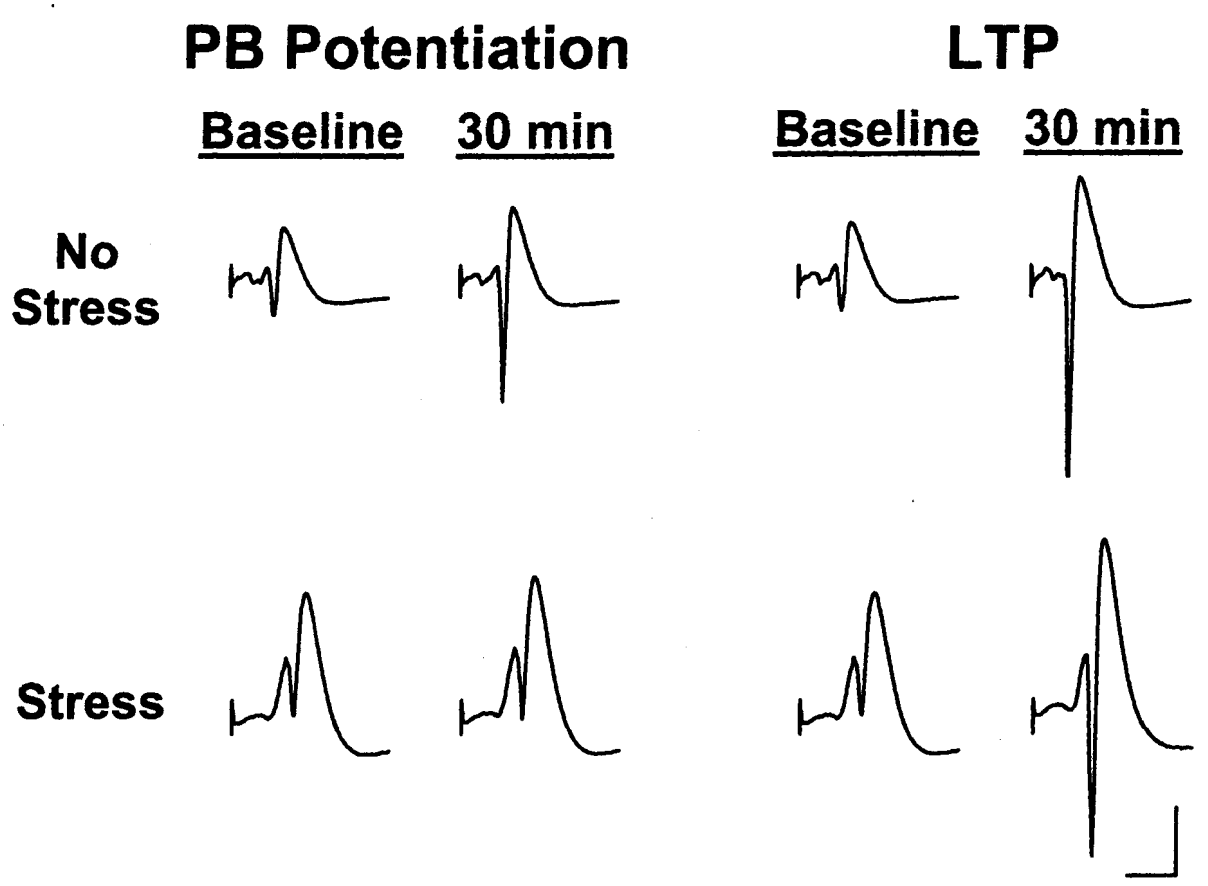

FIGURE 5 Off-line waveform averages of population spike recordings in two different recording sessions in animals given DHEAS under non-stress conditions (top; "No Stress") or under stress conditions (bottom; "Stress"). The top 4 waveforms are average responses (left to right) during the 10 minute pre-PB stimulation baseline, 21–30 minutes after PB stimulation, the 10 minute pre-LTP stimulation baseline and 21–30 minutes after LTP stimulation. The top 4 averages show an increase in response to both PB and LTP stimulation. The lower 4 averages show no change in response to PB stimulation and an increase in response to LTP stimulation under stress conditions. Calibration bar at the lower right indicates 2 mV and 10 msec

current levels across the groups was 79 – 113 μA. ANOVA on the 5 groups demonstrated that there was no significant effect of treatment on the amount of current used to evoke the baseline test responses ($F_{(4,32)}$= 0.52, P > 0.5). Similarly, ANOVA on the baseline magnitude of evoked responses during baseline testing (range 1.8 – 2.0 mV) also showed no treatment effect ($F_{(4,32)}$ = 0.39, P > 0.5). These findings indicate that, within the limitations of these extracellular baseline measurements, the stress and DHEAS manipulations did not produce a gross change in hippocampal excitability.

There was no clear connection among DHEAS treatment, corticosterone levels during anesthesia, and PB potentiation. Although DHEAS enhanced PB potentiation in the laboratory acclimated rats (Figure 2), the levels of corticosterone did not differ between the saline and DHEAS-injected groups (48 (± 2.8) vs. 50 (± 2.8) μg/dl, t-test, p > 0.05).

We did find one significant interaction between the DHEAS manipulations and corticosterone levels. The group given DHEAS before they were stressed (Protocol 2) had lower corticosterone levels (35.0 (± 2.8) μg/dl vs. 48 – 52 μg/dl) than the other groups

($F_{(4,32)} = 3.23$, $P < 0.05$, Student Newman Keuls Test, $P < 0.05$). Thus, administration of DHEAS prior to stress and the induction of anesthesia reduced the urethane-induced increase in corticosterone levels. However, the importance of this small reduction in corticosterone levels in this one group is not clear. While 35.0 μg/dl may be lower in magnitude compared to the other groups, it is still within the range of high stress levels of corticosterone.

More importantly, corticosterone data obtained from two groups (n=8/group) of awake rats confirmed that transport to the laboratory was stressful, and that 2–5 days of housing within the laboratory was a sufficient amount of time to acclimate the rats to the laboratory environment. The group that spent 2–5 days in the laboratory had significantly lower levels of corticosterone than the group that was in the laboratory for only 20 minutes (1.5 ± 0.3 μg/dl vs. 22.5 ± 3.1 μg/dl, respectively; $t = 18.3$, 14 df, $p < .001$). Hence, the conditions that produced an increase in corticosterone levels, i.e., transport to the laboratory and exposure to an unfamiliar environment, were the same conditions that blocked the DHEAS-induced enhancement of PB potentiation.

DISCUSSION

The original purpose of this research program was to investigate the effects of dehydroepiandrosterone sulfate (DHEAS) on hippocampal primed burst (PB) and long-term (LTP) potentiation, two electrophysiological models of memory. To this end, we previously reported that DHEAS increased the magnitude of PB potentiation, but not LTP (Diamond *et al.*, 1996b). The novel finding we present here is that when rats were stressed prior to being given DHEAS, no enhancement of PB potentiation occurred. DHEAS enhanced PB potentiation only when it was administered to rats that had been sheltered from stress influences. This study provides the first evidence of an inhibitory effect of stress on neurosteroid action.

Interactions Among Stress, DHEAS and Hippocampal Plasticity

In pilot studies we found that intraperitoneal or subcutaneous injections of a broad range of DHEAS doses (6 – 100 mg/kg) given before, as well as during, anesthesia were ineffective at altering the magnitude of PB potentiation or LTP (data not shown). We then considered the possibility that the stress the rats experienced in being transported to the laboratory potentially interfered with the effect of DHEAS on hippocampal plasticity. Results of previous studies provide several points in support of this idea. First, stress produces rapid changes in the binding characteristics of the $GABA_A$ receptor (Schwartz *et al.*, 1987) which is also the site of DHEAS action (Majewska, 1992). Second, stress levels of corticosterone can block PB potentiation (Diamond *et al.*, 1992) thereby interfering with a potential DHEAS-induced enhancement of hippocampal plasticity. Third, corticosterone can antagonize DHEAS effects on neuromodulatory systems (e.g., serotonin (Singh *et al.*, 1994)). Fourth, removing a rat from its home environment is a stressor (Hennessy, 1991; Hennessy *et al.*, 1979) and this manipulation alone can block PB potentiation (Diamond *et al.*, 1990; Diamond *et al.*, 1994). We therefore developed the hypothesis that stress may have interfered with a potential DHEAS-induced enhancement of PB potentiation or LTP.

In the first test of the stress hypothesis, rats were brought to the laboratory and left undisturbed for 2–5 days until the acute recording took place (laboratory acclimated group). Acclimation to the laboratory was confirmed by our finding that corticosterone levels were elevated in rats that had been in the laboratory for only 20 minutes (indicating the presence of transport stress), but not after they had spent 2–5 days in the laboratory. In the laboratory-acclimated group, DHEAS increased the magnitude of PB potentiation (Figures 2 and 3), but not LTP (Figures 2 and 4). Protecting rats from environmental stress, therefore, enabled a DHEAS-induced increase in PB potentiation to occur. While studies have shown that LTP can be affected by stress (Foy *et al.*, 1987; Kim *et al.*,

1996; Shors *et al.*, 1989) and hormonal manipulations (Gold *et al.*, 1984; Kerr *et al.*,1994; Pavlides *et al.*, 1994; Pavlides *et al.*, 1995) our findings are consistent with other work showing that LTP is less sensitive than PB potentiation to modulation by behaviorally relevant influences such as aging (Moore *et al.*,1993), stress (Mesches *et al.*, 1998) and neurotransmitters (Corradetti *et al.*, 1992).

We then tested the stress hypothesis from a different perspective. Since removing environmental perturbations ***facilitated*** a DHEAS-induced enhancement of PB potentiation (described above), then the insertion of a stressful experience at the time of the DHEAS injection would be expected to ***block*** the DHEAS-induced enhancement of PB potentiation. To evaluate this possibility we explicitly stressed rats prior to injecting them with DHEAS. Rats were brought to the laboratory and placed in a novel chamber. This procedure reliably produces behavioral signs of stress, e.g., grooming and defecation, elevated levels of corticosterone, and blocks PB potentiation in behaving rats (Diamond *et al.*, 1990; Diamond *et al.*, 1994). Under these conditions, DHEAS did not enhance PB potentiation (Figure 3). This finding supported our hypothesis that the DHEAS-induced enhancement of hippocampal plasticity can be blocked by stressful experiences occurring prior to the DHEAS injection.

In the final manipulation we evaluated the relationship between the timing of the stress manipulation and the timing of the DHEAS injection. Work to this point indicated that when DHEAS was administered to stressed rats it was ineffective at enhancing PB potentiation. What would be the outcome if DHEAS was given first (under non-stress conditions) and then the rat was stressed? The last group of rats addressed this question. DHEAS was injected while the rats were in their home (non-stress) environment. After DHEAS was injected the rats were brought to the laboratory, placed in a novel environment (stress manipulation), and then anesthetized for the recording. In this experiment, DHEAS still produced a significant enhancement of PB potentiation (Figure 3). DHEAS, therefore, increased PB potentiation only when the hormone was administered either in the absence, or in advance, of a stress experience.

The basis of the stress-DHEAS interaction we have observed is unknown, given the novelty of our observations and the absence of experimental work directly addressing how stress can block neurosteroid actions. However, sufficient work has been accomplished on stress-plasticity interactions and neurosteroid physiology that limited speculation is warranted.

In our prior work (Diamond *et al.*, 1992; Diamond *et al.*, 1996b) as well as in the current study, we found that urethane-anesthetized animals have elevated (stress) levels of corticosterone. This observation is consistent with other work showing that urethane (Hamstra *et al.*,1984; Spriggs and Stockham, 1964) like other anesthetics (e.g., pentobarbital (Engstrom *et al.*, 1990)), produces sustained increases in corticosterone levels. The elevated corticosterone levels reported here (for the anesthetized animals) were therefore produced by urethane-induced activation of the pituitary-adrenal axis and do not reflect the influence of the pre-anesthesia stress or DHEAS manipulations.

We reported previously that there was an inverted-U relationship between the level of corticosterone and the magnitude of PB potentiation in urethane anesthetized rats. That is, maximal PB potentiation developed in animals that had intermediate (10–20 μg/dl) levels of corticosterone, and reduced PB potentiation occurred in animals with very low (0–9 μg/dl) or very high (>20 μg/dl) levels of corticosterone (Diamond *et al.*, 1992). In the current study, once again, corticosterone levels in control anesthetized animals were very high (>20 μg/dl) and the magnitude of PB potentiation was low. By contrast, in two of the DHEAS-injected groups (DHEAS before stress and DHEAS with no stress), PB potentiation was enhanced. It was therefore possible that, in these two groups, DHEAS attenuated the urethane-induced increase in corticosterone levels. If this attenuation did occur, then perhaps DHEAS reduced corticosterone levels into the optimal range (10–20 μg/dl) for enhancing PB potentiation. This explanation of the findings is therefore based on the idea that DHEAS enhanced PB potentiation indirectly, by

altering corticosterone levels. However, this hypothesis can be rejected because corticosterone levels in the DHEAS-injected groups were very high (all were greater than 20 μg/dl). Thus, DHEAS enhanced PB potentiation, despite the presence of high (stress) levels of corticosterone.

A second hypothesis is that the critical DHEAS and stress effects on the hippocampus developed while the animals were awake, and then those changes in hippocampal physiology were expressed hours later, while the animals were anesthetized. In this view, hormonal-induced changes in hippocampal physiology in the awake state are so durable that they can influence plasticity when subsequently studied in the anesthetized animal. This hypothesis is supported by prior findings utilizing two different methodological approaches. First, numerous studies have shown that behavioral and hormonal manipulations performed on behaving animals produce intrinsic changes to hippocampal physiology that can be studied *in vitro*. For example, Hesen and Joels (1996) showed that *in vivo* administration of corticosterone altered hippocampal cholinergic responsiveness, subsequently recorded *in vitro*. Other studies have shown that hippocampal slices obtained from stressed rats exhibit significantly reduced PB potentiation and LTP (Kim *et al.*, 1996; Mesches *et al.*, 1998; Shors *et al.*, 1989). These studies indicate that a stress experience produces changes in hippocampal processing that can be studied long after the structure is removed from hormonal and extra-hippocampal influences.

Second, and more germane to the current methodology, are the findings of Pavlides *et al.*, (1993) and Xu *et al.*, (1998). In these studies either corticosterone or stress was administered to awake rats and then LTP was recorded while the animals were anesthetized. Recordings from corticosterone-injected or previously stressed rats yielded significantly reduced LTP, and the stress effects were blocked by a corticosterone receptor antagonist (Xu *et al.*, 1998). Thus, stress or hormone manipulations performed on an awake animal can influence the expression of plasticity when the animal is subsequently anesthetized. Corticosterone, therefore, acting through steroid receptors, transcriptional factors and protein synthesis (Joels *et al.*, 1995; Karst and Joels, 1991) can produce long-lasting effects on hippocampal physiology and plasticity. In theory, the stress-induced rise of corticosterone blocked the enhancement of PB potentiation by DHEAS. Corticosterone acted functionally as a DHEAS antagonist, but only if the stress-induced rise of corticosterone levels preceded the administration of DHEAS.

Additional study using the combined awake/anesthetized and *in vitro* preparations can investigate further how DHEAS and stress (corticosterone) interact to influence hippocampal plasticity. It should be noted, however, that while the anesthetized and *in vitro* preparations are useful, ultimately, a thorough understanding of how stress and DHEAS affect hippocampal-function will require study of the intact hippocampus in behaving animals.

Relevance of the Current Findings Toward Understanding DHEAS Effects on People

The work presented here may be relevant toward understanding inconsistencies in DHEAS (or DHEA) effects on people. Some studies have shown that DHEAS enhances memory and a sense of well-being (Morales *et al.*, 1994), (Wolkowitz *et al.*, 1995; Wolkowitz *et al.*, 1997; Yen *et al.*, 1995) but other studies have not replicated these findings (Wolf *et al.*, 1997; Wolf *et al.*, 1998; Wolf *et al.*,1997). In fact, Wolf and Kirschbaum (1998) have questioned whether the evidence is strong enough to warrant routine replacement of DHEAS in the elderly. This issue will not be resolved here, but we can identify two potential confounding variables which may interfere with DHEAS effects in people. First, there is an inverted-U function between DHEAS and both, the enhancement of PB potentiation (Diamond *et al.*, 1996b) and learning (Diamond *et al.*, 1996a; Flood *et al.*, 1988b). It may therefore be necessary to identify the doses of DHEAS that are within the optimal range of an inverted-U dose-response function that presumably occurs in people. Second, our present work indicates that stress can block DHEAS effects on brain physiology. We have shown that DHEAS enhanced hippocampal function only when administered to rats

that were maintained in a "stress free" environment. Clearly, people weren't isolated in "stress free" environments while participating in the DHEAS studies, thereby inviting the possibility that any stress they experienced before and during the study period may have influenced the effectiveness of the DHEAS treatments. Subsequent studies in people may need to take into account the sensitivity of the dose-dependency of DHEAS effects on the brain, and the possibility that stress may antagonize the effects of DHEAS supplementation on mental health.

CONCLUSIONS

Our findings shed light on the complex effects of DHEAS on nervous system function. We have provided the first evidence that stress can block neurosteroid action. That is, DHEAS enhanced PB potentiation when given under non-stress conditions, but was ineffective when given to stressed rats. Our work indicates, therefore, that the timing between DHEAS administration and stress can have a profound effect on the capacity for DHEAS to enhance learning-related synaptic plasticity. Moreover, DHEAS enhanced a threshold form of plasticity (PB potentiation) and had no effect on a supra-threshold form of plasticity (LTP). That DHEAS enhanced a subset of forms of hippocampal plasticity under restricted behavioral conditions may prove to be relevant toward resolving conflicting observations of DHEAS effects on cognition and mood in people.

Acknowledgements

The authors thank Berrilyn Branch for assisting in the electrophysiological recordings. This work was supported by the Office of Naval Research (ONR N00014–91-J-1753; DMD) and Merit Review funding from the Veterans Administration (DMD and GMR).

References

Araneo, B. and Daynes, R. (1995a) Dehydropepiandrosterone functions as more than an antiglucocorticoid in preserving immunocompetence after thermal injury. *Endocrinology*, **136**, 393–401.

Araneo, B., Dowell, T., Woods, M. L., Daynes, R., Judd, M. and Evans, T. (1995b) DHEAS as an effective vaccine adjuvant in elderly humans – Proof- of-principle studies. *Annals of the New York Academy of Sciences*, **774**, 232–248.

Baulieu, E. E. (1996) Dehydroepiandrosterone (DHEA): A fountain of youth? *Journal of Clinical Endocrinology and Metabolism*, **81**, 3147–3151.

Baulieu, E. E. and Robel, P. (1990) Neurosteroids: a new brain function? *J. Steroid Biochem. Mol. Biol.*, **37**, 395–403.

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M. and Meaney, M. J. (1995) Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *Journal of Neuroscience*, **15**, 61–69.

Caramanos, Z. and Shapiro, M. L. (1994) Spatial memory and *N*-methyl-D-aspartate receptor antagonists APV and MK-801: Memory impairments depend on familiarity with the environment, drug dose, and training duration. *Behavioral Neuroscience*, **108**, 30–43.

Corradetti, R., Ballerini, L., Pugliese, A. M. and Pepeu, G. (1992) Serotonin blocks the long-term potentiation induced by primed burst stimulation in the CA1 region of rat hippocampal slices. *Neuroscience*, **46**, 511–518.

Davis, S., Butcher, S. P. and Morris, R. G. M. (1992) The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP *in vivo* at intracerebral concentrations comparable to those that block LTP *in vitro*. *J. Neurosci.*, **12**, 21–34.

Diamond, D. M., Bennett, M. C., Fleshner, M. and Rose, G. M. (1992) Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus.*, **2**, 421–430.

Diamond, D. M., Bennett, M. C., Stevens, K. E., Wilson, R. L. and Rose, G. M. (1990) Exposure to a novel environment interferes with the induction of hippocampal primed burst potentiation. *Psychobiology*, **18**, 273–281.

Diamond, D.M., Branch, B.J., Coleman-Mesches, K., Mesches, M.H. and Fleshner, M., DHEAS enhances spatial memory and hippocampal primed burst potentiation, *Soc. Neurosci. Abst.*, 22 (1996a) 140(Abstract)

Diamond, D. M., Branch, B. J. and Fleshner, M. (1996b) The neurosteroid dehydroepiandrosterone sulfate (DHEAS) enhances hippocampal primed burst, but not long-term, potentiation. *Neuroscience Letters*, **202**, 204–208.

Diamond, D. M., Branch, B. J., Fleshner, M. and Rose, G. M. (1995) Effects of dehydroepiandrosterone sulfate and stress on hippocampal electrophysiological plasticity. *Annals of the New York Academy of Sciences*, **774**, 304–307.

Diamond, D. M., Dunwiddie, T. V. and Rose, G. M. (1988) Characteristics of hippocampal primed burst potentiation in vitro and in the awake rat. *J. Neurosci.*, **8**, 4079–4088.

Diamond, D. M., Fleshner, M. and Rose, G. M. (1994) Psychological stress repeatedly blocks hippocampal primed burst potentiation in behaving rats. *Behavioural Brain Research*, **62**, 1–9.

Eichenbaum, H. (1995) Spatial learning. The LTP-memory connection [news]. *Nature*, **378**, 131–132.

Engstrom, D. A., Bennett, M. C., Stevens, K. E., Wilson, R. L., Diamond, D. M., Fleshner, M. and Rose, G. M. (1990) Modulation of hippocampal primed burst potentiation by anesthesia. *Brain Res.*, **521**, 148–152.

Ffrench-Mullen, J. M. and Spence, K. T. (1991) Neurosteroids block Ca2+ channel current in freshly isolated hippocampal CA1 neurons. *Eur. J. Pharmacol.*, **202**, 269–272.

Fleshner, M., Pugh, C. R., Tremblay, D. and Rudy, J. W. (1997) DHEA-S selectively impairs contextual-fear conditioning: support for the antiglucocorticoid hypothesis. *Behavioral Neuroscience,* **111,** 512–7.

Flood, J. F., Morley, J. E. and Roberts, E. (1992) Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc. Natl. Acad. Sci. U.S.A.,* **89,** 1567–1571.

Flood, J. F. and Roberts, E. (1988a) Dehydroepiandrosterone sulfate improves memory in aging mice. *Brain Res.,* **448,** 178–181.

Flood, J. F., Smith, G. E. and Roberts, E. (1988b) Dehydroepiandrosterone and its sulfate enhance memory retention in mice. *Brain Res.,* **447,** 269–278.

Foy, M. R., Stanton, M. E., Levine, S. and Thompson, R. F. (1987) Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behavioral & Neural Biology,* **48,** 138–149.

Frye, C. A. and Sturgis, J. D. (1995) Neurosteroids affect spatial/reference, working, and long-term memory of female rats. *Neurobiology of Learning & Memory,* **64,** 83–96.

Gold, P. E., Delanoy, R. L. and Merrin, J. (1984) Modulation of long-term potentiation by peripherally administered amphetamine and epinephrine. *Brain Res.,* **305,** 103–107.

Hamstra, W. N., Doray, D. and Dunn, J. D. (1984) The effect of urethane on pituitary-adrenal function of female rats. *Acta Endocrinol. (Copenh.),* **106,** 362–367.

Hargreaves, E. L., Côté, D. and Shapiro, M. L. (1997) A dose of MK801 previously shown to impair spatial learning in the radial maze attenuates primed burst potentiation in the dentate gyrus of freely moving rats. *Behavioral Neuroscience,* **111,** 35–48.

Hennessy, M. B. (1991) Sensitization of the plasma corticosterone response to novel environments. *Physiol. Behav.,* **50,** 1175–1179.

Hennessy, M. B., Heybach, J. P., Vernikos, J. and Levine, S. (1979) Plasma corticosterone concentrations sensitively reflect levels of stimulus intensity in the rat. *Physiology & Behavior,* **22,** 821–825.

Hesen, W. and Joëls, M. (1996) Cholinergic responsiveness of rat CA1 hippocampal neurons *in vitro:* Modulation by corticosterone and stress. *Stress,* **1,** 65–72.

Holscher, C. (1997) Long-term potentiation: a good model for learning and memory?. [Review] [69 refs]. *Progress in Neuro. -Psychopharmacology & Biological Psychiatry,* **21,** 47–68.

Izquierdo, I. (1994) Pharmacological evidence for a role of long-term potentiation in memory. *FASEB Journal,* **8,** 1139–1145.

Jarrard, L. E. (1995) What does the hippocampus really do?. [Review] [42 refs]. *Behavioural Brain Research,* **71,** 1–10.

Joels, M., Hesen, W. and de Kloet, E. R. (1995) Long-term control of neuronal excitability by corticosteroid hormones. [Review] [72 refs]. *Journal of Steroid Biochemistry & Molecular Biology,* **53,** 315–323.

Karst, H. and Joels, M. (1991) The induction of corticosteroid actions on membrane properties of hippocampal CA1 neurons requires protein synthesis. *Neuroscience Letters,* **130,** 27–31.

Kerr, D. S., Huggett, A. M. and Abraham, W. C. (1994) Modulation of hippocampal long-term potentiation and long- term depression by corticosteroid receptor activation. *Psychobiology,* **22,** 123–133.

Kim, J. J., Foy, M. R. and Thompson, R. F. (1996) Behavioral stress modifies hippocampal plasticity through N- methyl-D-aspartate receptor activation. *Proceedings of the National Academy of Sciences of the United States ofAmerica,* **93,** 4750–4753.

Larson, J. and Lynch, G. (1986) Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. *Science,* **232,** 985–988.

Lephart, E. D., Baxter, C. R. and Parker, C. R., Jr. (1987) Effect of burn trauma on adrenal and testicular steroid hormone production. *Journal of Clinical Endocrinology & Metabolism,* **64,** 842–848.

Majewska, M. D. (1992) Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. [Review] [127 refs]. *Progress. in Neurobiology.,* **38,** 379–395.

Maren, S. and Baudry, M. (1995) Properties and mechanisms of long-term synaptic plasticity in the mammalain brain: Relationships to learning and memory. *Neurobiol. Learning Memory,* **63,** 1–18.

Mesches M. H., Rose G. M., Fleshner M., Heman K. L. and Diamond D. M., Exposing rats to a predator blocks hippocampal primed burst potentiation in vitro, *Proceedings of the 27th Annual Society for Neuroscience Meeting,* (In Press)

Moore, C. I., Browning, M. D. and Rose, G. M. (1993) Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged Fischer 344 rats. *Hippocampus,* **3,** 57–66.

Morales, A. J., Nolan, J. J., Nelson, J. C. and Yen, S. S. (1994) Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *J. Clin. Endocrinol. Metab.,* **78,** 1360–1367.

Morris, R. G. (1989) Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D- aspartate receptor antagonist AP5. *J. Neurosci.,* **9,** 3040–3057.

Orentreich, N., Brind, J. L., Rizer, R. L. and Vogelman, J. H. (1984) Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *Journal of Clinical Endocrinology and Metabolism,* **59,** 551–555.

Pavlides, C., Kimura, A., Magarinos, A. M. and McEwen, B. S. (1994) Type I adrenal steroid receptors prolong hippocampal long-term potentiation. *Neuro Report,* **5,** 2673–2677.

Pavlides, C., Watanabe, Y., Magarinos, A. M. and McEwen, B. S. (1995) Opposing roles of type I and type II adrenal steroid receptors in hippocampal long-term potentiation. *Neuroscience,* **68,** 387–394.

Pavlides, C., Watanabe, Y. and McEwen, B. S. (1993) Effects of glucocorticoids on hippocampal long-term potentiation. *Hippocampus.,* **3,** 183–192.

Regelson, W. and Kalimi, M. (1994a) Dehydroepiandrosterone (DHEA)--The multifunctional steroid. II. Effects on the CNS, cell proliferation, metabolic and vascular, clinical and other effects. Mechanism of action. *Annals of the New York Academy of Sciences,* **719,** *564–575.*

Regelson, W., Loria, R. and Kalimi, M. (1994b) Dehydroepiandrosterone (DHEA)--The "mother steroid". I. Immunologic action. *Annals of the New York Academy of Sciences,* **719,** 553-563.

Roberts, E., Bologa, L., Flood, J. F. and Smith, G. E. (1987) Effects of dehydroepiandrosterone and its sulfate on brain tissue in culture and on memory in mice. *Brain Res.,* **406,** 357–362.

Rose, G. M. and Dunwiddie, T. V. (1986) Induction of hippocampal long-term potentiation using physiologically patterned stimulation. *Neurosci. Lett.,* **69,** 244–248.

Saucier, D. and Cain, D. P. (1995) Spatial learning without NMDA receptor-dependent long-term potentiation. *Nature,* **378,** 186–189.

Schwartz, R. D., Wess, M. J., Labarca, R., Skolnick, P. and Paul, S. M. (1987) Acute stress enhances the activity of the GABA receptor-gated chloride ion channel in brain. *Brain Research.*, **411,** 151–155.

Shafagoj, Y., Opoku, J., Qureshi, D., Regelson, W. and Kalimi, M. (1992) Dehydroepiandrosterone prevents dexamethasone-induced hypertension in rats. *American Journal of Physiology,* **263,** E210–3.

Shors, T. J., Seib, T. B., Levine, S. and Thompson, R. F. (1989) Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science,* **244,** 224–226.

Singh, V. B., Kalimi, M., Phan, T. H. and Boadle-Biber, M. C. (1994) Intracranial dehydroepiandrosterone blocks the activation of tryptophan hydroxylase in response to acute sound stress. *Mol. Cell Neurosci., 5,* 176–181.

Spriggs, T. L. B. and Stockham, M. A. (1964) Urethane anaesthesia and pituitary-adrenal function in the rat. *J. Pharm. Pharmacol.,* **16,** 603–610.

Tsien, J. Z., Huerta, P. T. and Tonegawa, S. (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell,* **87,** 1327–1338.

Wolf, O. T. and Kirschbaum, C. (1998) Wishing a dream came true: DHEA as a rejuvenating treatment?. [Review] [30 refs]. *Journal. of Endocrinological. Investigation.,* **21,** 133–135.

Wolf, O. T., Köster, B., Kirschbaum, C., Pietrowsky, R., Kern, W., Hellhammer, D. H., Born, J. and Fehm, H. L. (1997) A single administration of dehydroepiandrosterone does not enhance memory performance in young healthy adults, but immediately reduces cortisol levels. *Biological Psychiatry,* **42,** 845–848.

Wolf O. T., Naumann E., Hellhammer D. H. and Kirschbaum C., Effects of dehydroepiandrosterone (DHEA) replacement in elderly men on event related potentials (ERPs), memory and well-being, *Journal of Gerontology,* (In Press)

Wolf, O. T., Neumann, O., Hellhammer, D. H., Geiben, A. C., Strasburger, C. J., Dressendorfer, R. A., Pirke, K. M. and Kirschbaum, C. (1997) Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly women and men. *Journal. of* Clinical. *Endocrinology & Metabolism,* **82,** 2363–2367.

Wolkowitz, O. M., Reus, V. I., Roberts, E., Manfredi, F., Chan, T., Ormiston, S., Johnson, R., Canick, J., Brizendine, L. and Weingartner, H. (1995) Antidepressant and cognition-enhancing effects of DHEA in major depression. *Annals of the New York Academy of Sciences,* **774,** 337–339.

Wolkowitz, O. M., Reus, V. I., Roberts, E., Manfredi, F., Chan, T., Raum, W. J., Ormiston, S., Johnson, R., Canick, J., Brizendine, L. and Weingartner, H. (1997) Dehydroepiandrosterone (DHEA) treatment of depression. *Biological Psychiatry,* **41,** 311–318.

Xu, L., Holscher, C., Anwyl, R. and Rowan, M. J. (1998) Glucocorticoid receptor and protein/RNA synthesis-dependent mechanisms underlie the control of synaptic plasticity by stress. *Proceedings of the National Academy of Sciences of the United States of America,* **95,** 3204–3208.

Yanase, T., Fukahori, M., Taniguchi, S., Nishi, Y., Sakai, Y., Takayanagi, R., Haji, M. and Nawata, H. (1996) Serum dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) in Alzheimer's disease and in cerebrovascular dementia. *Endocrine Journal,* **43,** 119–123.

Yen, S. S. C., Morales, A. J. and Khorram, O. (1995) Replacement of DHEA in aging men and women – Potential remedial effects. *Annals of the New York Academy of Sciences,* **774,** 128–142.

Zola-Morgan, S. M. and Squire, L. R. (1990) The primate hippocampal formation: evidence for a time-limited role in memory storage. *Science,* **250,** 288–290.