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Behavioral stress modifies hippocampal plasticity through *N*-methyl-D-aspartate receptor activation

(learning/memory/long-term potentiation/long-term depression/learned helplessness)

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ABSTRACT **Behavioral stress has detrimental effects on subsequent cognitive performance in many species, including humans. For example, humans exposed to stressful situations typically exhibit marked deficits in various learning and memory tasks. However, the underlying neural mechanisms by which stress exerts its effects on learning and memory are unknown. We now report that in adult male rats, stress (i.e., restraint plus tailshock) impairs long-term potentiation (LTP) but enhances long-term depression (LTD) in the CA1 area of the hippocampus, a structure implicated in learning and memory processes. These effects on LTP and LTD are prevented when the animals were given CGP39551 (the carboxyethylester of CGP 37849; DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid), a competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, before experiencing stress. In contrast, the anxiolytic drug diazepam did not block the stress effects on hippocampal plasticity. Thus, the effects of stress on subsequent LTP and LTD appear to be mediated through the activation of the NMDA subtype of glutamate receptors. Such modifications in hippocampal plasticity may contribute to learning and memory impairments associated with stress.**

It is now well-documented that behavioral stress impairs an organism's subsequent ability to acquire and retain information, a phenomenon known as "learned helplessness" (1, 2). When events are perceived to be uncontrollable, the organism learns that its behavior and outcomes are independent; this learning seems to produce cognitive, emotional, and motivational deficits (for review, see ref. 3). For instance, Vietnam combat veterans diagnosed with posttraumatic stress disorder exhibit marked deficits in immediate, delayed, and long-term recall tasks when compared with other military enlistees not diagnosed with posttraumatic stress disorder (4, 5). In laboratory settings, dogs, cats, rats, and even fish have shown learned helplessness after exposure to a series of inescapable electric shocks (3). It now appears that stress interferes with performance in hippocampal-dependent tasks such as Olton's radial-arm maze (6, 7), but facilitates performance in hippocampal-independent tasks such as delay eyeblink conditioning in both rats (8) and humans (9).

Rats exposed to uncontrollable stress (restraint plus shock) also show an impairment in long-term potentiation (LTP) in the hippocampus (10). Interestingly, rats able to control shock schedule do not show LTP impairment unlike "yoked" animals receiving the identical shock schedule without control (11). LTP refers to a sustained enhancement of synaptic transmission that follows a brief tetanic stimulation of afferent fibers (12, 13). In addition to longevity, LTP is rapidly induced, strengthened by repetition, demonstrates specificity and associativity, and occurs prominently in the hippocampus, a structure implicated in learning and memory processes (14). Because of these properties, LTP is widely regarded as a potential synaptic mechanism underlying information storage (15, 16). Other forms of stress, such as exposure to a novel environment, have been shown to block primed-burst potentiation (a low threshold form of LTP) in the hippocampus (17, 18). It is thus possible that learning and memory deficits associated with stress may in part be due to LTP impairment in the hippocampus. However, the mechanism by which stress impairs LTP is not well-understood.

It is conceivable that stress occludes subsequent LTP by elevating the basal synaptic transmission level within the hippocampus. LTP is known to be saturable (14); if stress produces LTP or LTP-like changes in the hippocampus, then the ensuing LTP can be occluded. To test this hypothesis, we first examined whether stress affects another form of plasticity, known as homosynaptic long-term depression (LTD). LTD is characterized by a decrease in synaptic efficacy following low-frequency stimulation of afferent fibers and, like LTP, has several properties desirable for information storage (e.g., longevity and input specificity) (19–21). Previous induction of LTP "primes" synaptic transmission such that the induction of LTD (or depotentiation) is enhanced (22). Thus, if stress produces LTP or LTP-like changes in the hippocampus, then LTD should likewise be enhanced. Additionally, because the induction of LTP in the hippocampus (e.g., the CA1 area) requires activation of NMDA receptors (23, 24), we tested whether CGP39551 (the carboxyethylester of CGP 37849; DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid), a competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist known to block LTP (25), can prevent the stress effects on subsequent LTP.

MATERIALS AND METHODS

Stress, LTD, and LTP. Adult male Long-Evans rats (290–350 gm) received 60 tailshocks (1 mA for 1 sec, 30–90 sec apart) while restrained in a Plexiglas tube. Control animals remained in their homecages. Promptly after stress, animals were killed and hippocampal slices were prepared in a standard manner. In brief, transverse hippocampal slices (400 μm) were maintained in an interface recording chamber continuously perfused ($\approx$2 ml/min) with 95% O_2 and 5% CO_2 saturated artificial cerebrospinal fluid (124 mM NaCl/3 mM KCl/1.25 mM KH_2PO_4/3 mM $CaCl_2$/1 mM $MgCl_2$/26 mM $NaHCO_3$/10 mM glucose). The temperature in the recording chamber was kept at 32°C ± 0.5°C. After at least 1 hr of incubation, the Schaffer collateral/commissural fibers were stimulated by concentric bipolar electrodes that delivered

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Abbreviations: LTP, long-term potentiation; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate; f-EPSP, field excitatory postsynaptic potential; LFS, low frequency stimulation; APV, DL-2-amino-5-phosphonovaleric acid; CGP39551, the carboxyethylester of CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid).

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100-μsec pulses. Glass electrodes filled with 2 M NaCl were placed in stratum radiatum in CA1 to record field excitatory postsynaptic potentials (f-EPSPs). Test stimulus intensity was adjusted to produce a response that was 50% of the maximum evoked responses for all experiments. Baseline synaptic transmission was monitored for 10 min before delivering 900 pulses of 1 Hz stimulation, a low frequency stimulation (LFS) known to produce LTD (19–21). A competitive NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV, 40 μM) (Sigma) was later used in the chamber perfusate to verify that the induction of LTD following LFS was NMDA-receptor mediated (19–21).

Stress and NMDA Antagonist CGP39551. Animals were administered i.p. with either 30 mg/kg of CGP39551, a competitive NMDA receptor antagonist that crosses the blood brain barrier, or saline 2 hr before stress. Another group of animals was injected with the anxiolytic drug diazepam (5 mg/kg i.p.) 30 min before undergoing stress. Hippocampal slices were prepared and electrophysiological recordings were obtained in the manner described. The tetanus used to induce LTP consisted of 5 trains of 100 Hz, each lasting 200 msec at an intertrain interval of 10 sec.

Data Analysis. Data were collected and analyzed with programs written in AXOBASIC/QUICKBASIC. The initial slope of f-EPSPs was used in all statistical analyses. In all studies, only those slices that exhibited a stable baseline for 10 min were included in the analysis. The change in f-EPSPs after LFS and tetanus was averaged across slices for each rat. The magnitudes of LTD and LTP were measured between 20 and 30 min after the LFS and tetanus, respectively.

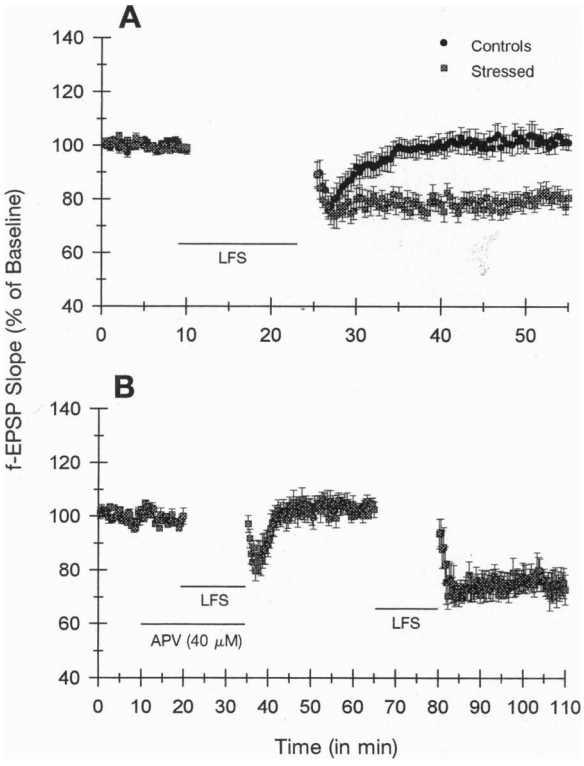

FIG. 1. Synaptic strength is expressed as a percentage of the average pretetanus f-EPSP over time. Test pulses were given every 20 sec. LFS consisted of 900 pulses given at 1 Hz. (*A*) Hippocampal slices obtained from stressed rats ($n = 10$) showed LTD following LFS of the Schaffer collateral-commissural pathway, whereas slices from control rats ($n = 10$ animals) did not. (*B*) The LTD observed in slices from stressed animals is NMDA-dependent as evidenced by APV (40 μM) blockade. Once APV was washed away from the recording chamber, LTD was observed following LFS.

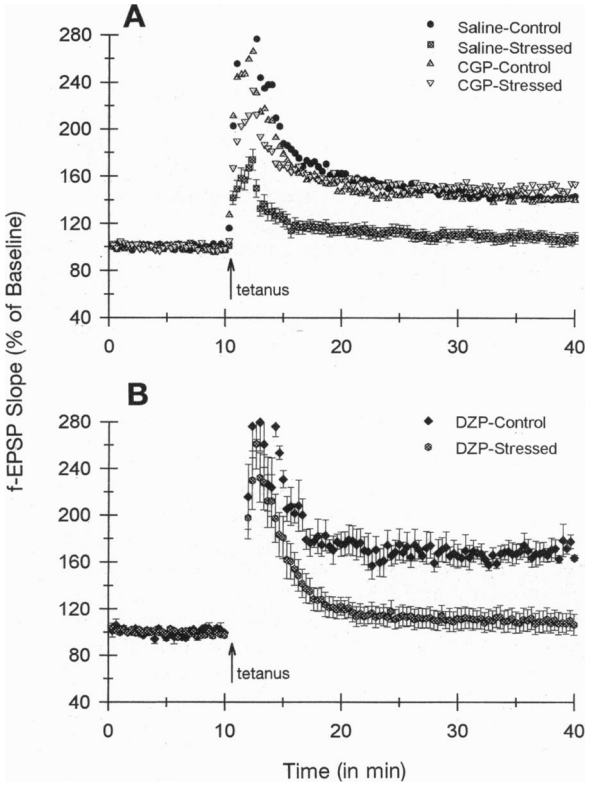

FIG. 2. Stress impairs subsequent LTP in the CA1 area of the hippocampus by NMDA receptor activation. Tetanus consisted of 5 trains of 100 Hz, each lasting 200 msec at an intertrain interval of 10 sec. (*A*) After tetanus, LTPs exhibited by saline-control ($n = 6$), CGP39551-control ($n = 6$), and CGP39551-stressed ($n = 8$) animals were robust and did not statistically differ from each other. In contrast, LTP exhibited by saline-stressed ($n = 8$) animals was significantly impaired. (*B*) Animals that received diazepam plus stress ($n = 6$) were impaired in LTP in comparison with animals that received diazepam only ($n = 6$).

RESULTS

As shown in Fig. 1*A*, LTD was not evoked by LFS in hippocampal slices obtained from control rats; synaptic efficacy was transiently depressed and returned to the prestimulation baseline level within 30 min (101.6 ± 3.1%; pre- versus poststimulation paired *t* test, $P > 0.1$). In contrast, LTD was observed for at least 30 min (80.7 ± 3.3%; $P < 0.01$) after LFS in the hippocampal slices obtained from stressed rats. The induction of LTD was blocked by a competitive NMDA antagonist APV, indicating that the development of depression displayed by slices from stressed animals is NMDA receptor-dependent (Fig. 1*B*).

Although the slices from control animals failed to show LTD, they exhibited LTD or depotentiation (reversal of LTP) if LFS was applied 30 min after LTP was established (normalized f-EPSP slopes, baseline = 100.0 ± 1.1%; after tetanus, 156.1 ± 8.8%; after first LFS, 133.7 ± 10.7%; after second LFS, 118.1 ± 11.1%; $n = 5$). The LTD, or depotentiation, observed from the potentiated state is NMDA receptor-dependent because it was blocked by APV.

When LTP was assessed after tetanus, slices from stressed animals administered saline exhibited impaired LTP (107.9 ± 4.9%) in comparison with slices from control animals administered saline (141.1 ± 6.4%), slices from control animals administered CGP39551 (140.4 ± 8.0%), and slices from stressed animals administered CGP39551 (151.1 ± 9.8%) [$F(3, 27) = 9.23$; $P < 0.01$; Newman–Keuls test] (Fig. 2*A*). When administered before stress, CGP39551 also blocked LFS-induced LTD (baseline, 99.9 ± 0.9%; after LFS, 94.4 ± 2.1%;

n = 4). Thus, hippocampal recordings from slices obtained from stressed animals injected with CGP39551 were similar to those obtained from unstressed controls in terms of hippocampal plasticity (showing LTP but not LTD). In slices prepared from animals injected with the anxiolytic drug diazepam (5 mg/kg i.p.) and then stressed, LTP was significantly impaired following tetanus (108.9 ± 9.0%) (Fig. 2*B*). In contrast, slices obtained from diazepam-unstressed animals showed robust LTP (169.8 ± 7.1%).

While the slices from stressed animals did not exhibit LTP, they showed NMDA-dependent LTP if tetanus was given after LTD was first induced (baseline, 100.5 ± 0.9%; after LFS, 79.6 ± 2.0%; after tetanus in the presence of APV, 82.0 ± 2.4%; after tetanus in the absence of APV, 96.7 ± 2.3%; n = 4).

DISCUSSION

In the present study, we have replicated and extended the original findings of the stress-induced impairment of LTP (Table 1). Specifically, our results indicate that during stress, NMDA receptor-dependent changes occur in area CA1 of the hippocampus that lead to alterations in the inducibility of subsequent LTP and LTD. We propose that stress modulates ensuing hippocampal plasticity by elevating the basal synaptic transmission level to bias LTD induction over LTP induction. For example, if LTP or LTP-like processes occur in the hippocampus during stress, then subsequent LTP may be impaired due to its occlusion or saturation. However, processes that decrease synaptic strength, such as LTD or depotentiation, should be facilitated after stress. Consistent with this view, we find that stress impairs the induction of LTP but promotes the induction of LTD in the hippocampus. This is in contrast to hippocampal slices obtained from unstressed controls that show robust LTP but not LTD. Interestingly, in slices from stressed animals, LTP can be demonstrated when tetanus is applied after LTD has been previously established. Moreover, slices from control animals display LTD (or depotentiation) when LFS is applied after LTP is first established. A recent study (22) also reports that although LTD is not evoked by LFS from baseline in hippocampal slices prepared from adult rodents, NMDA receptor-dependent depotentiation (a process similar to LTD) is observed when LFS is applied from the LTP state. It appears that the dynamic range of LTP and LTD inducibility is linked, and our findings suggest that stress modulates subsequent hippocampal plasticity by altering the basal synaptic transmission.

The competitive NMDA receptor antagonist CGP39551, when administered before stress, blocks the stress effects on hippocampal plasticity. Recordings from slices obtained from CGP39551-stressed animals are identical to those obtained from unstressed controls, i.e., they exhibit LTP but not LTD. The effect of CGP39551 on subsequent plasticity is unlikely due to an anxiolytic property associated with the drug since the anxiolytic drug, diazepam, did not block the stress effect on LTP. Similarly, diazepam did not eliminate or reduce the magnitude of the escape-learning deficit produced by the inescapable shock in rats (26). Thus, CGP39551 prevents the effect of stress on LTP and LTD by specifically blocking the NMDA receptors. Pharmacological blockade of NMDA receptors during stress also prevents a stress-induced facilitation of classical eyeblink conditioning in rats (27).

Table 1. Summary of data from stress studies

Hippocampus (area CA1)*	LTP	LTD
Controls	+	−
Stressed	−	+
Controls + APV	−	NA
Stressed + APV	NA	−
Controls (from LTP state)	NA	+
From LTP state + APV	NA	−
Stressed (from LTD state)	+	NA
From LTD state + APV	−	NA
Stressed with CGP39551	+	−

+, Present or enabled; −, absent or attenuated; NA, not applicable.
*Hippocampal slices from adult rodents.

Other lines of evidence suggest that LTP or LTP-like changes occur in the hippocampus during behavioral stress. Both *in vivo* LTP and exposure to inescapable tailshock in rats have been shown to increase the ligand binding of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subclass of glutamate receptors in the hippocampus (28–30). While NMDA receptors are critical for the induction of LTP, the expression of LTP appears to be mediated largely through AMPA receptors (14). It is conceivable that the change in AMPA receptors (e.g., an increase in affinity or an increase in number) after stress may hinder further LTP development.

Other effects associated with behavioral stress have also been shown to be mediated through activation of NMDA receptors. For instance, a prolonged exposure to stress or corticosterone contributes to neuronal loss in the hippocampus (31, 32). Although yet to be tested with stress, the NMDA receptor antagonist APV seems to protect the hippocampus from glucocorticoid-induced endangerment (33).

Our results suggest that stress modulates subsequent hippocampal LTP and LTD processes by elevating basal synaptic transmission. Such changes in hippocampal synapses produced by stress may affect later learning and memory capabilities. Presently, the possibility of stress effects on other parts of the brain or other forms of plasticity (e.g., heterosynaptic LTD) are not known and need to be investigated. Understanding the mechanisms for how behavioral stress modifies synaptic plasticity in the hippocampus and subsequent learning and memory processes may suggest approaches, both pharmacological and behavioral, to better aid in the treatment of individuals whose cognitive performances are impaired following stressful events.

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