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# Amygdala Is Critical for Stress-Induced Modulation of Hippocampal Long-Term Potentiation and Learning

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Stress is a biologically significant factor shown to influence synaptic plasticity and memory functioning in the hippocampus. This study examined the role of the amygdala, a brain structure implicated in coordinating stress behaviors and modulating memory consolidation, in mediating stress effects on hippocampal long-term potentiation (LTP) and memory in rats. Electrolytic lesions of the amygdala effectively blocked the adverse physiological and behavioral effects of restraint and tailshock stress, without impeding the increase in corticosterone secretion to stress. Physiologically, hippocampal slices from stressed animals exhibited impaired LTP relative to slices from unstressed control animals, whereas hippocampal slices from stressed animals with amygdalar lesions exhibited normal LTP. Behaviorally, stressed animals were impaired in retention of a hippocampal-dependent hidden platform version of the Morris water maze task, and this impairment was blocked by amygdalar lesions. In a fixed location–visible platform water maze task that can be acquired by independent hippocampal and nonhippocampal memory systems, stress enhanced the use of nonhippocampal-based memory to acquire the task. These results indicate that an intact amygdala is necessary for the expression of the modulatory effects of stress on hippocampal LTP and memory.

*Key words: hippocampus; learning; fear; emotion; glucocorticoids; corticosterone; synaptic plasticity*

It is well documented that adverse effects on cognitive functioning generally accompany stress (Maier and Seligman, 1976). Although the acute response to stress (e.g., heightened cognition) is an adaptive mechanism, excessive stress, in particular uncontrollable stress, can have severe repercussions ranging from impairments in learning and memory to enhanced susceptibility to neuronal cell death (for review, see McEwen and Sapolsky, 1995; Kim and Yoon, 1998).

The hippocampus, as part of a system necessary for the formation of stable memory (Scoville and Milner, 1957; Eichenbaum et al., 1992; Squire and Zola, 1996), is enriched with receptors for corticosteroids (the principal glucocorticoid secreted by the adrenal cortex in response to stress; cortisol in humans, corticosterone in rats) and participates in terminating the stress response via the glucocorticoid-mediated negative feedback of the hypothalamus–pituitary–adrenal axis (McEwen and Sapolsky, 1995). In the rat hippocampus, corticosterone has been shown to regulate metabolic, physiologic, and genomic functions of neurons (Sapolsky, 1992). As a result, certain hippocampal functions appear to be susceptible to stress, possibly linking the effects of glucocorticoids to cognitive functions such as learning and memory. For example, stress and corticosterone have been shown to impair hippocampal-dependent forms of verbal memory in humans (Bremner et al., 1993; Newcomer et al., 1999) and spatial memory in rats (Diamond et al., 1992; Luine et al., 1994; Bodnoff et al., 1995; de Quervain et al., 1998). Consistent with these behavioral data, both *in vitro* and *in vivo* electrophysiological studies indicate that stress impairs hippocampal LTP (Foy et al., 1987; Shors et al., 1989; Diamond et al., 1992; Shors and Dryver, 1994; Kim et al., 1996; Xu et al., 1997), a putative cellular mnemonic mechanism (Morris et al., 1990; Bliss and Collingridge, 1993) (but see Shors and Matzel, 1997). If the notion that changes in synaptic efficacy are essential for learning and memory [e.g., Hebb's postulate; Hebb (1949)] is correct, then it is possible that the LTP impairment associated with stress might be one neural basis for stress-induced alterations in learning.

Considerable evidence indicates that the amygdala is critically involved in mediating stress-related effects on behavior and modulating hippocampal function. For example, amygdalar lesions and/or pharmacological manipulations have been shown to (1) prevent stress-induced gastric erosion (Henke, 1981, 1990) and analgesia (Helmstetter, 1992), (2) block memory modulatory effects of intrahippocampally administered drugs (Roozendaal et al., 1996, 1998; Packard and Chen, 1999), and (3) impair *in vivo* dentate gyrus LTP in the hippocampus (Ikegaya et al., 1994, 1995, 1996). In addition, the amygdala has been implicated in emotional learning (Kim et al., 1993; LeDoux, 1994; Maren and Fanselow, 1996) and attention (Gallagher and Schoenbaum, 1999; Holland et al., 2000). Anatomically, the amygdala projects to several hippocampal regions (including the CA1 area) (Krettek and Price, 1977; Aggleton, 1986), providing various routes by which it may potentially influence hippocampal function. Therefore, the present series of experiments examined the possibility that the amygdala is involved in mediating stress effects on hippocampal LTP and hippocampal-dependent learning, using a hidden platform version of the Morris water maze task. In view of evidence that memory is organized in multiple brain systems (Packard et al., 1989; Squire and Zola, 1996; Thompson and Kim, 1996), we also examined whether stress might influence learning in a

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task in which both hippocampal-dependent and hippocampal-independent memory systems appear to be engaged (McDonald and White, 1994). Specifically, we hypothesized that a selective impairing effect of stress on hippocampal memory processes would enhance the use of hippocampal-independent y systemsk/GS2 gaonald

ANOVA; main effect of stress: $F_{(1,27)} = 78.9$, $p < 0.01$; main effect of lesion: $F_{(1,27)} = 2.3$, $p > 0.05$; lesion × stress interaction: $F_{(1,27)} = 1.3$, $p > 0.05$). Although there appears to be a trend of lesion-stress animals (49.7 ± 7.8 μg/dl) showing a lesser amount of stress-induced corticosterone elevation than sham-stress animals (65.0 ± 7.6 μg/dl), this difference was not statistically reliable, ($p > 0.05$, Newman–Keuls). This indicates that amygdalar lesions do not affect stress-induced elevations in corticosterone levels.

In a hippocampal-dependent hidden platform version of the water maze task, all groups significantly decreased their latencies to find the hidden platform during the eight training trials (Fig. 3*A*). The rate of acquisition was comparable among the four groups (two-way ANOVA with trials as a repeated measure; main effect of lesion: $F_{(1,31)} < 1.0$, $p > 0.05$; main effect of surgery: $F_{(1,31)} = 2.7$, $p > 0.05$; lesion × stress × trials interaction: $F_{(7,245)} < 1.0$, $p > 0.05$). On the retention (probe) test a day later, however, the lesion animals required significantly shorter latencies to swim to the original location of the platform than the sham animals, irrespective of stress (two-way ANOVA; $F_{(1,34)} = 13.5$, $p < 0.01$). Although neither the main effect of stress nor lesion × stress interaction was significant (two-way ANOVA; $F_{(1,34)} = 3.0$, $p > 0.05$, and $F_{(1,34)} = 1.4$, $p > 0.05$, respectively), a simple planned comparison analysis indicated that the sham-stress animals (39.1 ± 8.4 sec) exhibited significantly longer l03.8(four7759T0 (anal)85.2(and)]TJ/F15 1 Tf4.5aur77590a3Tf7rTf7 0 -n/F1,)Tj/ 0 TD(p)Tj/F

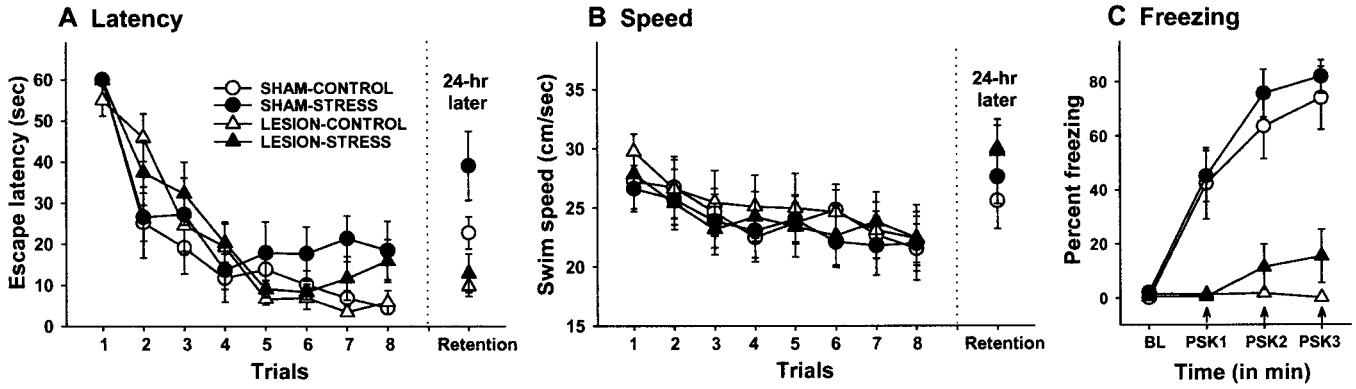


*Figure 3.* Effects of amygdalar lesions and stress on spatial memory and fear conditioning. *A*, Mean (±SE) latencies to find a submerged platform from sham-control (*open circles*, $n = 8$), sham-stress (*filled circles*, $n = 9$), lesion-control (*open triangles*, $n = 9$), and lesion-stress (*filled triangles*, $n = 9$) animals during acquisition and a single retention test. *B*, Mean (±SE) swim speed (centimeters per second) of four groups during acquisition and a single retention test. *C*, Mean (±SE) percentage postshock (*PSK*) freezing during the 1 min baseline (*BL*) and during the three 1 min intershock intervals.

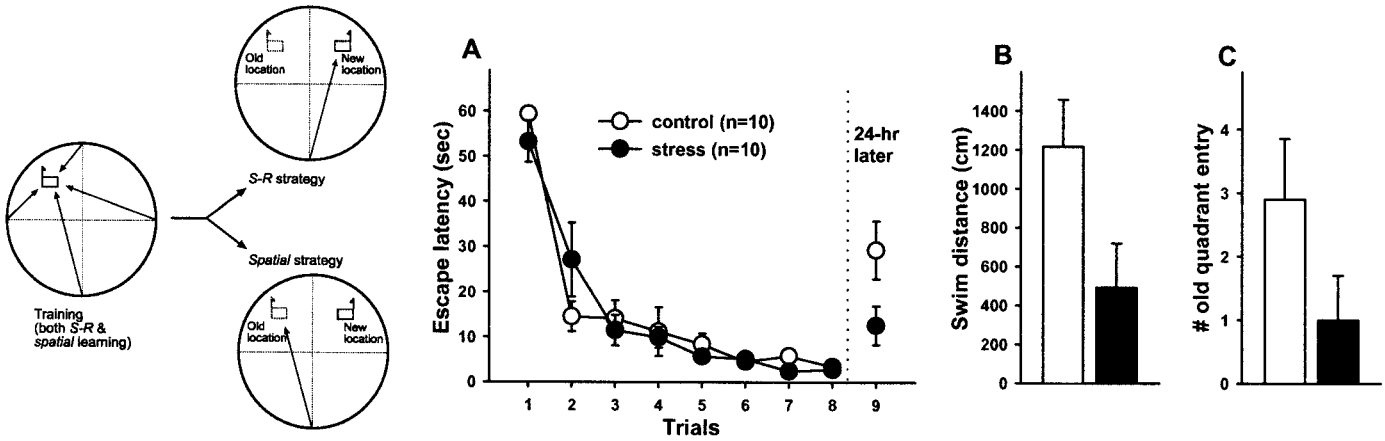


*Figure 4. Left*, Fixed location–visible platform water maze paradigm for assessing stress effects on the relative use of S-R and spatial memory. *A*, Mean (±SE) latency to find a submerged platform marked with a visually salient pole from control (*open circles*, $n = 10$) and stress (*filled circles*, $n = 10$) animals during the acquisition trials (*1–8*) and on a single test trial (*9*). *B*, Mean (±SE) distance to find a submerged platform marked with a visually salient pole on a single test trial. *C*, Mean number of old quadrant entry (where the platform was located during training).

(10 of 10) initially swam to the original platform location (preferentially using a spatial strategy) before swimming to the visible platform now located in a new quadrant. In contrast, 5 of 10 stress animals swam directly to the new platform location [preferentially using a stimulus–response (S-R) strategy], whereas the remaining 5 animals swam to the original platform location before the new platform location (preferentially using a spatial strategy). The swim distance to the new platform location and the number of old quadrant entry measures (Fig. 4*B*,*C*) also indicate that stress enhances the use of an S-R strategy in this task.

## DISCUSSION

The present findings demonstrate that amygdalar lesions effectively block stress effects on hippocampal LTP and hippocampal-dependent memory and are consistent with previous reports that amygdalar lesions prevent other effects of stress, including gastric erosion (Henke, 1990) and analgesia (Helmstetter, 1992). Specifically, we found that hippocampal slices obtained from sham animals exposed to stress exhibited LTP impairments in the CA1 area, whereas slices from sham animals not exposed to stress demonstrated robust LTP, replicating earlier *in vitro* and *in vivo* findings of stress-induced impairment of LTP (Foy et al., 1987; Shors et al., 1989; Diamond and Rose, 1994; Kim et al., 1996; Xu et al., 1997). In contrast, LTP was observed reliably in hippocampal slices prepared from amygdala-lesioned animals, regardless of whether or not they experienced stress. Similarly, we observed that amygdalar lesions also blocked stress-induced memory impairments when rats were tested in a hidden platform water maze task that has previously been shown to be hippocampus-based (Packard et al., 1994; Packard and Teather, 1998). Thus, our findings that the amygdala is critically involved in mediating stress effects on hippocampal LTP and hippocampal-dependent memory are consistent with the view that one function of the amygdala is to modulate memory processes in other brain structures, such as the hippocampus (Gallagher and Kapp, 1978; Ikegaya et al., 1994; Packard et al., 1994; Cahill and McGaugh, 1998; Packard and Teather, 1998; Roozendaal et al., 1998; Packard and Chen, 1999; McGaugh, 2000).

In the present study, sham lesion animals exposed to 1 hr of uncontrollable stress (60 tailshocks and restraint) before undergoing water maze training (pretraining stress effects) exhibited impairments in spatial memory when tested 24 hr later. In another study (de Quervain et al., 1998), a relatively milder three footshock stress (lasting <1 min) that was presented before a retention test (pretesting stress effects) impaired performance in

a water maze spatial task in a time-dependent manner (i.e., retention was impaired 30 min poststress but not 2 min or 4 hr poststress) that corresponds to the corticosterone levels at the time of testing. It appears then that pretraining exposures to a relatively intense and longer-lasting stress (used in the present study) can affect spatial memory in a manner that does not directly correspond to the corticosterone levels at the time of testing (24 hr later).

Because there is no evidence that three footshock stress influences hippocampal plasticity (i.e., LTP), it would be important to investigate whether or not these two different magnitudes of stress produce similar pretraining and pretesting effects on hippocampal-dependent memory.

Interestingly, using the present training–testing procedures, lesioning the amygdala per se seems to enhance the performance in the hidden platform water maze task. This finding differs from a previous study (Sutherland and McDonald, 1990) that found neither enhancing nor impairing effects of amygdalar lesions on a spatial version of the water maze task when animals were trained across several days. It is conceivable that high levels of stress hormones (such as epinephrine and glucocorticoids) are released during the eight massed water maze training trials, which might normally produce memory impairing effects in the amygdala-intact animals. Thus, this finding is consistent with the accumulating evidence indicating that amygdala function is necessary for intrahippocampally administered drugs to modulate (enhance or impair) consolidation of hippocampal-dependent (e.g., spatial) memory and for mediating memory modulatory effects of stress hormones (Cahill and McGaugh, 1991; Packard et al., 1994; Roozendaal and McGaugh, 1996, 1997; Roozendaal et al., 1998; Packard and Teather, 1998; Packard and Chen, 1999; McGaugh, 2000).

It is also significant that amygdalar lesions did not affect Schaffer collateral–commissural-CA1 LTP in hippocampal slices from unstressed animals. Recent studies suggest that the amygdala influences LTP in the hippocampus. For instance, electrolytic lesions to the basolateral (but not central) nuclei of the amygdala have been shown to significantly attenuate perforant path–dentate gyrus LTP *in vivo* (Ikegaya et al., 1994), whereas high-frequency stimulation of the amygdala augmented LTP (Ikegaya et al., 1996). It now appears that stimulation of the amygdala induces a time-dependent biphasic effect on hippocampal LTP (an immediate excitatory effect and a longer-lasting inhibitory effect) (Akirav and Richter-Levin, 1999). Additionally, intra-amygdala infusions of NMDA receptor antagonists have been found to impair dentate gyrus LTP (without affecting the baseline synaptic response), suggesting that NMDA receptors in the amygdala might be involved in influencing LTP (Ikegaya et al., 1995). In the present study, however, although amygdalar lesions (which included both central and basolateral nuclei) blocked stress effects on CA1 LTP *in vitro*, the lesions did not affect LTP in unstressed animals. Thus, it is possible that the amygdala may differentially influence synaptic plasticity in different regions of the hippocampus.

Although stress impaired retention of hippocampal-dependent memory in a hidden platform water maze task, the same stress enhanced the relative use of hippocampal-independent S-R memory in a fixed location–visible platform water maze task in which both hippocampal-dependent and caudate-dependent memory systems are engaged (McDonald and White, 1994). The effects of stress on behavior in this task are similar to those of fornix lesions, which also result in enhanced use of S-R behavior relative to normal animals (McDonald and White, 1994). Thus, both stress (presumably via impairing hippocampal LTP) and fornix lesions (via disrupting hippocampal afferent-efferent pathways) impair the use of spatial information and facilitate the use of S-R information in the acquisition of an escape response to a visible platform in a fixed location. Similarly, stress (Shors et al., 1992; Shors and Mathew, 1998) and hippocampal lesions (Schmaltz and Theios, 1972; Port et al., 1985) have been shown to facilitate the acquisition of hippocampal-independent (but cerebellar-dependent) delay eyeblink conditioning (Kim et al., 1995; Kim and Thompson, 1997). It has also been reported that infusions of NMDA receptor antagonists into the amygdala before stress effectively block stress-induced facilitation of eyeblink conditioning (Shors and Mathew, 1998). Thus, it would be important to test whether NMDA receptor antagonists in the amygdala would also block stress-induced enhancement of hippocampal-independent S-R memory as well as stress-induced impairment in hippocampal LTP and spatial memory. At any rate, our findings are consistent with the general notion that amygdala activation can influence both hippocampal-dependent and hippocampal-independent memory (Packard et al., 1994; McGaugh, 2000)

It is generally viewed that there are multiple memory systems that are subserved by different brain substrates (Packard et al., 1989, 1994; Packard and McGaugh, 1992; Squire and Zola, 1996; Thompson and Kim, 1996). Under normal conditions, however, competition for control of learned behavior may arise among these systems. For example, although the hippocampus is not essential for delay eyeblink conditioning (Schmaltz and Theios, 1972; Kim et al., 1995), hippocampal lesions can facilitate the acquisition of delay eyeblink conditioning (Port et al., 1985), pretraining LTP saturation in the hippocampus accelerates the rate of delay eyeblink conditioning (Berger, 1984), and PKC$\gamma$ mutant mice (deficient in the $\gamma$ isoform of protein kinase C) with a moderate impairment in hippocampal LTP (Abeliovich et al., 1993) exhibit facilitated acquisition of delay eyeblink conditioning (Chen et al., 1995). In addition, lesions of the hippocampal system facilitate the acquisition of caudate-dependent S-R learning in a win-stay radial maze task (Packard et al., 1989; McDonald and White, 1993). Together, these results indicate that during hippocampal-independent learning (e.g., delay eyeblink conditioning, S-R learning), the hippocampus may be engaged in processing information (e.g., context) (Good and Honey, 1991; Kim and Fanselow, 1992; Phillips and LeDoux, 1992) that might interfere with the formation or expression of hippocampal-independent memory. Thus, stress-induced alterations in synaptic plasticity that selectively affect hippocampal memory processes may inhibit the competitive interference between hippocampal-dependent and hippocampal-independent memory systems and thereby enhance performance in nonhippocampal learning tasks.

With regard to stress effects on hippocampal LTP, it has been reported previously that there is a biphasic relationship between level of corticosterone and magnitude of LTP (Diamond et al., 1992), with both low (via adrenalectomy) and high (via exogenous administration) levels of corticosterone impairing LTP. In addition, corticosterone has been shown to affect the intrinsic properties of hippocampal neurons (e.g., prolonging the afterhyperpolarization) (Joels and De Kloet, 1989; Kerr et al., 1989) that would reduce cell excitability. Behaviorally, rats that were administered corticosterone at doses comparable with those observed during natural stress were found to be impaired in spatial learning (Bodnoff et al., 1995). Given these findings, it is surprising that amygdalar lesions effectively blocked stress effects on hippocam-

pal LTP and spatial memory without significantly affecting the increase in corticosterone secretion in response to stress. Our results suggest that this increase in corticosterone levels is not a sufficient condition to mediate stress effects on hippocampal plasticity and learning. This view is also supported by findings that LTP is reduced further in adrenalectomized rats after stress and is not restored by exogenous administration of corticosterone (Shors et al., 1990), and that in normal animals administered with dexamethasone (a synthetic glucocorticoid that blocks the HPA axis activity), stress-induced impairments in LTP nonetheless occurred (Foy et al., 1990). Collectively, these data indicate that multiple factors (in addition to glucocorticoids) mediate stress effects on hippocampal functioning.

In conclusion, the current findings suggest that alterations in hippocampal plasticity subsequent to stress might be caused by excessive modulatory effects of the amygdala during the stress experience. If amygdalar modulation of hippocampal physiology occurs during stress, then this effect must have a long duration because it was observed in hippocampus isole. It is now of interest to characterize the neuroanatomical–neurochemical projections from the amygdala to the hippocampus to further elucidate the modulating mechanisms of stress on neur.5(on.9(s)-220-on.9(s)-2o)-24.9(ge9(gest)-o)-24.9(h49(it)-o)-249(it)(it)-oh49to ftheed i1(i)-2ions

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