In this study we present experiments that address this question. Using behaving mice as subjects, we show that Pavlovian fear conditioning to contextual conditioned stimuli (CSs), but not previous exposure to the physical stressor (unconditioned stimulus, US), interferes with the induction but not the maintenance of LTP in the LS.

**METHOIDS**

**Subjects and surgery**

The experiments were performed with the use of young (4–6 mo) male mice of the C57 BL/6 JI Co strain weighing between...
FIG. 1. Example of unit activation during fimbrial-stimulation-evoked septal field potential. Traces were recorded with same electrode with different filter settings adjusted to reveal either field potential or single-unit activity. Latency of single-unit activity coincided with peak latency of N3 component of field potential.

Experimental protocol

The baseline was established over a 4-day period (1 recording session per day). For each recording session, animals were maintained in their home cages, which were transferred from the animal room to the room used for electrophysiological tests. Immediately after the last recording session on day 4, animals were randomly divided into 4 groups. Animals from the first group (HFS/FS-cc group, n = 7) received HFS of the fimbria in the home cage before being submitted to contextual fear conditioning with the use of footshocks (FSs). Twenty-four hours later, these animals were reexposed to the conditioning chamber (cc) for electrophysiological recording as described below. Animals from the second group (FS/HFS-cc group, n = 6) were first submitted to contextual fear conditioning and received HFS of the fimbria the following day, i.e., during reexposure to the conditioning chamber. The two other groups were submitted to the same regimen as the second one except that for the third group (NFS/HFS-cc group, n = 6), no FS was delivered in the conditioning chamber, whereas animals of the 4th group (FS/HFS-hc group, n = 6) that had received FSs in the conditioning chamber were not reexposed to the chamber the day after but were maintained in their home cages for LTP induction. For all groups, HFS of the fimbria consisted of three trains (0.2 Hz) of 200 pulses at 200 Hz that produced significantly larger LTP than a train of 100 pulses over a 1-s period (100 Hz, see Fig. 3A, left).

FSs were administered in a gray plastic chamber (30 x 24 x 22 cm) equipped with a stainless steel grid floor connected to a shock generator. Two minutes after being placed in this conditioning chamber, mice were subjected to 4 FSs (0.6 mA, 2 s in duration, 20 s apart), each of which was capable of inducing the startle reflex and sometimes the emission of a vocalization. Immediately afterward, mice were replaced in their home cages and transferred to the animal room. Twenty-four hours later, animals were placed back into the conditioning chamber (except for the 4th group, see above) for a period of ~1 h, during which the animals were left undisturbed. For the HFS/FS-cc group, field potentials were recorded at the beginning (20 s and 4 min) and at the end (1 h) of the session. For the three other groups (i.e., FS/HFS-cc, NFS/HFS-cc, and FS/HFS-hc), two recording sessions were performed during the first 10 min that preceded the delivery of HFS and, as for the HFS/FS-cc group (see Fig. 3A, left), at 15 min, 1 h, and 24 h after that stimulation (see Fig. 3B, right).

Histology and data analysis

After completion of experiments, all mice were given an overdose of pentobarbital sodium and perfused with saline (0.9%) followed by Formalin (10%). The exact placement of the electrodes was then verified by conventional histological methods.

Changes in the N3 wave amplitude are presented as the means ± SE of the individual values of animals from each group. Statistical analysis of the data was performed with the use of analysis of variance (ANOVA).

RESULTS

Lateral septal responses to fimbria stimulation

Fimbria stimulation evoked characteristic field responses in the LS (Garcia and Jaffard 1992, 1993, 1996; Garcia et al. 1993, 1996). Single stimuli delivered to fimbria evoked single action potentials in LS neurons, the latency of which (varying from 6 to 10 ms, mean = 7.5 ms, n = 14) coincided with the excitatory negative N3 component of the field potentials (Fig. 1). Moreover, the majority of such neurons...
Effects of tetanus frequency

The amplitudes of negative components (N2 and N3) of the field potential were measured according to the method described previously (Garcia and Jaffard 1992, 1993, 1996). Only the N3 component displayed an increase in amplitude following the stimulation train (see Fig. 2). As shown in Fig. 3A, left, HFS paradigm at 200 Hz resulted in a larger increase in the amplitude of N3 (LTP) than at 100 Hz. A two-way ANOVA with recording periods (7 levels) as within-subjects factor and frequency (200 vs. 100 Hz) as between-groups factor indicated a significant period x group interaction \( F(6,66) = 11.3, P < 0.001 \). In the 200-Hz group, one-way ANOVA indicated that the amplitude of N3 changed significantly during the recording sessions \( F(6,36) = 36.2, P < 0.001 \). Post hoc Fischer tests revealed that significant LTP first occurred following (15 min, 1 h, and 24 h) the 200-Hz stimulation (all \( P \) values <0.001).

The same analysis performed on data from the 100-Hz train revealed that although there were significant changes in the N3 amplitude during recording sessions \( F(5,30) = 3.26, P < 0.05 \), the changes that followed the 100-Hz train did not reach statistical significance (Fisher tests, all \( P \) values >0.05).

Effects of conditioned fear stress on the maintenance of LTP

Twenty-four hours after the induction of LTP, animals were transferred from their home cages (where pre- and post-LTP recordings were performed) to the conditioning chamber for contextual fear conditioning. As shown in Fig. 3A, right, the day after conditioning, when animals were reexposed to the conditioning chamber, the amplitude of N3 remained slightly but not significantly above baseline. Importantly, however, the amplitude of N3 was significantly greater (HFS/FS-cc group: 20 s, 104.6 ± 5.6%, mean ± SE; 4 min. 105.1 ± 7.2%) than that recorded at the same time periods in the FS/HFS-cc group (see Fig. 3B, right), which was submitted to the same regimen but had not yet been subjected to HFS \( [74.0 ± 9.3% \text{ and } 81.3 ± 7.2%] \). A two-way ANOVA performed on data from these two groups (4 baselines + 2 periods x 2 groups) also indicated a significant period x group interaction \( F(5,55) = 6.6, P < 0.001 \).

Effects of contextual fear conditioning on the induction of LTP

Results are summarized in Fig. 3B. A two-way ANOVA indicated that before conditioning, the amplitude of N3 was stable across the 4 days of baseline recording sessions in home cages, with no significant between-group difference nor group x session interaction (all \( P \) values <1, see Fig. 3B, left). When reexposed to the conditioning chamber (Fig. 3B, right), animals that had received FSs the day before (the FS/HFS-cc group) displayed a reduction (20–25%) in the amplitude of N3 with respect to animals that had not received the FSs (NFS/HFS-cc group). Although of weaker magnitude, there was also a tendency for animals that received FSs but that were maintained in their home cages (the FS/HFS-hc group) to exhibit a reduction in the amplitude of N3 (≈10% with respect to the NFS/HFS-cc group). A two-way ANOVA performed on these data indicated that, with respect to baseline, there was a significant session x group interaction \( F(10,75) = 3.79, P < 0.001 \), with, as revealed by repeated-measures ANOVA, no significant change in the amplitude of N3 in the NFS group \( F(5,25) = 0.53 \) but significant changes in both the FS/HFS-cc group \( F(5,25) = 7.0, P < 0.001 \) and the FS/HFS-hc group \( F(5,25) = 3.41, P < 0.05 \). To compensate for individual differences in the degree of conditioned-fear-induced decreases in N3 amplitude (in both the FS/HFS-cc and the FS/HFS-hc groups), the intensity of subsequent tetanic stimulation was individually adjusted (increased) to evoke an N3 wave amplitude as close as possible to baseline. The intensity of pulses used for subsequent posttetanic recordings was readjusted to the pretetanic value. As shown in Fig. 3B, right, a stimulation train at 200 Hz resulted
in an increase in the amplitude of N3 (LTP) in both the NFS/HFS-cc and FS/HFS-hc groups but not in the FS/HFS-cc group. A two-way ANOVA with recording periods in the conditioning chamber (5 levels) as within-subjects factor and the three groups as between-groups factor indicated a significant period \times group interaction \( F(8,60) = 5.83, P < 0.001 \). Specifically, in both the NFS/HFS-cc and FS/HFS-hc groups, one-way ANOVA indicated that the amplitude of N3 changed significantly during the recording sessions \( F(4,20) = 19.0 \) and 19.6, respectively, \( P \text{ values} < 0.001 \). Post hoc Fisher tests revealed that in both groups, significant LTP first occurred following (i.e., 15 min, 1 h, and 24 h) the 200-Hz train (all \( P \text{ values} < 0.01 \)). The same analysis performed on data from the FS/HFS-cc group did not reveal any significant changes in the amplitude of N3 \( F(4,20) = 1.90, P > 0.15 \). Finally, it may be noted that, with respect to baseline, the magnitude and time course of LTP in the three groups that displayed LTP (including the group that was subjected to HFS before conditioning, see Fig. 3A, left) were highly similar with respect to baseline [2-way ANOVA, session (7 levels) \times group interaction: \( F(12,96) = 0.77 \)].

**Discussion**

The major finding in the present study is that conditioned fear, elicited by reexposure to the specific context (condi-
tioning chamber) wherein animals had received FSs 24 h previously, totally blocked the induction of lateral septal LTP. However, once induced, conditioned fear does not affect its maintenance. Moreover, the presently observed blockade of LTP cannot be a simple result of previous exposure to the FSs themselves, because animals that were not reexposed to the conditioning chamber but that were maintained in their home cages (the FS/HFS-he group) displayed normal levels of LTP.

In the present experiments, no significant changes were observed for the N2 wave component of the field potentials evoked by fimbria stimulation. Our previous recording data on depth profiles of these field potentials with the use of anesthetized mice revealed that 1) the N2 and N3 waves are mediated by depolarization of target cells that 2) represent the responses of the dorsal (N3) and ventral (N2) populations of neurons located in the medial part of the LS, and that 3) only the amplitude of the N3 component displayed a long-lasting enhancement following HFS of the fimbria (Garcia and Jaffard 1992, 1993). Combining these observations with the present findings and those discussed below, mediadorsal LS synapses thus appear to be more prone to exhibit both HFS-induced and behaviorally induced plastic changes than the more ventral synapses. It must be noted that in previous in vivo and in vitro studies on LTP in the LS of the rat (Racine et al. 1983; Van den Hooff et al. 1989), the authors did not distinguish the two N2 and N3 components. Although we used a tetanic stimulation paradigm (i.e., 3 trains at 0.2 Hz of 200 pulses at 200 Hz) different from the one used in our previous studies carried out in anesthetized mice (20 trains at 0.2 Hz of 8 pulses each at 400 Hz) (Garcia and Jaffard 1992, 1993), our present data again show that only the amplitude of the N3 wave displayed a significant increase. Single action potentials recording performed in the present study are consistent with the electrophysiological findings of McLennan and Miller (1974) showing that the latency of the action potentials caused by fimbrial stimulation coincided with the N3 wave. This result and the ability of the N3 component to follow a relatively high frequency of stimulation (see Garcia and Jaffard 1992) suggest that this component of the field potential is monosynaptic and generated by cell firing.

Recent experiments have provided evidence that the acquisition of several learning tasks in mice (Garcia et al. 1993, 1995; Jaffard et al. 1996) and rats (Urban et al. 1995) is associated with long-term changes in synaptic efficacy in the LS. Specifically, depending on the form of memory under investigation (i.e., spatial reference vs. working memory) and/or the ability of each individual to master the task (Garcia et al. 1993; Jaffard et al. 1996; Urban et al. 1995), both testing-induced enhancement (LTP) and depression (long-term depression, LTD) of synaptic transmission in the LS have been reported. Of particular interest in the present context are the recent findings of Urban et al. (1995) showing that, in rats, high shuttle box (active avoidance) performance is associated with LTD induction in the LS, whereas low shuttle box performance is associated with LTD. Indeed, it was suggested that the low performance in the shuttle box task may be, at least partially, due to a conflict between conditional freezing behavior to the CSs and running to the safe compartment. Accordingly, it is possible that an increase in activity of LS neurons via LTD may facilitate the initiation of the active conditioned response to the tone conditioned stimulus (CS) by reducing contextual conditioned fear. Such a hypothesis seems congruent with several findings. First, it has been shown that conditioned inhibitors of fear produce an increase in unit activity in the LS (Thomas 1988; Thomas et al. 1991). Second, recent experiments (Garcia and Jaffard 1996) in which Pavlovian fear conditioning was used both to CSs and to a phasic auditory cue (CS) showed that when the tone CS was made predictive of the shock US (by pairing the tone CS and the US), subsequent reexposure to the context alone did not produce the decrease in septal excitability that was otherwise observed either when the CS and US were unpaired or when, as presently observed (see Fig. 3B, right, before tetanus), the tone CS was omitted. Third, vasopressin, which has been shown both to increase the transmission between fimbria fibers and LS neurons and to facilitate maintenance of LTP in the LS (Van den Hooff et al. 1989), is known to markedly improve active avoidance learning when administered either peripherally (De Wied 1991) or intraseptally (Engelman et al. 1992).

Taken as a whole, these data suggest that the level of excitability of LS neurons might play a role in attention to more significant (i.e., predictive) events. More specifically, an increase in lateral septal cell excitability would favor attention to the more (predictive) tone CS while overshadowing the less predictive (background) context, whereas a decrease in septal excitability would be associated with attention to the less pertinent contextual cues (see Garcia and Jaffard 1996). Accordingly, the presently observed blockade of induction of lateral septal LTP would prevent (or slow down) subsequent instrumental learning tasks using discrete unimodal CS predictive of the occurrence of the US (i.e., “learned helplessness”) (Maier and Seligman 1976; Overmaier and Seligman 1967). Along with this assumption, adding a CS that is consistently paired with the US to the protocol we have presently used should release lateral septal LTP from blockade. Although this possibility remains to be directly tested, it seems congruent with previous experiments showing that signaled FSs preclude the occurrence of subsequent impairment of escape learning (Jackson and Minor 1988), and it is reminiscent of previous experiments conducted by Shors et al. (1989) showing that inescapable but not escapable shocks block LTP in the rat hippocampus. Beyond the fact that this phenomenon was described in the Schaffer collateral–CA1 hippocampal synapses, there are, nevertheless, important differences between the findings of Shors et al. and our present data. First, LTP was measured in vitro in hippocampal slices so that the observed blockade of LTP induction was clearly not related to contextual conditioning as presently demonstrated. Second, because subjects were not presented with a CS before the occurrence of the shock US, it may only be assumed that the controllability and not the predictibility of the US modulates subsequent synaptic plasticity.

In conclusion, the present experiment shows that contextual conditioned fear blocks the induction of LTP by high-frequency electrical stimulation in the LS. Because previous experiments have shown that these synapses exhibit long-lasting changes in efficacy that are associated with the rate of acquisition of certain learning tasks (i.e., “behavioral
LTP/LTD’), this phenomenon offers a unique opportunity to identify the specific conditions in which a stressful stimulus may produce a detrimental effect on learning, via concomitant alterations in training-induced changes in synaptic efficacy in the LS. Experiments aimed at testing this hypothesis are currently in progress. More widely, the present results again emphasize the functional importance of plasticity of LS glutamatergic synapses, the alteration of which (see also Garcia and Jaffard 1993) may compromise, through impaired feedback regulation of hippocampal activity, specific learning and memory processes (see Jaffard et al. 1996).

We thank Dr. T. P. Durkin for helpful comments on the manuscript. This work was supported by the Centre National de la Recherche Scientifique and by the Conseil Regional d’Aquitaine.

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Received 18 October 1996; accepted in final form 18 March 1997.

REFERENCES


