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Long-term potentiation in freely moving rats reveals asymmetries in thalamic and cortical inputs to the lateral amygdala

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Abstract

Long-term memory underlying Pavlovian fear conditioning is believed to involve plasticity at sensory input synapses in the lateral nucleus of the amygdala (LA). A useful physiological model for studying synaptic plasticity is long-term potentiation (LTP). LTP in the LA has been studied only *in vitro* or in anaesthetized rats. Here, we tested whether LTP can be induced in auditory input pathways to the LA in awake rats, and if so, whether it persists over days. In chronically implanted rats, extracellular field potentials evoked in the LA by stimulation of the auditory thalamus and the auditory association cortex, using test simulations and input/output (I/O) curves, were compared in the same animals after tetanization of either pathway alone or after combined tetanization. For both pathways, LTP was input-specific and long lasting. LTP at cortical inputs exhibited the largest change at early time points (24 h) but faded within 3 days. In contrast, LTP at thalamic inputs, though smaller initially than cortical LTP, remained stable until at least 6 days. Comparisons of I/O curves indicated that the two pathways may rely on different mechanisms for the maintenance of LTP and may benefit differently from their coactivation. This is the first report of LTP at sensory inputs to the LA in awake animals. The results reveal important characteristics of synaptic plasticity in neuronal circuits of fear memory that could not have been revealed with *in vitro* preparations, and suggest a differential role of thalamic and cortical auditory afferents in long-term memory of fear conditioning.

Introduction

Long-term potentiation (LTP) in the lateral nucleus of the amygdala (LA) is a candidate mechanism underlying the acquisition of Pavlovian fear conditioning and the maintenance of a long-term memory trace of the conditioning experience (Fendt & Fanselow, 1999; Maren, 1999; LeDoux, 2000; Blair *et al*. 2001; Schafe *et al*., 2001). In auditory fear conditioning, the auditory stimulus reaches the LA both directly from the auditory thalamus (areas of the medial geniculate body and associated regions), or indirectly via thalamo-cortical and cortico-amygdala connections (Romanski & LeDoux, 1992), and both thalamic and cortical inputs converge in the LA (Romanski & LeDoux, 1993a; Romanski & LeDoux, 1993b; Li *et al*., 1996; Shi & Cassell, 1997; Doron & LeDoux, 1999). Both inputs, tested by stimulation of the internal and external capsule, respectively, have been shown to express LTP *in vitro* (Chapman *et al*., 1990; Watanabe *et al*., 1995; Huang & Kandel, 1998; Weisskopf *et al*., 1999; Huang *et al*., 2000). When induced in an *in vivo* anaesthetized preparation, successful LTP has been shown at thalamic inputs to the LA (Clugnet & LeDoux, 1990; Rogan & LeDoux, 1995; Yaniv *et al*., 2001), but failures have been reported at cortical inputs (Yaniv *et al*., 2001). In the latter case, however, the stimulation sites were aimed at the perirhinal cortex and not at the auditory association cortex (area TE3).

Primarily based on studies in the hippocampus, three properties of LTP have emerged that make it suitable as a model for learning and memory: associativity, synapse specificity, and longevity (Bliss & Gardner-Medwin, 1973; Bliss & Collingridge, 1993; Martin *et al*., 2000). The first two properties have been demonstrated in the LA (Huang & Kandel, 1998; Weisskopf & LeDoux, 1999; Weisskopf *et al*., 1999), but only in an *in vitro* preparation. As LTP in the LA has never been studied in awake animals, its longevity, and thus its suitability as a storage mechanism of fear memories in the amygdala, has not been established.

In the present study, we used an awake, freely moving preparation to test for the first time whether LTP can be induced in the LA at both thalamic and cortical auditory inputs, and, if so, whether it persists over days. As the amount of LTP could differ not only because of the pathways stimulated, but could also critically depend on the actual locus of recording, we chose an experimental design that allowed us to compare the amount of LTP between the two pathways in the same animal with the same recording placement (Doyère *et al*., 1997b; Heinbockel & Pape, 2000). The design of the experiment also allowed us to examine whether the coactivation of cortical and thalamic inputs could possibly benefit the induction of LTP in either pathway alone.

Materials and methods

Subjects

Adult male Sprague–Dawley rats (Hilltop Laboratories, Scottdale, PA) weighing 320–380 g at the time of surgery were used as subjects. They were housed individually in plastic Nalgene cages with food and water *ad libitum* in a temperature-controlled room and on a

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12-h light : 12-h dark cycle. All experiments took place during the light phase of the cycle. All procedures were in accordance with the National Institutes of Health *Guide for the Care and Use of Experimental Animals* and approved by the New York University Animal Care and Use Committee.

Surgery

Rats were prepared for chronic implants under Nembutal anaesthesia (50 mg/kg, i.p., supplemented with 10 mg/kg as necessary), using conventional surgical techniques and coordinates according to (Paxinos & Watson, 1986). Three recording electrodes (nichrome, 65 μm in diameter) attached to a guide tube and extending from it by ≈4.5 mm, were aimed at the left LA at different antero-posterior levels (AP −2.8/−3.8 mm; L 5.3–5.5 mm; DV 5.5–6.0 mm below dura). Two bipolar concentric stimulating electrodes (250 μm, SNEX-100, Rhodes Medical Instruments) were positioned ipsilaterally in the medial division of the medial geniculate and the posterior intralaminar nuclei (MGm/PIN, AP −5.4 mm; L −3.2 mm; DV 5.5–6.0 mm below dura) and in the auditory association cortex (TE3, AP −5.8 mm; L −6.5 mm; DV 3.5–4.0 mm below dura). Final depths of the electrodes were determined using electrophysiological guidance to maximize the evoked responses for both pathways on at least one of the three recording channels. The tube served as a reference, and a cortical silver ball, placed contralaterally, served as a ground. Surgical screws and dental cement were used to anchor the electrodes and the multichannel connecting device for chronic recordings. Rats were given at least five days to recover in their home cage before experimental procedures.

Electrophysiological recording procedures

Rats were habituated to the recording chamber and to the recording-headstage system, which consisted of flexible recording and stimulating cables passing through a multichannel rotating commutator at the top of the recording chamber. Rats received 30 min of habituation each day for 3 days before the beginning of the experiments. Recording always started 20 min after the rats were placed in the chamber. Evoked potentials (EPs) evoked by stimulation delivered to the MGm/PIN and TE3 (monophasic square pulse, 100 μs) were recorded through unity-gain operational amplifiers placed on the connecting sockets. Signals were amplified (× 1000), filtered (bandpass 0.1 Hz−1 kHz), digitized at 10 kHz and stored on disk for off-line analyses. Stimulation of the two pathways and recordings of the three channels were automated using Experimenter's Workbench 32 software (Datawave Technologies, Longmont, CO).

On the third day of habituation to the chamber, polarities of stimulation were chosen for individual pathways and input/output curves (I/O) were generated by collecting responses to a series of 7–9 increasing current intensities (40–800 μA) in an ascending manner to prevent any effect of higher intensities on subsequent tests at lower intensities. Field potentials were evoked alternately on the two pathways at 15 s intervals, for a total of four times at each intensity starting at the lowest intensities. Test intensities to be used in subsequent phases of the experiments were set to evoke an EP of approximately 50% of its maximum amplitude for each pathway.

A schematic of the experimental design is depicted in Fig. 1A. For baseline recordings, field potentials were evoked alternately on the two pathways at 15 s intervals for 20 min (40 pulses/pathway, Fig. 1B) each day for 3 days to ensure a stable recording for at least 2 days. On the second day of stable baseline, after the baseline was taken and I/O curves generated, tetanic stimulation was delivered to the MGm/PIN, and baseline recordings were resumed for 60 min immediately after the last tetanus. Tetanic stimulation consisted of three series of theta-burst stimulation [TBS: 10 trains (100 Hz, 100 ms) at 5 Hz] at test intensity, with a 1-min interseries interval (Fig. 1C). The next day, the same procedure was repeated, but the TBS was applied to TE3. Baseline recordings were followed for an additional 5 days. On the eighth day, TBS was applied to both pathways with a delay of 5 ms between TE3 and MGm/PIN activation (Fig. 1D). In this condition, stimulation of the two pathways during the TBS was equally spaced, thus allowing an equivalent opportunity for the cortical input to benefit the thalamic input, and for the thalamic input to benefit the cortical input. Baseline was again taken 24 h later. I/O curves were recorded systematically before and 24 h after each LTP induction. The animal's behaviour was carefully watched and EEG derived from the LA recordings was monitored on an oscilloscope during all sessions to ensure the absence of electrically induced afterdischarges and that the animals were in a still-alert state. In a separate control experiment, animals underwent the exact same protocol, with the exception that the TBS on each single pathway on day 2 and day 3 were omitted. These animals were, however, given the combined stimulation protocol on the eighth day.

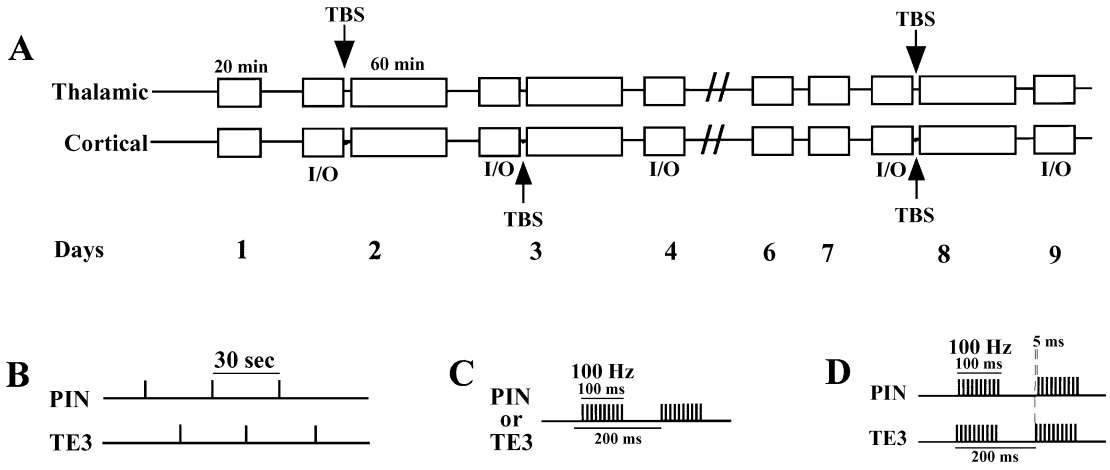

FIG. 1. Experimental protocol. (A) Schematic of the experimental design over the 9 days of recording (see Materials and methods). (B) Pattern of stimulation used for baseline recordings, with alternate stimulation on the two pathways at 15 s intervals for 20 min (40 pulses/pathway). (C) Theta-burst stimulation (TBS) applied for LTP induction to either the MGm/PIN on day 2 or TE3 on day 3. The full tetanization protocol consisted of three series of TBS [10 trains (100 Hz, 100 ms) at 5 Hz] at test intensity, with a 1 -min interseries interval. (D) Combined TBS on both MGm/PIN and TE3 for LTP induction on day 8.

Analyses

The onset and peak latencies, as well as amplitudes were measured for each EP for each pathway. Values of EP amplitude were normalized for each animal with respect to the mean values obtained either before each tetanus or before the first tetanus (days 1–2). They were averaged by time periods for illustrative purposes or statistical comparisons. For I/O curves, the four values obtained at each intensity were first averaged. For specific comparisons between I/O curves, each averaged value of EP amplitude and each intensity were expressed as a ratio of the maximum value obtained for the given comparison for each pathway, in each animal. Values reported in the text or represented in figures are mean ± SEM. Effects were analysed by Student's *t*-tests. I/O curves were fitted by Boltzmann sigmoid functions (using GraphPad Prism software package), providing a set of parameters (plateau, I_{50} = intensity at 50% of maximum amplitude, slope) that characterizes individual I/O curves and allows statistical comparisons.

Histology

Histological verification of recording and stimulating electrode placements was performed in all rats. At the end of the experiment, rats were anaesthetized with an overdose of chloral hydrate (600 mg/kg, i.p.) and perfused transcardially with 10% buffered formalin. The brains were postfixed in 10% buffered formalin. Coronal sections were cut at 30–40 μm on a cryostat or microtome, and mounted onto gelatin-coated glass slides. Sections were then stained for Nissl using Cresyl violet, and examined using light microscopy for electrode placement.

Data analysis selection criterion

Rats were included in the present study if they had (i) correct placements of both, thalamic and cortical, stimulating electrodes, and correct placement of the recording electrode, and (ii) stability of the shapes of the field potentials at all recording placements over the entire experiment to guarantee no sliding shift in the preparation. In some cases, there was more than one successful recording site for a given animal. We choose to consider these as different, because they were separated by a distance of at least 300 μm, making overlap in recording area unlikely. Of the initial 13 implanted rats, seven fulfilled the criteria for data analysis with a total of nine successful recording sites in the LA. For the control group, four out of seven rats fulfilled the criteria, and seven sites were successfully recorded over 8 days.

Results

Characteristics of field potentials evoked in the LA by auditory thalamic and cortical stimulation in the awake, freely moving rat

The LA, and more specifically its dorsolateral subnucleus (LAd), is the primary recipient of auditory inputs in the amygdala. Anatomical tracing studies have shown that both auditory thalamic afferents, originating from the medial division of the medial geniculate (MGm) and the posterior intralaminar nuclei (PIN), and auditory cortical inputs, originating from the auditory association cortex (TE3), project in an overlapping fashion to the LAd (LeDoux *et al*., 1991; Romanski & LeDoux, 1993a; Shi & Cassell, 1997; McDonald, 1998; Doron & LeDoux, 1999). Electrophysiological studies, using both extracellular and intracellular recordings *in vivo*, have confirmed the convergence in LAd, showing a monosynaptic activation of the same cell by the two inputs (Li *et al*., 1996). In agreement with these studies, we found placements that when stimulated evoked field potentials by either thalamic or cortical stimulation at the same recording site in the LA in the same animal. Histological verifications indicated that recordings in the more dorsal part of the LA, especially in the posterior positions, were larger and more reliable.

Stimulating and recording sites are represented in Fig. 2. Stimulation sites depicted in the figure are the deepest points in each track (the

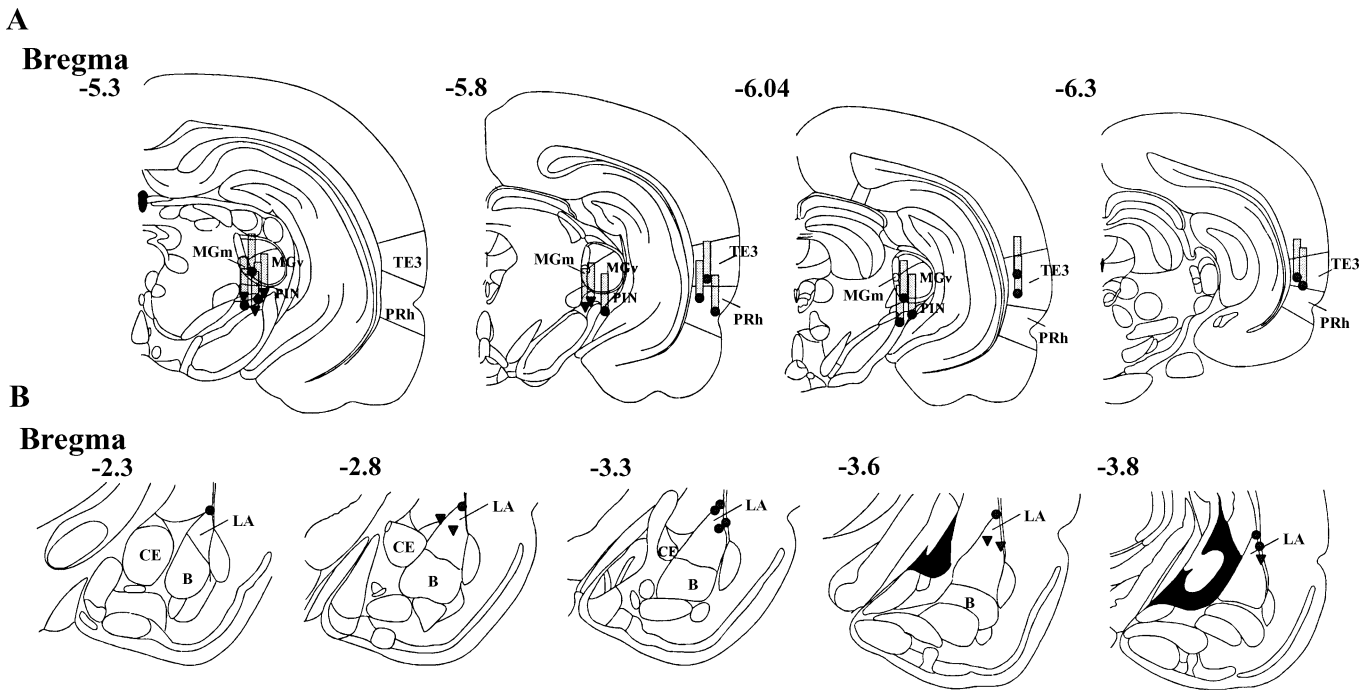

FIG. 2. Electrode placements for the 7 rats (circles) that had stable recordings for the entire experiment, and for the 6 rats (triangles) of the supplementary experiment that had stimulating electrodes only in the MGm/PIN (see text). (A) Placements of the tip of the stimulating electrodes aimed at activating the MGm/PIN and the auditory association cortex TE3. Note the distance between the tip and the base of the bipolar stimulating electrodes is 1 mm (grey bar). (B) Placements of the tip of the recording electrodes. Note that there are all confined to the dorsal part of the LA.

tips of the stimulating electrodes). The actual maximal effective area of stimulation, however, could be anywhere between this point and the base of the bipolar concentric stimulating electrode, i.e. 1 mm higher (depicted by grey bars). In fact, in most cases, the field potentials evoked by thalamic stimulation were of similar amplitude for both polarities of stimulation, suggesting that the effective area stimulated was between the tip and the base.

The characteristics of the field potentials evoked by both cortical and thalamic stimulation can be viewed in Fig. 3. Stimulation of MGm/PIN and TE3 evoked responses in the LA of similar shapes, with the peak negativity corresponding to the peak of multiunit firing (Fig. 3A). This suggests that these potentials were locally generated, and that the negative wave reflects a summation of both EPSPs and synchronized action potentials (population spike component). The EPs of the two pathways differed clearly with regard to their onset and peak latencies, which were not dependent on intensity (Fig. 3D and E). The difference in latency was 3.2 ms on average, consistent with the values reported previously in the latency of spike activity in the LA elicited by stimulation of the MGm/PIN and TE3 (Li *et al*., 1996). The latencies of the potentials evoked by MGm/PIN stimulation were also in agreement with the ones reported in other *in vivo* studies carried out in anaesthetized animals (Rogan & LeDoux, 1995; Yaniv *et al*., 2001). However, the latencies of the potentials evoked by cortical stimulation in our experiment were much shorter (by 4 ms) than the one reported by Yaniv *et al*. (2001). This might represent an inherent difference between the two cortical pathways, from the dorsal perirhinal (dPRC) vs. from the auditory association cortex (TE3). Alternatively, this might suggest that our coordinates targeted preferentially a monosynaptic pathway, whereas in the other study a polysynaptic response may have been recorded.

The amplitudes of the EPs increased with the intensity, with an overall tendency for the thalamic pathway to evoke potentials of greater amplitude than for the cortical pathway (Fig. 3F and G). As the amplitude of the negativity and the peak-to-peak amplitude were correlated, the amplitude taken from the peak negativity to a tangent line from the EP onset to the end of the negativity was analysed as representative of the potential (see drawing in Fig. 3C). This measure showed less variability from test to test than the amplitude of the negativity, and the results obtained with it were comparable to those obtained with the measure of the negativity only. The results obtained by measuring the slope of the negativity would typically be similar to those obtained when measuring the amplitude. The slope measure is, however, more sensitive to variability and noise in the signal, making it more difficult to analyse in freely moving animals. We therefore chose to analyse amplitude in the present study.

The test intensities chosen for the two pathways (Fig. 3B) did not differ significantly ($P > 0.05$), and evoked EPs of similar amplitudes (Fig. 3C, $P > 0.05$). The averaged onset and peak latencies were 2.02 ± 0.16 ms and 5.28 ± 0.37 ms (ranging from 4.85 to 8.00) for the thalamic pathway, and 4.47 ± 0.56 ms and 8.49 ± 0.39 ms (ranging from 7.34 to 10.92) for the cortical pathway, respectively.

Input-specific long-term potentiation in the LA at auditory thalamic afferents

The effects of tetanic stimulation applied to the MGm/PIN are summarized and illustrated in Fig. 4. At the thalamic afferents, there was an immediate increase of the evoked potentials, which declined rapidly, but remained at a potentiated level for 1 h. The increase was also evident 24 h later (Fig. 4A). Increases in EP amplitudes were significant at the three important time periods (Fig. 4B): during the

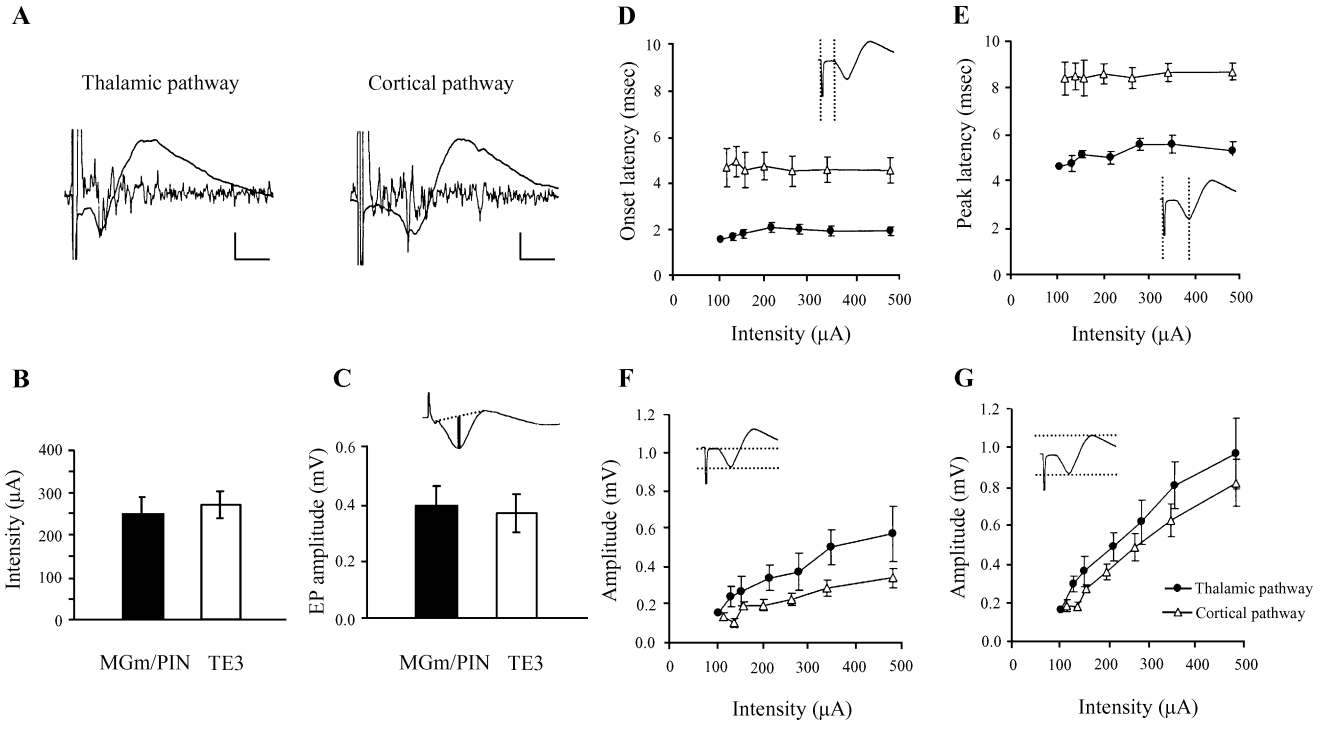

FIG. 3. Characteristics of the field potentials evoked by thalamic (MGm/PIN) and cortical (TE3) stimulation. (A) Simultaneous recording of multiunit responses and field potentials in the LA evoked by stimulating MGm/PIN (left) or TE3 (right). Scale bars, 0.4 mV, 5 ms. (B) Mean (± SEM) test intensity used for MGm/PIN and TE3 stimulation. (C) Mean (± SEM) amplitude of the evoked potentials (EP) during baseline recordings at test intensities for PIN and TE3 stimulations. (D) Mean (± SEM) onset latency (ms), measured as depicted by the drawing, of field potentials evoked in the LA by thalamic (filled circles) and cortical (open triangles) stimulation of increasing intensities during I/O curve recordings. (E) Mean (± SEM) peak latency (ms). (F) Mean (± SEM) amplitude (mV) of the first component. (G) Mean (±SEM) amplitude of the second component (mV). Note that the field potentials evoked by activation of the cortical pathway show longer onset and peak latencies.

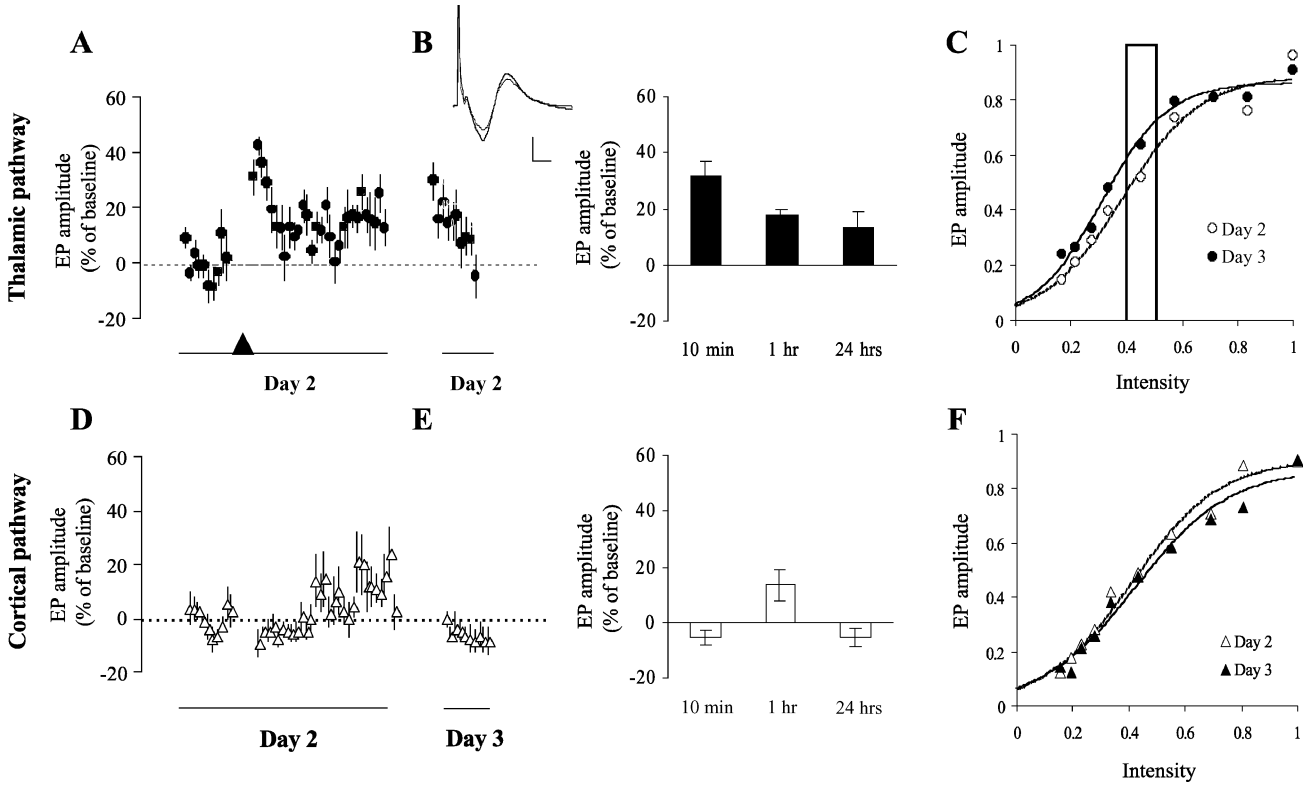

FIG. 4. Input-specific LTP in the LA at auditory thalamic afferents. Effects of theta burst stimulation applied to the MGm/PIN for the thalamic pathway (top panels A–C), and for the cortical pathway in the same animals (bottom panels D–E). (A and D) Plots of the mean percentage change (± SEM) in the amplitude of the evoked potentials during the 20 min baseline, during the 1 h following the tetani on the MGm/PIN (black harrow-head), and during the 20-min test 24 h later. Each point represents a group mean of averages by 2-min periods (four test stimuli). Inset: Superimposed traces of averages of 40 potentials evoked by thalamic test stimulation during the 20-min baseline (thin line) and 24 h after the tetani (thick line). Scale bars, 0.2 mV, 5 ms. (B and E) Mean (± SEM) percentage change in EP amplitude for three time periods, during the first 10 min after the tetani, at 40–60 min after the tetani, and during the 20-min test period taken 24 h later. (C and F) Plots of I/O curves generated before the tetani on day 2 (open symbols), and 24 h after on day 3 (filled symbols). For each pathway, EP amplitudes and intensities were normalized, then averaged, and approximated by a Boltzmann function (see Materials and methods). The width of the white bar in C shows the mean (± SEM) of the intensities used for test and tetanic stimulation of the MGm/PIN. Note that TBS of the MGm/PIN induced an enduring increase in field potentials and a shift to the left of the I/O at thalamic afferents, whereas the cortical pathway showed no systematic change.

first 10 min, which represents short-term potentiation ($t_8 = 5.00$; $P < 0.001$), and at both 1 and 24 h ($P < 0.001$ and $P < 0.05$, respectively). The latter two time points show that LTP was enduring and thus that it fulfilled the long-lasting criterion of LTP as a model of memory. A comparison of I/O curves performed before and 24 h after the tetani shows that the increase in EP amplitude seen at the test intensity was also observable over a range of intensities. There was an apparent shift of the curve to the left with no increase of the saturation level (Fig. 4C). This was confirmed statistically by a significant change in the slope and I_{50} parameters of the Boltzmann functions (Table 1, both, $P < 0.05$) and no significant change in the plateau ($P > 0.05$).

At the same time, there were no systematic heterosynaptic effects at the cortical afferents (Fig. 4D–F). There was a slight tendency for a depression to be seen both immediately and 24 h later. The depression reached significance only at 24 h ($t_8 = 2.25$; $P < 0.05$), but there was also a significant increase in EP amplitude at 1 h ($P < 0.05$). In fact, this tendency for a slow developing, but transitory, increase at 1 h was observed in parallel on both pathways and almost every time a very long recording session was performed (Figs 4, 5 and 8). Thus, this does not seem to represent any specific heterosynaptic effect, but rather some increase of the responses with time as it becomes harder to keep the animals in a stable, still alert behavioural state. Analyses of the I/O curves confirmed there was no systematic change over a range of intensities (Fig. 4F), and no significant change in any of the parameters of the Boltzmann function was detected (Table 1, all $P > 0.05$). Overall, these data show that the LTP induced in the LA by tetanization of MGm/PIN was input-specific, i.e. restricted to the activated pathway. This extends to the *in vivo* preparation that has previously been demonstrated *in vitro* (Weisskopf & LeDoux, 1999).

Although significant, the average magnitude of LTP observed at thalamic afferents appeared lower than what has been previously reported for the same pathway, using this protocol of tetanization in anaesthetized animals (Yaniv *et al*., 2001). In an independent, supplementary experiment ($n = 6$; data not shown), we tested whether the magnitude of LTP observed at the thalamo-amygdala afferents could possibly be increased to a higher level of saturation by repeating the tetanic stimulation paradigm three times at 10-min intervals. As in the first series of experiments LTP was induced successfully after the first series of tetani, and was still significant 24 h later ($P < 0.05$). However the magnitude of LTP both shortly after tetani and at 24 h did not appear to benefit from the repetition of tetani. In a few additional cases ($n = 2$), we tried another protocol of tetanization using a higher frequency (300 Hz), a protocol that has been used successfully in anaesthetized animals (Rogan & LeDoux, 1995). There was a tendency for this protocol to be more effective, but this could not be explored systematically due to behavioural side-effects (strong motor reactions) during the tetani. To date, the available data suggest that LTP at thalamic inputs to the LA may be more easily inducible in

TABLE 1. Changes in I/O functions of the thalamic and cortical projections to the LA after LTP induction

TBS	Pathway tested	Testing day	Slope	I_{50}	Plateau
MGm/PIN	Thalamic	Day 2 (baseline)	0.148 ± 0.016	0.418 ± 0.028	0.957 ± 0.025
		Day 3 (24 h)	0.105 ± 0.010	0.332 ± 0.023	0.884 ± 0.034
	Cortical	Day 2 (baseline)	0.143 ± 0.012	0.396 ± 0.030	0.901 ± 0.050
		Day 3 (24 h)	0.152 ± 0.015	0.439 ± 0.030	0.906 ± 0.045
TE3	Thalamic	Day 3 (baseline)	0.106 ± 0.011	0.332 ± 0.023	0.868 ± 0.041
		Day 4 (24 h)	0.105 ± 0.010	0.358 ± 0.020	0.914 ± 0.027
	Cortical	Day 3 (baseline)	0.151 ± 0.016	0.438 ± 0.029	0.820 ± 0.053
		Day 4 (24 h)	0.120 ± 0.013	0.415 ± 0.035	0.948 ± 0.029
Long-term	Thalamic	Day 2 (baseline)	0.157 ± 0.015	0.443 ± 0.022	0.886 ± 0.048
		Day 3 (24 h)	0.111 ± 0.011	0.349 ± 0.021	0.803 ± 0.059
		Day 8 (long-term)	0.118 ± 0.012	0.379 ± 0.025	0.887 ± 0.022
	Cortical	Day 3 (baseline)	0.147 ± 0.013	0.416 ± 0.029	0.781 ± 0.036
		Day 4 (24 h)	0.123 ± 0.013	0.408 ± 0.035	0.910 ± 0.040
		Day 8 (long-term)	0.133 ± 0.020	0.470 ± 0.058	0.868 ± 0.055
Combined vs. single	Thalamic	Day 2 (baseline)	0.149 ± 0.017	0.421 ± 0.029	0.851 ± 0.042
		Day 3 (single)	0.105 ± 0.011	0.333 ± 0.023	0.786 ± 0.048
		Day 9 (combined)	0.127 ± 0.020	0.396 ± 0.030	0.965 ± 0.031
	Cortical	Day 3 (baseline)	0.148 ± 0.014	0.419 ± 0.033	0.748 ± 0.057
		Day 4 (single)	0.120 ± 0.014	0.415 ± 0.035	0.859 ± 0.063
		Day 9 (combined)	0.099 ± 0.015	0.395 ± 0.029	0.914 ± 0.027

Individual I/O curves recorded before and 24 h, or 5–6 days, after tetanization of either pathway alone or after a combined tetanization of both pathways were normalized for each comparison (see Materials and methods), and then fitted by Boltzmann sigmoid functions ($Y = \text{Plateau}/1 + \exp(I_{50} - X/\text{Slope})$). Values are mean ± SEM for the three parameters that describe the whole I/O curve. Note that Slope describes the steepness of the curve, with a smaller value denoting a steeper curve.

urethane-anaesthetized animals. The level of inhibition has been shown to be critical in the induction of LTP in hippocampus (Steward *et al.*, 1990). A recent anatomical study has shown that thalamic afferents synapse directly onto inhibitory neurons in LA (Woodson *et al.*, 2000), providing a neural network for feed-forward control of the level of inhibition, known to exert a powerful control in the amygdala (Lang & Paré, 1997; Szinyei *et al.*, 2000). It is conceivable that during the TBS, the direct activation of inhibitory cells provided a higher level of inhibition in awake animals than in urethane-anaesthetized animals, and this may have resulted in a reduction of LTP at thalamic inputs to the LA in our conditions.

Input-specific long-term potentiation in the LA at auditory cortical afferents

The effects of tetanic stimulation applied to the auditory association cortex (TE3) are summarized and illustrated in Fig. 5. Similar to the findings obtained on the thalamic input pathway, tetanic stimulation applied to TE3 induced an input-specific LTP in the LA (Fig. 5). There was an immediate increase of the amplitude of the EPs evoked by the activation of the cortical pathway, an increase that remained at the same level for an hour and was still evident 24 h later (Fig. 5D). The potentiation was significant at the three time periods tested, showing both short-term potentiation during the first 10 min ($t_8 = 4.50$; $P < 0.001$), and long-term potentiation both at 1 h and at 24 h (all $P < 0.05$). The analyses of I/O curves showed a faster increase to a higher plateau (Fig. 5F), as confirmed statistically by a significant change in the slope ($P < 0.01$), with no change in the I_{50} ($P > 0.05$) of the Boltzmann functions. The change in the plateau just reached significance ($P = 0.05$), mainly due to a higher variability of this variable, especially in this case for the I/O taken during baseline (Table 1).

There was no heterosynaptic effect of the cortical tetanization, as revealed by the fact that the EPs evoked by the thalamic stimulation in the same animals did not change (Fig. 5A–C). No significant change was obtained at the test intensity at any of the three time periods tested (all $P > 0.05$), or at the other intensities (all $P > 0.05$ for the three parameters of the Boltzmann function).

Overall, these data show that tetanization of TE3 in the awake rat induces LTP in the LA, which is input-specific, i.e. restricted to the activated pathway. *In vitro* studies have shown that 'cortical' inputs undergo LTP (Weisskopf *et al.*, 1999; Huang *et al.*, 2000). In contrast, *in vivo* studies are less clear about LTP in cortical pathways to LA, with some sites showing LTP and others not (Yaniv *et al.*, 2001). In the latter case, the stimulation sites were aiming at the perirhinal cortex and not at the auditory association cortex TE3. In fact, there was an increased propensity to show some LTP as the stimulation sites were moved from the ventral part to the dorsal part of the perirhinal cortex. This could suggest a very specific input-related capacity for LTP in the LA, with a higher capability for TE3 afferents to potentiate.

Differential longevity of LTP in the LA between auditory thalamic and cortical afferents

The design of the present study allowed us to compare different characteristics of LTP for two sets of inputs in the same animals. Unlike previous studies, which have performed this over a matter of minutes or hours (Rogan & LeDoux, 1995; Huang *et al.*, 2000; Yaniv *et al.*, 2001), LA recordings in our experiments were measured over days. The changes seen in the EPs for both pathways over the entire experiment are presented for both the experimental group (Fig. 6A and B), and the control group (Fig. 6C and D). These findings, along with those in Fig. 7, clearly indicate that LTP at the thalamic afferents was of smaller amplitude, but longer lasting than LTP at cortical afferents.

Indeed, after single pathway tetanization for the experimental group or after combined tetanization of the two pathways in the control group, the amplitude of potentiation for the thalamic pathway was significantly smaller than the one for the cortical pathway (Fig. 7A), both during the first 10 min (STP) and at 1 h after the tetanization (all $P < 0.05$). Although the same tendency was still observed at 24 h, it was no longer significantly different ($P < 0.10$).

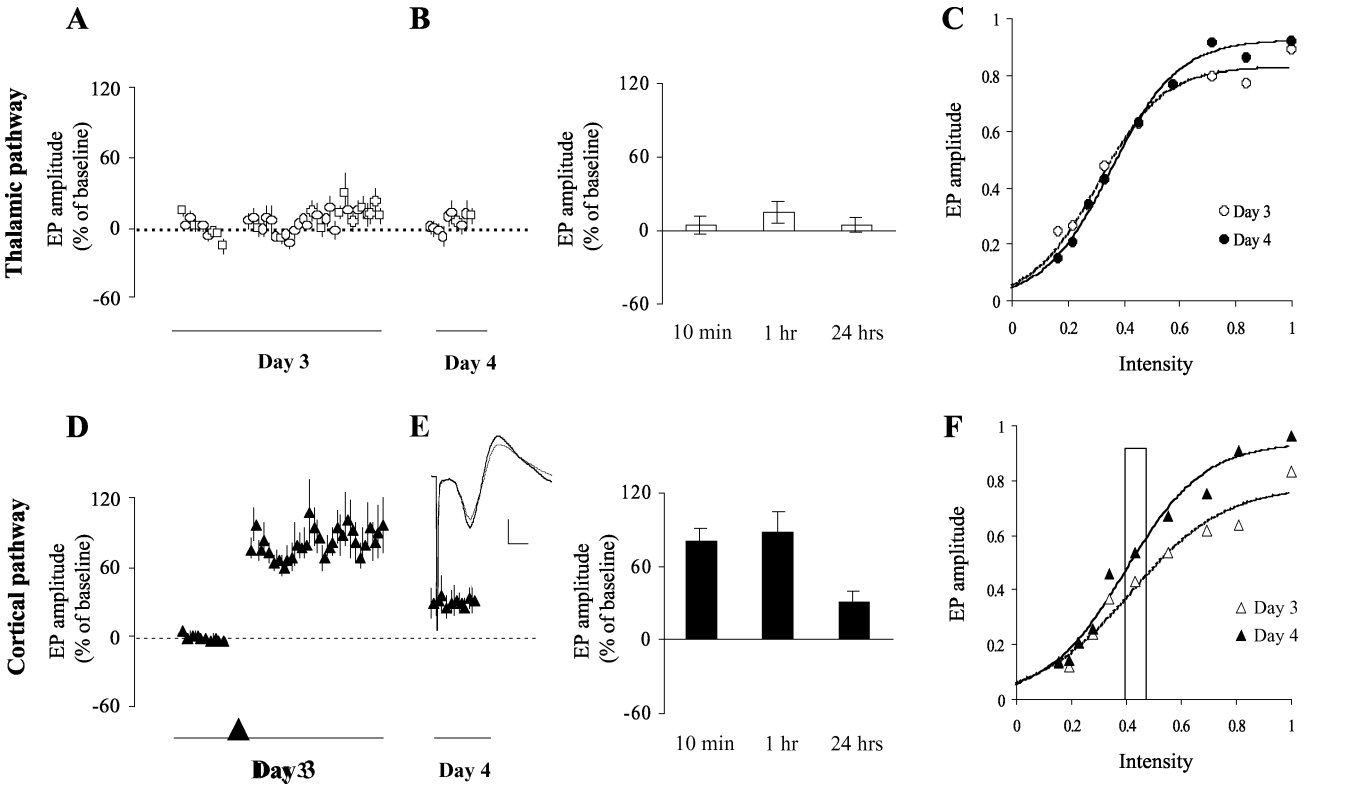

FIG. 5. Input-specific LTP in the LA at auditory cortical afferents. Effects of theta burst stimulation applied to the auditory association cortex TE3 observed for the thalamic pathway (top panels A–C), and for the cortical pathway in the same animals (bottom panels D–F). (A and D) Plots of the mean percentage change (± SEM) in the amplitude of the evoked potentials during the 20-min baseline, during the 1 h following the tetani in TE3 (black harrow-head), and during the 20-min test 24 h later. Each point represents a group mean of averages by 2 -min periods (four test stimuli). Inset: Superimposed traces of averages of 40 potentials evoked by cortical test stimulation during the 20-min baseline (thin line) and 24 h after the tetani (thick line). Scale bars, 0.2 mV, 5 ms. (B and E) Mean (± SEM) percentage change in EP amplitude for three time periods: during the first 10 min after the tetani, at 40–60 min after the tetani, and during the 20-min test period 24 h later. (C and F) Plots of I/O curves generated before the tetani on day 3 (open symbols), and 24 h after on day 4 (filled symbols). For each pathway, EP amplitudes and intensities were normalized, then averaged, and approximated by a Boltzmann function (see Material and methods). The width of the white bar in F shows the mean (± SEM) of the intensities used for test and tetanic stimulation of TE3. Note that TBS on TE3 induced an enduring increase in field potentials and a shift to the left of the I/O with an increased level of the plateau at cortical afferents, whereas the thalamic pathway showed no systematic change.

Despite being smaller in amplitude, however, the change at thalamic inputs was much longer lasting. The amplitude of EPs evoked by MGm/PIN stimulation remained significantly potentiated for as long as 6 days after the tetanization (all $P < 0.05$), while LTP for the cortical pathway decayed back to baseline by 3 days. Importantly, this was confirmed by the comparison of the I/O curves taken on day 8, with the I/O curves taken before and 24 h after each LTP induction (Fig. 7C and D). The data of the I/O curves from one rat were unusable on day 8 because of recording problems, and thus analyses could only be performed on the eight remaining recordings. The analysis indicated that the shift to the left seen on day 3 in the I/O curve for the thalamic pathway was still observable on day 8 (Fig. 7C), as shown by the still significant change in the I_{50} and slope parameters of the Boltzmann functions 6 days after the tetanization (Table 1, both $P < 0.05$). In contrast, the I/O curve for the cortical pathway had returned to its baseline level (Fig. 7D), no longer showing a significant change in either the plateau, or the slope parameters of the Boltzmann function (both $P > 0.05$). Moreover, LTP amplitudes 4–5 days after their respective induction were also significantly different between the two pathways (Fig. 7A, L-LTP, $t_{16} = 2.16$; $P < 0.05$). This difference was even more pronounced when the potentiation for each pathway was expressed as a percentage of its maximum potentiation, thus taking into account the differences in the initial levels of potentiation and showing in a direct manner the decay functions of LTP on both pathways (Fig. 7B). The initial LTP for the cortical pathway showed a substantial decrease within 24 h, and then completely decayed back to baseline within three days after its induction. In contrast, LTP for the thalamic pathway showed an initial decrease within the first 24 h, but remained stable at that level for the next six days of recording.

Importantly, control rats that received repeated test stimulation, but not LTP-inducing stimulation, over the 8-day recording sessions did not show significant changes in EP amplitudes in either pathway (Fig. 6C and D). This was observed not only for the test intensity, but also when EP amplitudes were measured across a range of intensities with the I/O curve (all $P > 0.05$ for the I/O-test parameters; data not shown). Thus, the differential longevity observed in the two pathways following LTP cannot simply be attributed to repeated stimulation of each pathway over many days. Finally, one could argue that the longevity of LTP in the cortical pathway may have been affected by the preceding LTP-induction on the thalamic pathway. Although we cannot completely rule out this possibility, we think it is unlikely for two reasons. First, shortly after tetanization, the LTP induced in the cortical pathway was of a much higher amplitude than that of the thalamic pathway, an outcome that would not be expected if prior tetanus on the thalamic pathway compromised the LTP of the cortical pathway. Second, there were a few cases in which we failed to induce LTP at thalamic inputs. Nonetheless, the amplitude

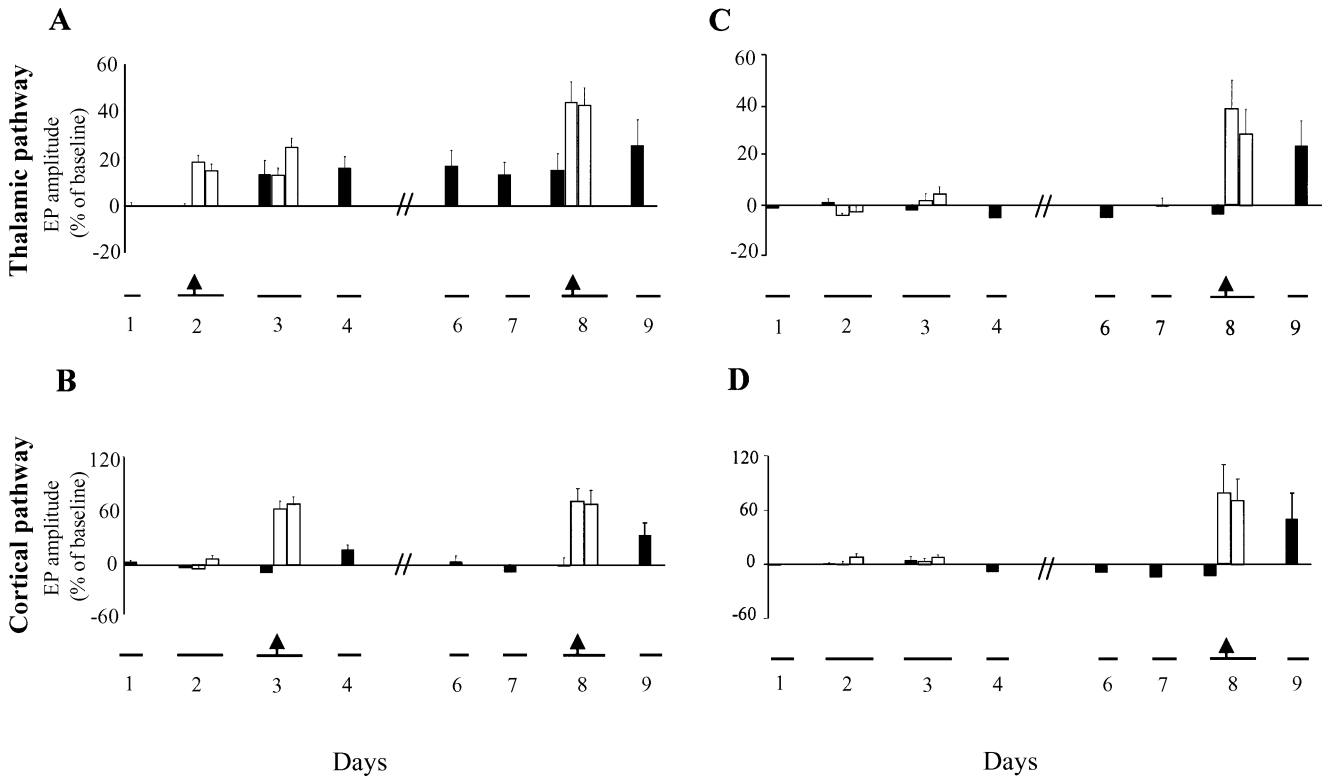

FIG. 6. Summary over days of the LTP time course in the LA. Mean percent change (± SEM) in field potential amplitude for the thalamic pathway (top row) and the cortical pathway (bottom row) in the same animals during the 9 days of the experiment for the experimental group (A and B) and for the control group (C and D). Each black bar represents the group mean of averaged responses to 40 test stimuli (20-min recordings). White bars represent averaged responses to 60 supplementary test stimuli (30-min recordings) given on days 2, 3 and 8, when tetanization protocol were applied to some of the animals (black arrowheads). The 20-min recordings on days 1 and 2 (before tetanization of MGm/PIN) constitute the baseline to which amplitude values were normalized.

and duration of LTP at the cortical inputs in these cases was similar to that reported here (data not shown). For these reasons, we think that it is very unlikely that the differences in LTP duration were due to the design of the experiment.

Long-term potentiation in the LA after combined tetanization of auditory thalamic and cortical afferents

Associative LTP can be induced in the LA *in vitro* by pairing a weak stimulation of either input with strong postsynaptic depolarization of LA neurons (Huang & Kandel, 1998; Weisskopf *et al.*, 1999), thus mimicking the coactivation of convergent inputs. The associative property of LTP has never been tested in the LA in an *in vivo* preparation. In the hippocampus *in vivo*, this has been demonstrated by showing that coactivation of two sets of strong inputs leads to a strengthening of LTP over that observed following the tetanization of either pathway alone (McNaughton *et al.*, 1978; Levy & Steward, 1979). Here, we approached this question similarly by testing whether simultaneous tetanic stimulation of thalamic and cortical inputs to LA would lead to a strengthening of LTP on either pathway. This was tested both in the same animals that underwent single tetanization a week earlier, and in the naïve control group. This latter group consisted of five channels from three animals (two recording channels from one animal were lost because of recording problems).

Following the simultaneous tetanization of both cortical and thalamic inputs, both pathways again showed significant LTP (Fig. 8), which lasted for 1 h and remained increased 24 h later. Statistical analyses confirmed that there was a significant short-term potentiation for both pathways during the first 10 min after the tetani ($t_8 = 2.69$; $P < 0.05$ and $t_8 = 8.70$; $P < 0.001$, respectively, for the thalamic and cortical pathways), as well as a significant long-term potentiation at the two other time points (1 h and 24 h, all $P < 0.05$). This same pattern of results was also observed when the combined protocol was performed on 'naive' pathways in the control group (all $P < 0.05$).

At first glance, the amount of STP and LTP induced by the combined protocol appeared to be of similar amount than that observed following tetanization of single pathways alone (compare Fig. 8B vs. Fig. 4B, and Fig. 8E vs. Fig. 5E). Paired comparisons confirmed that for both pathways, there was no significant difference in the amount of STP, nor in the amount of LTP at 1 h or at 24 h between combined vs. single pathway conditions (all $P > 0.05$). However, as the LTP on the thalamic pathway had not decayed back to the baseline level, this supplementary potentiation resulted in a higher amount of LTP reached after the combined tetanization (Fig. 6A). When compared in naïve pathways, the amount of STP and LTP induced by the combined protocol appeared to be larger than that observed following tetanization of single pathways alone. This was especially true for the cortical pathway. However, at this single test intensity, no clear benefit was observed for the combined procedure (Fig. 7A). In contrast, analyses of the I/O curves showed shifts after combined tetanization that were different from those observed after single pathway tetanization (Fig. 8C and F). As discussed previously, single pathway tetanization of the thalamic pathway produced a shift in the slope of the Boltzmann function, indicating a potentiation in the lower range of intensities. After combined tetanus, however, a significant increase of the plateau level was observed, indicating a potentiation at the higher range of intensities (Fig. 8C, Table 1). In contrast, for the cortical pathway, the single-pathway tetanization induced a significant increase in the plateau level, which was not further modified by the combined-

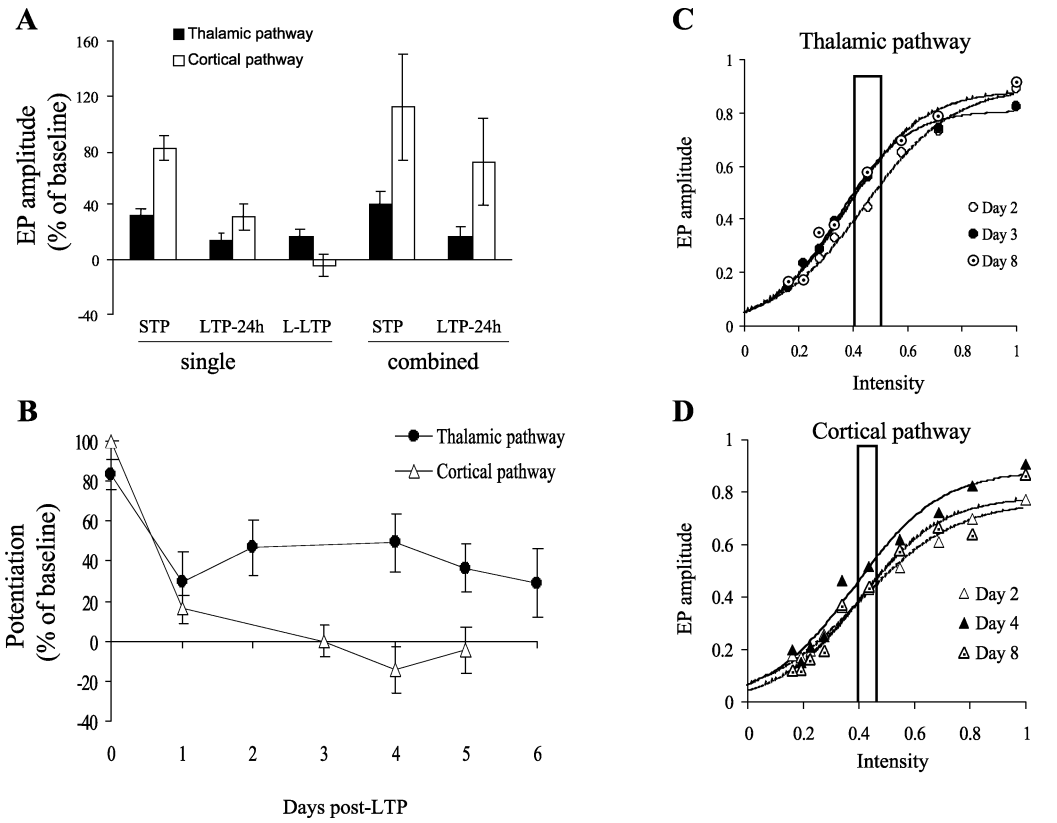

FIG. 7. Comparison of LTP in the LA at auditory thalamic and cortical afferents. (A) Mean (± SEM) amount of potentiation obtained for the thalamic (black bars) and the cortical (white bars) pathway at different time periods after the tetanization of either pathway alone in the experimental group, or after the combined tetanization of both pathways in the control group. STP = the first 10 min after the tetani, LTP-24 h, L-LTP = 4–5 days after the tetani. (B) Decay of LTP over days after the tetanization for the thalamic pathway (closed circles) and the cortical pathway (open triangles). Each point represents the mean (± SEM) of the field potential amplitude, as expressed as a percentage of the maximum potentiation for each pathway. (C) Plots of I/O curves generated for the thalamic pathway during the baseline on day 2 (open circles), and 24 h after the tetanization of the thalamic pathway alone on day 3 (filled circles), and many days later on day 8 (circles with dots). (D) Plots of I/O curves generated for the cortical pathway during the baseline on day 2 (open triangles), and 24 h after the tetanization of the cortical pathway alone on day 4 (filled triangles), and days later on day 8 (triangles with dots). For each pathway, EP amplitudes and intensities were normalized, then averaged, and approximated by a Boltzmann function (see Materials methods). The width of the white bars show the mean (± SEM) of the intensities used for test and tetanic stimulation.

pathway tetanization protocol (Fig. 8F, Table 1). Thus, the I/O analysis revealed differences that could not be seen at single test intensities (width of the white bar in Fig 8C and F). Importantly, a similar picture emerged in the I/O curves after combined tetanization in the naïve control group (data not shown). For the thalamic pathway, we observed a tendency for an increase of the plateau ($P = 0.07$) with no change in the I_{50} or the slope. For the cortical pathway, there was a significant decrease in the I_{50} ($P < 0.05$), but no change in the observed plateau level. Thus, the combined stimulation protocol produced changes different from those observed following single pathway tetanization. However, these differences emerged only after careful consideration of EP amplitudes over a range of intensities.

Discussion

LTP in the amygdala may underlie auditory fear conditioning (LeDoux, 2000; Maren, 1999; Martin *et al.*, 2000). In the present study, we compared the induction of LTP in the two main routes of auditory transmission to the LA, the sensory interface of the amygdala. We show for the first time in awake animals that both the thalamic and cortical afferents undergo input-specific LTP that lasts for more than 24 h, thus exhibiting the longevity properties necessary for a cellular mechanism of long-term memory storage. The use of double pathway stimulation in an awake preparation allowed us to reveal important differences between the two pathways. LTP at the thalamic afferents was of smaller amplitude, but outlasted LTP at cortical afferents by at least 3 days. Comparisons of I/O curves indicated that the two pathways may rely on different mechanisms for the maintenance of LTP and may benefit differently from their coactivation. The results suggest a differential role of thalamic and cortical auditory afferents in long-term memory of fear conditioning that could not have been appreciated with an *in vitro* model.

Auditory thalamic and cortical afferents to the LA differ in multiple ways

LTP in the amygdala has never been tested in awake animals. In the present study we show that the same pattern of stimulation induces LTP in both auditory pathways in the awake rat, but that the LTP in the two pathways has different characteristics. Soon after its induction, the level of potentiation observed was much higher for the cortical than for the thalamic pathway, a difference that faded over a 24-h period. Repeating the tetanization protocol three times at 10-min intervals did not increase the level of potentiation at the thalamic inputs further. I/O curves indicated that LTP at thalamic inputs induces a shift to the left of the I/O function, with no change in the saturation level at high intensities, whereas LTP at cortical afferents preferentially increases the saturation level of the I/O function. To date, the biophysics of I/O curves are poorly understood, and it is hard to make inferences about

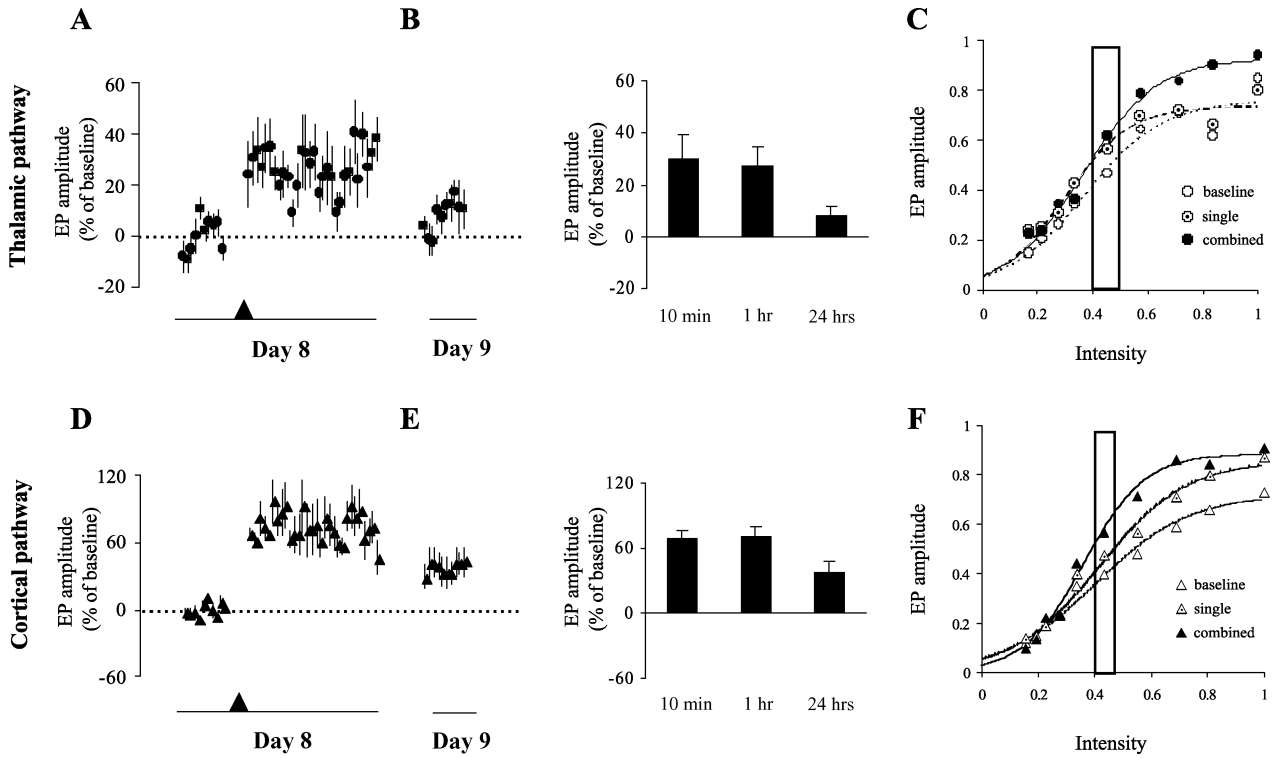

FIG. 8. LTP in the LA following combined tetanization of auditory thalamic and cortical afferents. Effects of theta burst stimulation applied simultaneously to the MGm/PIN and TE3 at thalamic afferents (top panels, A–C), and at cortical afferents (bottom panels D–F) in the same animals. (A and D) Plots of the mean percentage change (± SEM) in the amplitude of the evoked potentials during the 20-min baseline, during the 1 h following the tetani on MGm/PIN and TE3 (black harrow-heads), and during the 20-min tests 24 h later. Each point represents a group mean of averages by 2-min periods (four test stimuli). (B and E) Mean (± SEM) percentage change in EP amplitude for three time periods: during the first 10 min after the tetani, at 40–60 min after the tetani, and during the 20-min test period 24 h later. (C) Plots of I/O curves generated for the thalamic pathway during the baseline period (open circles), 24 h after its tetanization (circles with dots), and 24 h after combined tetanization with the cortical pathway (filled circles). (D) Plots of I/O curves generated for the cortical pathway during the baseline period (open triangles), 24 h after its tetanization (triangles with dots), and 24 h after combined tetanization with the thalamic pathway (filled triangles). For each pathway, EP amplitudes and intensities were normalized, then averaged, and approximated by a Boltzmann function (see Materials and methods). The width of the white bars shows the mean (± SEM) of the intensities used for test and tetanic stimulation.

the changes underlying this differential expression of LTP between the two pathways. A relationship between I/O slope and spine density, and receptor binding has been reported in the hippocampus (Woolley *et al*., 1997). Differences in the ratio of AMPA vs. NMDA receptors have been described between these two pathways to the LA, the NMDA component in the basal synaptic transmission being much more prevalent for the thalamic pathway than for the cortical one (Li *et al*., 1995; Weisskopf & LeDoux, 1999; Zinebi *et al*., 2001). An increase in the number of AMPA receptors would thus prominently affect the I/O function of the cortical pathway, while an increase in sensitivity of NMDA receptors would prominently affect the I/O function of the thalamic pathway. Other hypotheses in terms of differential involvement of inhibitory networks by the two pathways, or differential EPSP/IPSP balance are also credible, but further experiments would be needed to address these possibilities.

There has been only one study attempting to analyse the duration of LTP in the LA, and unfortunately, this was an *in vitro* study (Huang *et al*., 2000), thus precluding the assessment of the longevity of LTP beyond a few hours. With the chronic preparation used in the present study it was possible to reveal differences in the duration of LTP at thalamic and cortical inputs, with a surprising twist between different time points. At one hour (the maximum time used in most studies), larger STP and LTP were seen in the cortical pathway. In the same animal and at the same recording site, a different conclusion emerged at later test times. LTP faded within 3 days at the cortical inputs, but remained stable until at least 6 days at the thalamic inputs. It is quite possible that other parameters of stimulation, for example closer to the natural pattern of firing of the origin cells in the MGm/PIN and in TE3 (Dobrunz & Stevens, 1999; Edeline, 1999; Paulsen & Sejnowski, 2000), could have given rise to LTP with different characteristics than those reported here. Nevertheless, the differential characteristics of LTP we have observed in the present study may actually represent a true intrinsic difference between the two sets of auditory inputs to the LA that may be functionally relevant.

Another variable that could play a critical role in the amplitude and/or duration of LTP in the LA is the precise anatomical placement of the recording sites. Indeed, a recent single-unit study has suggested that LA cells have different conditioning behaviour depending on their dorso-ventral location within the LAd (Repa *et al*., 2001). For example, cells in the ventral part of the LA exhibit sustained, extinction-resistant conditioned responses after fear conditioning, while those in the more dorsal part of the LA exhibit transient, extinguishable conditioned responses (Repa *et al*., 2001). In the present study, we could thus have expected a different duration of LTP depending on the dorso-ventral position of the recordings. One limit of the field potential technique, however, is the fact that the area of recording is much broader than in the case of single units. Further, in the unit recording studies, there are generally only 30% of the cells that show a change in firing after conditioning, and the areas delimited in the paper of Repa *et al*. (2001) are very small, as they are within the LAd subdivision. It is

thus very likely that each field potential would represent a summation of cells that are either, not modified, modified transiently, or modified 'permanently' in the ventral part. Such a question, though critical for a better understanding of the functional role of LTP in the LA, would require a correlative study with many more animals than in the present study, with variable placements throughout the LA, and with a different technique that allows for recordings of field potentials within a more restricted area.

Associative LTP

Single neurons in LA have been shown to respond to both thalamic and cortical stimulation both *in vivo* (Li *et al*., 1996) and *in vitro* (Mahanty & Sah, 1999; Weisskopf & LeDoux, 1999), suggesting that such a convergence might promote associative interactions between these afferents and facilitate synaptic plasticity. *In vitro* studies have shown that associative LTP can be induced at putative 'thalamic' and 'cortical' synapses in LA by pairing weak stimulation of either input with strong postsynaptic depolarization of LA neurons (Huang & Kandel, 1998; Weisskopf *et al*., 1999). These protocols were designed to mimic the coactivation of convergent inputs, fulfilling the well-known associative rule of Hebbian LTP (Teyler & Discenna, 1984; Brown *et al*., 1990; Bliss & Collingridge, 1993). Such protocols are, however, impractical in an *in vivo* preparation, particularly in awake animals. In the hippocampus, the associative property of LTP has been demonstrated *in vivo* by showing that coactivation of two sets of strong inputs lead to a strengthening of LTP above that observed following tetanus of each pathway alone (Levy & Steward, 1979; McNaughton *et al*., 1978). In that study, it was suggested that the combined tetanus protocol was effective due to its ability to recruit weaker inputs within the stimulated pathways that otherwise would not have potentiated.

Here, we took the opportunity in our experimental design to begin to approach the question of associativity by giving simultaneous tetanic stimulation of thalamic and cortical pathways. The results indicated that both pathways may benefit from this coactivating procedure. However, this benefit was only observed after EP amplitudes were considered over a range of intensities. Interestingly, the I/O curves revealed differences in the two pathways after combined tetanus. For the thalamic pathway, we observed a tendency for an increase of the plateau with no change at lower intensities. For the cortical pathway, conversely, there were significant increases at lower intensities, but no change in the observed plateau level. These observations could represent different thresholds for LTP induction reached because of the higher frequency with which postsynaptic cells in the LA were activated (i.e. 200 Hz as opposed to 100 Hz). Alternatively, this could reflect the involvement of distinct mechanisms in the maintenance of LTP following different stimulation protocols, as has been shown for the differential involvement of NMDA receptors and voltage-gated calcium channels in LTP induced by pairing or tetanic stimulation, respectively, *in vitro* (Weisskopf *et al*., 1999; Bauer *et al*., 2002). Further experiments will be necessary to distinguish between these two possibilities and to explore the question of associativity at thalamic and cortical inputs to the LA in greater detail.

Functional implications

Three properties of LTP have made it an attractive model for the synaptic plasticity induced during learning: associativity, input-specificity and longevity (Bliss & Collingridge, 1993; Martin *et al*., 2000). Establishing a link between LTP in the hippocampus and hippocampal-dependent memory has proven to be challenging (Barnes, 1995; Eichenbaum, 1995; Shors & Matzel, 1997; Martin *et al*., 2000). Somewhat more progress has been made in relating LTP in the amygdala to learning and memory (Bauer *et al*., 2001; Rogan & LeDoux, 1996; Stevens, 1998; Maren, 1999; LeDoux, 2000; Schafe *et al*., 2001). Two of the key properties of LTP – associativity and input specificity – have been shown in the LA *in vitro* (reviewed in Blair *et al*., 2001). However, the *in vitro* preparation is not ideal for testing longevity as the measurement of synaptic changes over days is not possible. Here, we report for the first time in the awake animal that LTP at auditory inputs to the LA is input-specific, has associative properties, and can last for at least one week.

LTP at both auditory thalamic and cortical pathways to the LA exhibited input-specificity. Not only was there no general increase in amygdala excitability, but also no obvious heterosynaptic LTD or depotentiation, in contrast to what has been shown at some hippocampal synapses (Levy & Steward, 1983; Doyère *et al*., 1997b). Functionally, this may represent the capacity for both pathways to support conditioning, as shown previously in lesion studies (Romanski & LeDoux, 1992). This may also suggest a low likelihood of convergent auditory information competing in the LA. In addition, our data suggest that associative interactions between these two sets of auditory afferents could lead to a mutual strengthening that could participate in the formation of CS–US associations. For example, a specific strengthening of the coactive auditory afferents in the LA during conditioning could have a facilitative effect for discriminative learning in normal conditions, by facilitating the sharpening of the generalization curves around the CS frequency. However, the extent to which these afferents interact, as well as the functional role of such convergence remains to be investigated.

Very stable LTP has been previously reported in the hippocampus (Staübli & Lynch, 1987; Doyère & Laroche, 1992), and, though few examples exist, correlational studies have suggested a role for the lasting component of LTP in the maintenance of memory (Barnes, 1979; Doyère & Laroche, 1992; Villarreal *et al*., 2002). Further, genetic and molecular studies have strongly suggested a role for stabilization mechanisms of amygdala LTP in memory consolidation of Pavlovian fear conditioning (Maren, 1999; Schafe *et al*., 2001). While Pavlovian fear conditioning is particularly resistant to forgetting, even after very few CS–US pairings (Campbell & Campbell, 1962; Gleitman & Holmes, 1967; Doyère *et al*., 1997a), the memory for more specific characteristics of the CS may fade with time as shown by a broadening of generalization gradients (Bouton *et al*., 1999; Riccio *et al*., 1992). Taken together, along with the hypothesis of the different contributions of thalamic and cortical inputs to the LA in auditory fear learning (Campeau & Davis, 1995; Corodimas & LeDoux, 1995; LeDoux, 2000; Duvel *et al*., 2001), the present results suggest that the more rigid, sustained encoding of the 'crude' aspects of fear memories may rely on the almost nondecremental plasticity at auditory thalamic afferents to the LA. Shorter lasting plasticity at cortical afferents may result in the more rapid loss of detailed, precise encoded information with time, but could provide some flexibility for adjustments in auditory fear memory processes.

Acknowledgements

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Abbreviations

EPs, evoked potentials; LA, lateral nucleus of the amygdala; LTP, long-term potentiation; I/O, input/output; MGm, medial geniculate; PIN, posterior intralaminar nuclei; TBS, theta-burst stimulation; TE3, auditory association cortex.

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