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Induction of Fos-like immunoreactivity and the maintenance of long-term potentiation in the dentate gyrus of unanesthetized rats

K.J. Jeffery[1], W.C. Abraham[1], M. Dragunow[2] and S.E. Mason[1]

[1]*Department of Psychology and the Neuroscience Research Centre, University of Otago, Dunedin (New Zealand) and* [2]*Department of Pharmacology and Clinical Pharmacology, School of Medicine, University of Auckland (New Zealand)*

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Memory formation in the mammalian central nervous system may require long-lasting alterations in gene expression. However, it is not yet known whether the candidate memory mechanism long-term potentiation (LTP) requires alterations in gene expression for its maintenance, nor the extent to which the time course of LTP can be manipulated at the time of induction. In this study we influenced the time course of LTP decay for the perforant path input to the dentate gyrus in awake rats by manipulating conditions at the time of induction, and correlated the outcome with the induction of c-*fos* protein(s) (Fos), as measured immunohistochemically in the dentate gyrus of separate animals 2 h post-tetanization. Sodium pentobarbital, which blocks the induction of Fos-like immunoreactivity (Fos-IR), also blocked a long-duration form of LTP maintained over weeks. On the other hand, two different patterns of delivery of 50 trains, that produced similar time courses of LTP decay, produced markedly different degrees of Fos-IR induction. In addition, while stimulation consisting of only 10 trains induced a sizable Fos response, it only produced LTP lasting a few days. When the 10-train stimulation was repeated on 3 or 5 consecutive days, there appeared to be no additional Fos-IR induction, yet the LTP decay time constant was considerably prolonged. Thus there is little correlation between the degree of Fos-IR induction and the subsequent durability of LTP.

INTRODUCTION

Long-lasting storage of information in the central nervous system may involve both short-term, transcription-independent events at the synapses, and longer-term activation of genes in the cell nucleus. Some immediate-early genes (IEGs), which are rapidly and transiently expressed following certain types of cell stimulation, are thought to be involved in the regulation of nuclear responses to cell-surface events through the action of their DNA-binding proteins[5,9,17,18,29]. In particular, a number of studies have shown that seizures, which are known to produce adaptive plastic responses in the nervous system such as kindling[16], induce the rapid and transient expression of the IEGs c-*fos*, c-*jun*, *Jun*-B and *zif/268*[14,23,24,28,31,32] in various limbic and neocortical brain regions, especially the hippocampus. It has been suggested therefore that c-*fos* may also play a part in more physiological forms of plasticity, such as the increase in synaptic strength (known as long-term potentiation, LTP[6]) which occurs after high-frequency, but non-seizure-inducing, tetanization of afferent pathways in the same regions.

Initial studies in urethane-anesthetized rats showed that LTP-inducing electrical stimulation of the perforant path failed to induce c-*fos* protein-like immunoreactivity (Fos-IR) postsynaptically in the dentate gyrus granule cells, unless repetitive firing of the granule cells was also elicited[12]. Recently though, we have demonstrated that c-*fos* mRNA and protein can be induced by LTP-inducing stimulation in unanesthetized rats[13]. This induction was blocked by NMDA receptor antagonists, and did not occur following low-frequency stimulation at 1 Hz. However, Fos-IR induction and LTP induction were separable since there was no significant correlation between the two events in individual rats, and two types of stimulation (patterned vs evenly-spaced trains of impulses) produced comparable amounts of LTP but very different amounts of Fos-IR.

It may be that IEGs such as c-*fos* regulate the maintenance of LTP rather than its induction, by producing a long-lasting modulation of the expression of other target genes. In support of this idea we have found, in preliminary experiments, that administration of tetanic stimulation under pentobarbital anesthesia produces only a short-lasting form of LTP[21] as well as blocking the induction of Fos-IR[13]. In this study we have looked in greater detail at the relationship between Fos-IR and LTP decay. Specifically, we have tested whether Fos-IR induction is at least qualitatively, if not quantitatively,

Correspondence: W.C. Abraham, Department of Psychology, University of Otago, P.O. Box 56, Dunedin, New Zealand.

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correlated with LTP decay as manipulated in three ways: (i) induction of LTP with spaced versus patterned tetanic stimulation, (ii) pentobarbital anesthesia during tetanization, and (iii) variation of the number of stimulus trains administered, and the number of days over which they were given.

MATERIALS AND METHODS

Surgical procedures

Male Sprague–Dawley rats (300–600 g) were anesthetized with sodium pentobarbital and implanted bilaterally, under electrophysiological guidance, with 75 μm Teflon-coated stainless-steel stimulating electrodes in the perforant path (4.5 mm lateral to lambda) and similar recording electrodes in the hilus of the dentate gyrus (3.8 mm posterior and 2.5 mm lateral to bregma), positioned to produce a maximal positive-going field EPSP (summed extracellular postsynaptic potentials) and population spike (summed extracellular action potentials). The electrodes, which had previously been soldered to gold amphenol pins, were connected to a plastic headcap and the whole assembly fixed to the skull with dental acrylic. At the end of the procedure, penicillin was administered i.m.

Chronic stimulation and recording

After a minimum 2 week recovery time, baseline recordings of the dentate evoked potentials were made. These were always obtained at the same time of day for each rat, and were conducted in the same room. The animals were generally still but awake during recording sessions. Signals were recorded through field effect transistors and thence by flexible wire leads to Grass P15 preamplifiers. After amplification and filtering (0.1 Hz to 3 kHz) the signals were sampled on-line at 10 kHz by a PDP micro-11 computer. A baseline session consisted of 60 diphasic test pulses of 150 μs duration at 0.05 Hz, current adjusted to give a 2–3 mV population spike. Baseline recordings were repeated at the same time on each of 3 days over the week prior to tetanization.

Tetanization

On the tetanization day, baseline recordings were made for 10 min, and then LTP-inducing stimulation was administered to one or both hemispheres according to one of the following paradigms ('train' = 10 pulses of 250 μs duration at 400 Hz):

AwBu(10) group — awake animals received 10 trains in two 'bursts' of 5 trains each, with 1 s between trains and 1 min between bursts;

AwBu(50) group — stimulation patterned as above but to a total of 50 trains;

AnBu(50) group — stimulation as for AwBu(50) but rats were anesthetized to a surgical level with sodium pentobarbital for the 4 h preceding tetanization. Recovery from the anesthesia took place over 1–2 h post-tetanization and subsequent recordings were made when the animals were awake;

AwSp(50) group — awake animals received 50 trains but all the trains were evenly spaced at 20 s intervals;

AnSp(50) group — stimulation as for AwSp(50) but rats were anesthetized to a surgical level with sodium pentobarbital for the 4 h preceding tetanization as for AnBu(50);

AwBu(5×10) group — 10 trains of patterned stimulation as for AwBu(10) but on 5 consecutive days.

After tetanization, recordings of the responses to test pulses were made for the following 20 min, and on post-tetanization days 1, 2, 4, 7 and weekly thereafter until either the baseline was regained or the recordings became unstable. Hemispheres where recordings returned to a stable baseline and remained so for 3–4 weeks were re-tetanized using a new paradigm.

Determination of LTP induction and decay

Offline analysis of the evoked waveforms consisted of measurement both of the slope of the rising phase of the positive-going EPSP and the vertical height of the population spike as previously described[22]. Because stimulation was begun immediately after connecting the rat to the stimulating leads and small variations in the responses sometimes occurred early in the recording session, only the mean values for the last 10 waveforms from each 20 min session were calculated. Except on the day of tetanization, all EPSP measures were expressed as percentage deviation from the mean of the 3 pre-tetanization baseline values. The corresponding population spike measures were expressed as the mean deviation (in mV) from the baseline values, to avoid the extremely large values that sometimes arise when a percentage transformation is used for these data. For the tetanization day, the post-tetanization measures were expressed relative to the mean of the last ten values immediately before tetanization.

EPSP and population spike data for each hemisphere, from day 1 post-tetanization onward, were plotted separately on a scattergram. A negative exponential curve was fitted to the points, where possible, according to the equation $y = Be^{-kt}$, where B is the y-intercept, k is the rate constant of decay and t is the number of days post-tetanization, as shown in Fig. 1. The decay time constant, τ, equals $1/k$ and represents the time taken (in days) for LTP to decay by 63%.

Determination of Fos-IR induction

For the Fos-IR assay, rats separate from those in the LTP decay study were used. They were implanted and tetanized, however, using the same protocols as described above. In addition, a further group was included, receiving stimulation as for AwBu(10) but repeated on 3 consecutive days (AwBu(3×10)).

Rats were anesthetized with ether 2 h after tetanization and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Fos was detected immunocytochemically using a polyclonal antibody to a synthetic 27 amino acid fragment corresponding to amino acids 127–152 of Fos, raised in rabbits and purified on peptide-conjugated affinity gels[10]. Sections (50 μm) were cut with a vibratome; during sectioning the brain was immersed in 0.01 M phosphate-buffered saline (PBS). Sections were washed 3 times (10 min per wash) with PBS, then incubated in 10% normal goat serum (Cappel) for 20 min. Sections were then incubated for

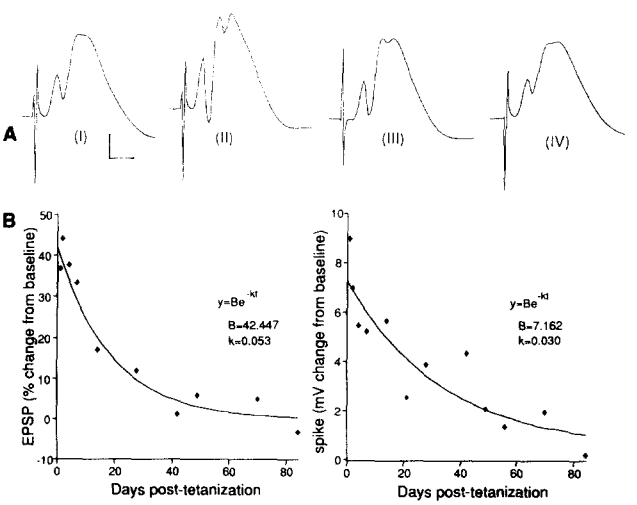

Fig. 1. A: waveforms for a single rat from the AwBu(50) group at successive stages of decay. I, just prior to LTP (i.e. one of several baseline responses used to calculate the mean baseline value); II, immediately post-tetanization; III, day 49 post-tetanization; IV, day 84 post-tetanization. Each waveform is the average of 10 responses. B: scattergram of the decay data for the same animal for both EPSP and spike. A single negative exponential curve was fitted to these points and the parameter k obtained as a measure of the rate of LTP decay. Calibration bar in A: 2 ms, 2 mV.

72 h with the primary c-*fos* antibody in 1% normal goat serum made up in 0.01 M PBS. Sections were again washed 3 times with PBS (10 min per wash) and incubated with biotinylated goat anti-rabbit (Vector labs.) for 1 h, using a modification of the avidin–biotin technique as described by Hsu et al.[20]. After washing the section 3 times in PBS they were incubated with the ABC solution (Vector) for 1 h, rinsed 3 times in PBS and placed in the chromagen diaminobenzidine for 5 min. The peroxidase reaction was visualized with the addition of H_2O_2. To control for specificity of immunostaining, some sections were incubated with c-*fos* antibody preadsorbed with its synthetic peptide. Fos-IR was quantified, as previously described[14] by counting the number of neurons showing positive immunostaining within 100 μm^2 and expressing the raw scores as a percentage increase over the contralateral control side. This use of the within-animal control hemisphere takes advantage of the fact that our stimulation procedures do not induce Fos-IR or LTP contralaterally. It does not, however, take into account small variations in the intensity of immunolabelling per labelled cell (an inherent problem with immunocytochemistry). In our previous study only neurons in the upper blade of the dentate gyrus were counted; however in this study we performed separate cell counts on both the upper and lower blades of the dentate. Since Fos-IR induction in the ventral blade occurred more consistently than in the dorsal blade, the ventral blade data are presented here.

RESULTS

The amounts of initial LTP arising from the different stimulation conditions for all animals (73 hemispheres) are shown in Fig. 2. Although the AwBu(10), AnBu(50) and AnSp(50) groups showed less LTP, these differences were not significant for either the EPSP or population spike. However, when the 50 train burst and spaced groups were combined, anesthesia produced a significant reduction in EPSP LTP (Student's *t*-test, one-tailed, $t_{46} = -2.261$, $P < 0.05$).

For the decay analysis, 6 hemispheres were discarded because they failed to show adequate LTP (defined as >10% increase in the EPSP or >2 mV increase in the spike). Another 8 hemispheres were discarded because their responses decayed below baseline (>10% or 2 mV below baseline for the EPSP and population spike, respectively) or showed a sudden large change in amplitude. An estimation of the decay function for each group was then made by plotting all the post-tetanization data points for the remaining hemispheres on a single scattergram and fitting the single negative exponential function (Table I, pooled data).

In order to statistically compare between groups, functions were also generated for those individual hemispheres that had data sufficiently consistent to permit accurate curve fitting. Because of the variability that is often seen in recordings made over weeks or months, this led to a considerable reduction in the number of hemispheres suitable for analysis (EPSPs, $n = 29$; spikes, $n = 39$).

The resulting distribution of the decay rates (k) was not normal but skewed towards the lower values, and for this reason log(k) was used in the statistical comparisons between groups presented below. The antilog of the mean log(k) was taken to represent the mean decay rate

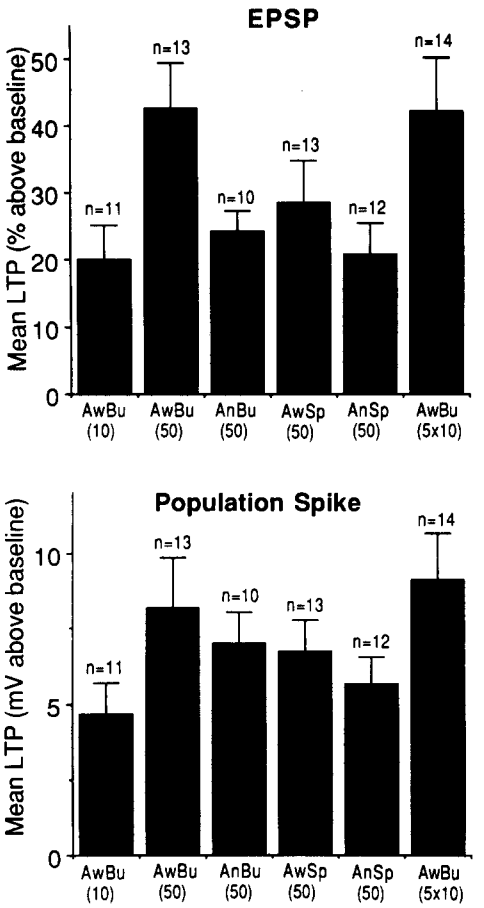

Fig. 2. Comparison of the amount of EPSP and spike LTP produced by all animals (73 hemispheres) in each of the 6 stimulation paradigms. Group designations are explained in the methods. Although groups AwBu(10), AnBu(50) and AnSp(50) showed less LTP than the others, these differences were not significant (one-way analysis of variance, $P > 0.05$). For this and subsequent histograms the values are means; the bars represent standard error of the mean. n represents number of hemispheres per group.

TABLE I

Comparison of the group decay constants resulting from two methods of estimation: (i) data pooled on a single scattergram and a single negative exponential curve fitted, and (ii) data plotted separately for individual hemispheres and a curve fitted to each

Group	$k_{(pooled\ data)}$				$k_{(mean\ of\ individual\ data)}$			
	n	*EPSP*	*n*	*Spike*	*n*	*EPSP*	*n*	*Spike*
AwBu(10)	9	0.530	9	0.542	5	1.060	6	0.673
AwBu(50)	11	0.059	11	0.061	5	0.072	7	0.044
AnBu(50)	8	0.484	8	0.444	5	0.402	7	0.422
AwSp(50)	10	0.039	10	0.068	5	0.074	7	0.074
AnSp(50)	9	0.338	9	1.219	3	0.094*	5	0.531
AwBu(5×10)	12	0.092	12	0.119	7	0.078	8	0.081

* Discrepancy from spike value probably due to small n.

for each group (Table I, mean of individual data). These decay rates were similar to those from the pooled data, showing that the selection of hemispheres for individual analysis did not greatly alter the estimate of the group decay rate.

The effect of patterned stimulation on Fos-IR induction and LTP decay

Although there was little difference in the amount of EPSP and population spike LTP produced by either the AwBu(50) or AwSp(50) conditions, the spaced paradigm resulted in significantly greater induction of Fos-IR (Fig. 3). Whereas Fos-IR following burst trains increased by 121% above the control side, Fos-IR following spaced trains increased by 368% (one-tailed *t*-test, $t_{10} = -2.242$, $P < 0.05$). This difference between groups, observed 2 h post-tetanization, is consistent with our previous results when Fos-IR was measured in the dorsal blade 30 min and 4 h post-tetanization[13].

The LTP decay rates for the two conditions were not significantly different, despite the difference in Fos-IR, with time constants (for EPSP and spike, respectively) of 13.9 and 22.7 days following burst trains and 13.5 and 13.5 days following spaced trains (Fig. 4).

The effect of anesthesia on Fos-IR induction and LTP decay

Administration of the same tetanization trains as above, but under barbiturate anesthesia, produced a reduction in EPSP LTP (see Fig. 1) and significantly reduced Fos-IR induction. This is consistent with our previous results[13], although greater LTP was induced in the present study, attributable at least in part to a lighter level of anesthesia and a more stable control of the animals' body temperature. A comparison of the effect of anesthesia on induction following spaced versus burst stimulation is shown in Table II. Combining across stimulation conditions, the mean Fos-IR induction in

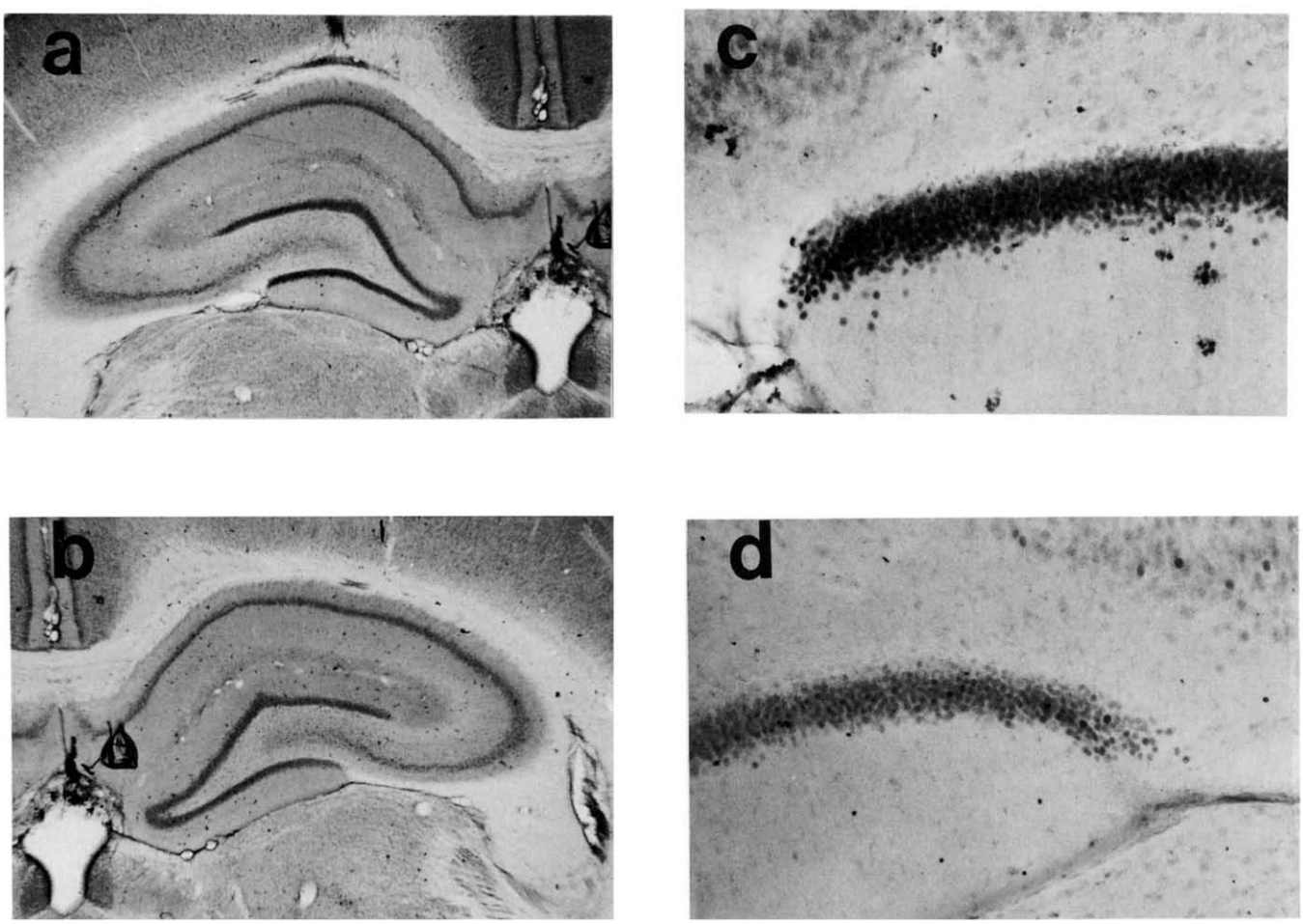

Fig. 3. a–d. Photomicrograph of Fos-IR induction in the dentate gyrus 2 h following sham stimulation (implanted control) or tetanic stimulation of the perforant path. a,b: low-power view of the dentate gyrus ipsilateral (a) and contralateral (b) to AwSp(50) stimulation. c,d: higher-power view of the ventral blades shown in a and b, respectively; e,f: higher-power view of the ventral blade ipsilateral (e) and contralateral (f) to AwBu(50) stimulation. Fos-IR-containing neurons appear as dark spots. The spaced stimulation induced significantly more Fos-IR than the burst stimulation in awake animals. Calibration bar: 1 mm low power, 100 μm higher power.

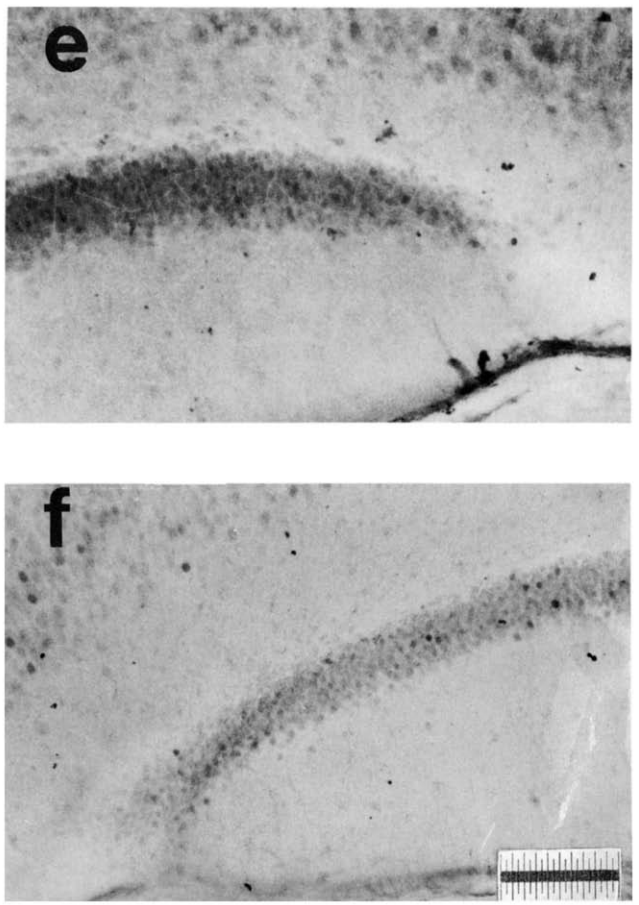

Fig. 3. e,f.

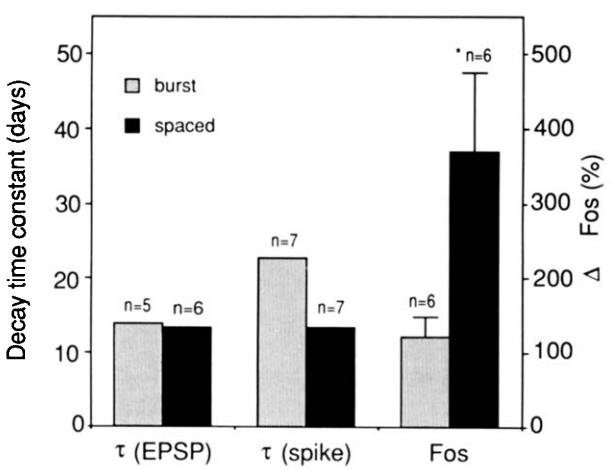

Fig. 4. Comparison of the EPSP decay time constant, spike decay time constant and Fos-IR (Fos) induction following 50 trains of either burst or spaced tetanic stimulation. * Significant difference ($P < 0.05$, Student's t-test). For ease of presentation the decay data are displayed as the inverse of the mean decay rate, i.e. the decay time constant ($1/k$). Thus no error bars are shown for these data. The t-tests for these decay data were performed on the normalized decay rates ($\log(k)$), and showed no significant differences between groups. Note there was a significant effect of stimulation pattern on Fos-IR induction.

TABLE II

Comparison of Fos-IR induction (% change from control hemisphere) following 50 burst or spaced tetanic trains in awake or anesthetized (anes.) animals

	Burst	*Spaced*	*Total*
Awake	121 ($n = 6$)	368 ($n = 6$)	245 ($n = 12$)
Anes.	−8* ($n = 6$)	3 ($n = 3$)	−5* ($n = 9$)

* Significantly different from awake group, $P < 0.05$ (Student's t-test).

awake rats was 245% and in anesthetized rats was −5% (one-tailed t-test, $t_{19} = 3.323$, $P < 0.01$).

LTP decay time was also significantly reduced by anesthesia (Fig. 5). Decay time constants were combined for the burst and spaced groups, and values for the EPSP and population spike respectively were 13.7 and 17.5 days in the awake condition, and 4.3 and 2.2 days in the anesthetized condition (one-tailed t-test, $t_{16} = 2.254$, $P < 0.05$ for the EPSPs, $t_{24} = 9.504$, $P < 0.01$ for the spikes).

The effect of train number and timing on Fos-IR induction and LTP decay

When the number of burst trains was varied, the greatest Fos-IR induction, 202%, was found in the AwBu(10) group. This was greater than, but not significantly different from the AwBu(50) group which showed 121% Fos-IR induction. Animals given 10 trains on consecutive days showed no additional Fos-IR induction on either the 3rd or the 5th day of trains (Fos-IR induction: AwBu(3×10) = −8%, AwBu(5×10) = −22%).

On the other hand, variation of the train number and

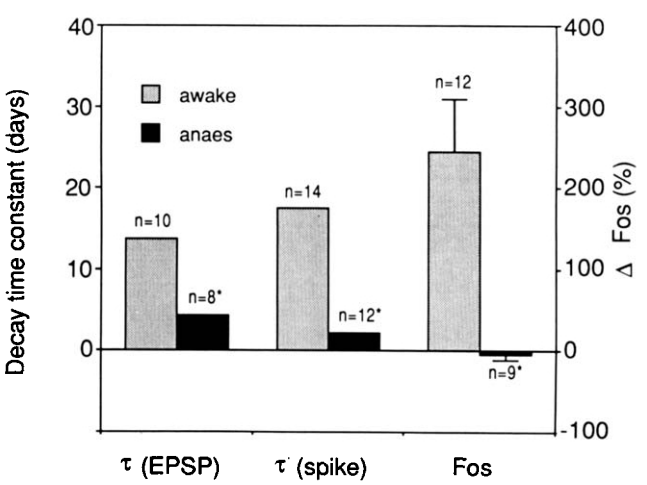

Fig. 5. Comparison of the EPSP decay time constant, spike decay time constant and Fos-IR (Fos) induction following 50 trains of tetanization (results for burst and spaced trains are combined), administered to awake or anesthetized animals. * Significant difference ($P < 0.05$, Student's t-test). The data are presented as in Fig. 4. Note the barbiturate effect on both LTP decay and Fos-IR induction.

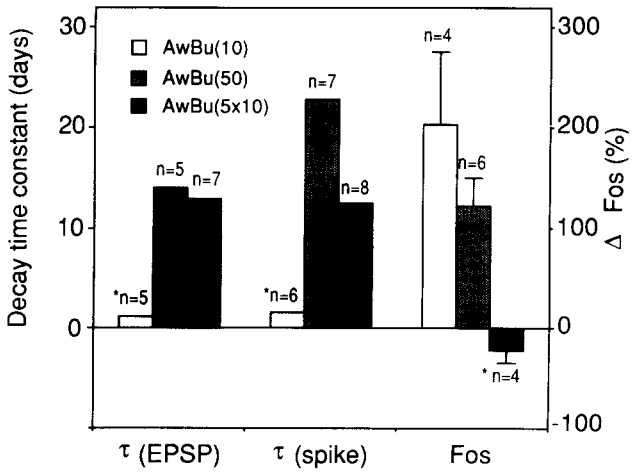

Fig. 6. Comparison of the EPSP decay time constant, spike decay time constant and Fos-IR (Fos) induction following burst stimulation consisting of either 10 trains, 50 trains or 10 trains repeated on 5 consecutive days. The Fos-IR response for the AwBu(5×10) group is that following the 5th and last day of tetanization. * Significant difference from the other two groups (Scheffe's post-hoc test, $P < 0.05$). Data are presented as for Fig. 4. Note the poor correlation between LTP decay and Fos-IR induction across the groups.

timing produced a significantly different decay rate across the three groups (analysis of variance: $F_{2,14} = 8.995$, $P < 0.01$ for the EPSPs, $F_{2,18} = 22.33$, $P < 0.01$ for the spikes). The LTP decay following only 10 trains of stimulation was significantly faster than that following 50 trains, whether given all on one day or over five days (Fig. 6). The AwBu(50) and AwBu(5×10) groups did not differ significantly for either EPSP or spike decay (post-hoc Scheffe test, $P > 0.05$).

For all groups, the decay time appeared to be independent of the initial amount of LTP, as comparison of individual decay rates with the corresponding amount of LTP for each hemisphere yielded a very low correlation ($R = -0.296$ for the EPSPs and -0.30 for the population spikes). Comparison of the mean LTP decay rate in each group with the mean initial LTP induced in that group did however yield a significant correlation for the spike ($R = -0.851$) but not for the EPSP ($R = -0.0572$).

DISCUSSION

The time course of LTP decay

It is clear from the findings reported here that the rate of decay of LTP is not fixed, but rather varies according to the conditions present at the time of induction. First, pentobarbital anesthesia caused a marked reduction of LTP maintenance. Second, 50 trains, whether given all at once or spread across five days, produced longer-lasting LTP than 10 trains alone. This latter effect is in agreement with Barnes[4], who found that a single episode of high-frequency stimulation induced EPSP LTP with a decay time constant of about 4.3 days, while repeated tetanizations on 3 consecutive days increased the time constant to about 30 days. De Jonge and Racine[11], on the other hand, induced LTP in 5 consecutive sessions, allowing varying amounts of time for LTP decay in 3 different groups, and found a constant rate of decay (for all sessions and all groups) of about 21 days. The reason for this discrepancy is not certain, but the stimulus parameters used in the Barnes study (15 trains) were more comparable to our ten-train paradigm, whereas De Jonge and Racine used 50 trains on each 5 days. It may be that with this strength of stimulation maximal duration LTP was induced on the first day.

Racine et al.[26], in a study of LTP throughout the limbic forebrain, found that LTP decay in the hippocampus was described by two separate components which had time constants of 1.5 h (LTP1) and 5 days (LTP2). On the basis of the distribution of decay constants elsewhere in the literature, we have suggested that there may also be a longer lasting component of LTP, termed LTP3, which decays with a time constant of about 24 days[2]. (This use of LTP1,2,3 terminology is confounded by its use also by Reymann et al.[27] to describe different LTP decay rates as defined biochemically. Since our determination is based on exponential decay functions, we have used terminology in accord with Racine et al.'s earlier work, also based on exponentials[26]. Clearly however, a unified terminology is called for.) In the present study we have provided additional evidence for the existence of an LTP3 by showing its repression by a barbiturate. The extent to which LTP3 involves mechanisms different from those for LTP2 is uncertain since both appear to be best fitted by single negative exponential functions (from day 1 post-tetanization onward). However because of the variability of evoked potentials in awake rats, we can not yet be certain that LTP3 is a unitary process with a single decay function.

The decay constants for the spike and EPSP were very similar across groups (cf. Table I), despite observations[6] that spike potentiation may persist for some time after EPSP potentiation has decayed. A possible explanation for this is that generally the spike shows a larger percentage increase than the EPSP following tetanization, and is even greater than that which can be explained by the increase in the EPSP alone, a phenomenon known as 'E-S potentiation'[1,3]. If spike LTP does in fact decay with the same rate as EPSP LTP then spike potentiation may seem to persist longer since, owing to its relatively greater magnitude, it is still measurable even after the EPSP appears to have regained baseline values.

Fos-IR and the maintenance of long-lasting LTP

Before discussing the Fos-IR results, it should be

pointed out that the antibody that we used to detect Fos protein also recognizes Fos-related molecules since the peptide sequence against which the antibody was generated is highly conserved[15]. Fos-related antigens (with different time courses of induction and decay) may therefore have been detected in addition to Fos after LTP. This fact, coupled with the difficulty in accurately quantifying the immunostaining, means that any conclusions relating Fos-IR to LTP decay are most safely made on the basis of the presence or absence of immunostaining changes following particular stimulation conditions. These conclusions will then apply equally to Fos and any Fos-related molecules that have been detected.

The hypothesis that the degree of Fos-IR induction accounts for the persistence of LTP is not supported by our data. For example, when the number of tetanization trains was varied, Fos-IR induction and LTP decay were not correlated, since 10 trains produced significant Fos-IR induction but only LTP2, whereas 10 trains repeated over 5 days, or 50 trains given on the same day, produced no additional Fos-IR and yet induced LTP3. Furthermore, LTP2 occurred independently of whether Fos-IR was induced (10-train condition) or not (anesthesia conditions). Thus it is clear that Fos-related proteins are not necessary for LTP2 induction, and correlate poorly with LTP3. It remains possible, however, that the Fos-related proteins induced on the first day of 10 trains play a permissive role for the induction of LTP3 on subsequent days of high-frequency stimulation. If so, this effect must be indirect, since Fos-IR is not evident 24 h after LTP-inducing stimulation[13]. Our data, therefore, do not completely rule out the possibility that Fos-IR induction is somehow necessary for LTP3.

It is surprising to note that when the 10 trains were repeated on 3 or 5 consecutive days, there was no additional Fos-IR induction. Morgan et al.[25] found that c-*fos* reinduction was markedly attenuated following repeated Metrazole-induced seizures, and suggested that c-*fos* induction is regulated by a negative feedback mechanism. However the refractory period for c-*fos* reinduction lasted only as long as Fos was still present following the initial dose of Metrazol. Our data indicate that once c-*fos* is induced, its further induction may be inhibited for at least several days. Recently described Fos-related molecules which decay with slower time courses than Fos and which also inhibit c-*fos* transcription might account for these results[30].

Administration of tetanic stimulation under anesthesia reduces LTP induction, and blocks both the Fos-IR response and LTP3. Since pentobarbital enhances synaptic inhibition, the effect on LTP induction is probably due to a decrease in membrane depolarization and consequent reduction in *N*-methyl-D-aspartate (NMDA) receptor activation[8] during the tetanus. Although individual decay time did not correlate with the initial amount of LTP, there was a significant correlation between the overall effect of a stimulation condition on the induction of spike LTP, and its effect on the persistence of LTP. Thus it may be that the effect of anesthesia on LTP3 induction is also due to reduced membrane depolarization but via an alternative mechanism, e.g. voltage-sensitive calcium channels.

The effect of anesthesia on Fos-IR induction may also have been due to reduced depolarization. Douglas et al.[12] showed that this anesthesia effect could be prevented if the dentate granule cells were made to fire repetitively in the presence of bicuculline. Fos induction in phaeochromocytoma cells is also caused by other factors producing membrane depolarization such as neurotransmitters[18] and ion flux[24].

Since it is clear that Fos protein(s) are not sufficient for LTP3 induction, it is possible that the blockade of LTP3 by pentobarbital is due to additional effects on other depolarization-sensitive IEGs. Recently Cole et al.[7] have reported that the expression of the IEGs c-*jun*, *jun*-B and *zif/268*, as well as c-*fos*, are induced in association with LTP under chloral hydrate anesthesia. Of these, *zif/268* was the most consistently induced, and its induction paralleled that of LTP in that it was blocked by NMDA receptor antagonists, and by the activation of convergent inhibitory synaptic inputs. Since the findings of our study suggest that the Cole et al. tetanization paradigm (12 trains) would only produce LTP2, it remains to be seen whether *zif/268* is crucially involved in LTP3 maintenance. In any event, it is clear that in order to determine which of the many biochemical events following tetanization are related to LTP maintenance, it is important to conduct decay studies in parallel with studies of the molecular correlates of LTP induction.

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