Developmental onset of long-term potentiation in area CA1 of the rat hippocampus.

K M Harris and T J Teyler

J. Physiol. 1984;346;27-48

J. Physiol. (1984), **346**, *pp.* 27–48
With 11 *text-figures*
Printed in Great Britain

DEVELOPMENTAL ONSET OF LONG-TERM POTENTIATION IN AREA CA1 OF THE RAT HIPPOCAMPUS

BY KRISTEN M. HARRIS* AND TIMOTHY J. TEYLER
From the Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272, U.S.A.

(*Received 23 November 1982*)

SUMMARY

1. Long-term potentiation (l.t.p.) was studied in area CA1 of rat hippocampal slices during development at post-natal days 1–8, 15 and 60. Tetanic stimulation at 100 Hz for 1 s was delivered to the fibres in stratum radiatum and the time course of potentiation was recorded in stratum pyramidale for 20 min after tetanus. L.t.p. was measured at 20 min post-tetanus as an increase in the amplitude of the population spike.

2. The time course and magnitude of post-tetanic potentiation (p.t.p.) differed with age. For 60-day-old animals p.t.p. was seen as a maximally potentiated response immediately post-tetanus that declined to a smaller potentiated response by 5 min post-tetanus. For animals younger than 15 days the response was also maximally potentiated immediately post-tetanus with subsequent decline. However, the duration of maximal potentiation was shorter and the magnitude was less. A different time course of p.t.p. was observed at 15 days. The maximal potentiation was approximately equal to that seen at 60 days, but instead of declining, the response remained maximally potentiated throughout the entire post-tetanus monitoring period.

3. L.t.p. was first observed at post-natal day 5, and by post-natal days 7 and 8 substantial levels of l.t.p. were seen consistently. The greatest magnitude of l.t.p. was found at 15 days, and was considerably more than that produced at 60 days.

4. When the duration of l.t.p. was monitored for longer than 20 min the response declined back to pretetanus levels by 1–1½ h for animals younger than 15 days. In 15-day-old rats the response remained maximally potentiated for the full 72 min that it was monitored, with no decline.

5. In control experiments of low-frequency stimulation (l.f.s.) at 1/15 s for 100 stimuli, hippocampal slices from 60-day-old animals showed response elevation. In contrast, l.f.s. resulted in response decrement over time for slices from 5–15-day-old animals.

6. Three measures of pretetanus excitability in area CA1 suggested an increase with age. The stimulus intensity required for field excitatory post-synaptic potential (e.p.s.p.) threshold declined, the magnitude of the maximal population spike amplitude increased, and the population spike latency decreased. These results suggest that the magnitude of l.t.p. is not strictly related to the pretetanus excitability of CA1 cells.

* Current address: Neurocytology Laboratory, Neurology Service, Massachusetts General Hospital, Boston, MA 02114 U.S.A.

A fourth measure of excitability, the slope of the field e.p.s.p. at population spike threshold, showed a larger value at 15 days than at 1–8 or 60 days.

7. A hypothesis is presented to show how a peak and decline in l.t.p. magnitude across ages might be mediated by the size of an available pool of 'plastic' synapses, which diminishes as more synapses become 'consolidated' by the production of l.t.p.

INTRODUCTION

Long-term potentiation (l.t.p.) is an enduring form of physiological plasticity that has been found in all the monosynaptic pathways of the hippocampus of adult animals (cf. Bliss & Lomo, 1970, 1973; Bliss & Gardner-Medwin, 1971, 1973; Douglas & Goddard, 1975; Alger & Teyler, 1976; Yamamoto & Chujo, 1978). It is of interest to know when l.t.p. first develops, and how it matures, because of its potential role in the process of long-term information storage in the brain (Swanson, Teyler & Thompson, 1982). Since rats are altricial animals, much of their behavioural repertoire develops post-natally, as does their capacity for long-term memory (Campbell & Coulter, 1976). If l.t.p. is a mechanism of learning and memory, then one would expect its development to parallel the development of learning and memory capacities.

In area CA1 a brief tetanic stimulation to stratum radiatum results in l.t.p. measured extracellularly as: (1) an increase in the amplitude of field potentials, (2) an increase in the probability of individual CA1 pyramidal cell firing, or (3) a decrease in the latency of individual cell firing. These responses are seen to persist for several hours in the *in vitro* slice preparation (e.g. Schwartzkroin & Wester, 1975; Andersen, Sundberg, Sveen, Swann & Wigstrom, 1980*b*). The specific underlying mechanism(s) of l.t.p. have not yet been discerned (for review see Swanson *et al.* 1982). It is likely that l.t.p. is mediated by a synaptic mechanism because the size of the afferent volley does not change following tetanic stimulation (Andersen, Sundberg, Sveen & Wigstrom, 1977) and because there is no generalized increase in the excitability of the post-synaptic cell (Andersen *et al.* 1980*b*). Furthermore, in area CA1, l.t.p. is specifically localized to the pathway that was tetanized and no heterosynaptic potentiation occurs (Dunwiddie & Lynch, 1978; Alger, Megela & Teyler, 1978; Anderson *et al.* 1980*b*). If l.t.p. is mediated by changes at the tetanized synapses, as these studies suggest, then further insight into the specific synaptic properties that are involved could be gained by studying l.t.p. when synaptogenesis is occurring. Synaptogenesis and dendritic development occur over an extended post-natal time period in area CA1 (Minkwitz, 1977; Loy, 1980; Bayer, 1980*a*, *b*; Pokorny & Yamamoto, 1981*a*, *b*; Schwartzkroin, Kunkel & Mathers, 1982); therefore, it should be possible to determine whether some form of 'synaptic maturation' must occur before l.t.p. can be produced.

L.t.p. has been observed before the second post-natal week in area dentata (Duffy & Teyler, 1978; Harris, Teyler & Cruce, 1980) and in area CA1 (Harris *et al.* 1980; Baudry, Arst, Oliver & Lynch, 1981) of the rat hippocampus. Our goal was to extend these studies in area CA1 to determine the exact developmental age when l.t.p. first occurs and to study its properties during development. Methods which produce maximal l.t.p. in area CA1 of the adult hippocampus were used to test for l.t.p. in area CA1 of developing animals. Measurements of the post-tetanus time course and

magnitude of potentiation observed during the first post-natal week were then compared with those observed in rats 2 weeks and 2 months old. A preliminary report of these results has been presented elsewhere (Harris & Teyler, 1983).

METHODS

Subjects

Male and female Long–Evans rats were maintained together to facilitate continuous production of litters. Breeders were monitored daily, and at birth or one day following birth, litters were culled; all the male pups were kept, plus enough females to give a total of ten pups in the litter. Sex was initially identified by examination of the external genitalia and later confirmed by dissection after hippocampal slices were prepared. For the majority of the experiments male pups were used; four females (one 1 day old, two 5 days old and one 7 days old) were also used. Experimental animals were randomly selected from a litter, every day from birth until a date when l.t.p. was reliably produced (1–8-day-old animals were tested). Then the potentiation observed in these young animals was compared with that seen in 15- and 60–64-day-old animals. A total of forty-three animals from sixteen litters were tested for l.t.p.

Preparation of hippocampal slices

Young animals (1–8 days old) were killed by decapitation; older animals (15 and 60–64 days) were first stunned by a blow to the back of the neck and then killed by a blow to the thoracic region dorsal to the heart. The left hippocampus was dissected free from surrounding brain tissue and placed on a tissue slicer (Stoelting Company: Duffy & Teyler, 1975). The hippocampus was oriented so that four slices (350 μm thick) could be obtained from the middle third of the hippocampus while preserving lamellar organization (Andersen, Bliss & Skrede, 1971; Skrede & Westgaard, 1971; Rawlins & Green, 1977). For each animal used in the l.t.p. experiments, one slice was selected for experimentation. Plate 1 illustrates representative slices from four different ages that had acceptable orientation and were used for experimentation. They show that area CA1 can be identified visually by post-natal day 4. It was more difficult to observe cell body layers in 1- and 2-day-old pups; instead, stratum pyramidale was identified by its response to orthodromic stimulation. Slices were transferred to a filter net located at the liquid–gas interface between medium (Earles balanced salts: 117 mM-NaCl, 5 mM-KCl, 0·9 mM-KH_2PO_4, 1·6 mM-$MgSO_4$, 1·8 mM-$CaCl_2$, 26 mM-$NaHCO_3$ and 10 mM-glucose) and 95 % O_2/5 % CO_2 bubbled through warm (33–34 °C) distilled water.

Selection of slices and positioning of electrodes

Slices were allowed to equilibrate for 1 h *in vitro*. Then stimulating and recording electrodes were placed in area CA1 under visual guidance as illustrated in Pl. 1 *E*. The concentric bipolar stimulating electrode had a central core conductor of Teflon-coated Pt–Ir wire protruding 50 μm from the surrounding 30-gauge stainless-steel tube, and was placed in stratum radiatum. It had a 13 kΩ resistance in saline. Stimulus voltages were varied, and the pulse duration was 0·1 ms. The recording electrode was a glass micropipette with an outside diameter of 18–20 μm filled with 2 M-NaCl, which was placed in stratum pyramidale. The electrodes were positioned so as to obtain a population response that was clearly separated from the stimulus artifact. To optimize size and stability of the population response the recording electrode was located between 80 and 100 μm beneath the slice surface – a depth comparable to where Andersen *et al.* (1980*a*) have also found the optimum response to occur.

If more than three electrode penetrations were necessary to obtain an acceptable population response, the slice was not used because it was assumed that the slice either was not cut parallel to the lamellae, or was not healthy. If an acceptable response was not obtained from the first slice, the positioning procedure was repeated on adjacent slices until a clear response was obtained. In only one experiment (at 3 days) were all four slices penetrated before a suitable response was obtained, and in only four experiments were three penetrations made (at 4 and 5 days).

With the recording electrode positioned at the optimum depth, the stimulus intensity was adjusted to give about a 1 mV population spike, and the response monitored at 1/min for 10 min. If the response was 'stable' (defined here as no consistent increase or decrease in the population spike

amplitude) the experiment began. If the response was not stable, monitoring continued for another 5 min. For all of the experiments reported here, the response was stable within 15 min of electrode entry.

Once an acceptable response was obtained, data were gathered as described in detail below, in the following order for each l.t.p. experiment: (1) field excitatory post-synaptic potential (e.p.s.p.) threshold; (2) ten pretetanus responses; (3) tetanic stimulation; (4) post-tetanus monitor; (5) ten post-tetanus responses; and (6) field e.p.s.p. threshold. In addition, control experiments of low-frequency stimulation (l.f.s.) were performed on adjacent slices or on slices from litter-mates. The data were stored on a PAR Scan recorder and plotted on a Houston $X-Y$ plotter while the experiments were in progress; this allowed immediate observation and analysis of wave form amplitudes so that proper stimulus intensities could be set. Wave forms were also stored on floppy disks of the Apple II microcomputer (Teyler, Mayhew, Chrin & Kane, 1982). This system allowed for later detailed measurements of the population spikes; they were measured as illustrated in Pl. 1*F* from the peak negativity to half-way between the two positive-going peaks. For these field potentials a sampling rate of 1059 or 1467 samples per second was used, and 512 or 350 sample points were collected for each wave form over 25–50 ms after the stimulus artifact. Wave forms presented in the Figures were traced by the Apple computer.

Test for field e.p.s.p threshold

The 'field e.p.s.p. threshold' is the stimulus intensity (in volts) required to produce the smallest observable field e.p.s.p. in response to stimulation of the fibres in stratum radiatum. The role of this test was to provide a physiological reference for detecting accidental electrode movement. In adult animals, electrode movement results in a measurable change in this parameter. In addition, this test provided an estimate of the minimal stimulus intensity necessary to stimulate the afferent axons and depolarize the CA1 cells at different ages. Starting below threshold, the stimulus voltage was increased in small increments (usually less than 0·05 V) until the field e.p.s.p. was first observed. Then, at least six suprathreshold responses were obtained.

Test for post-tetanic potentiation (p.t.p.) and long-term potentiation (l.t.p.)

The stimulus voltage was then readjusted to give a pretetanus population spike approximately 1 mV in amplitude. The average stimulus voltage ranged from 4–34 V for animals younger than 15 days, to 5–14 V at 15 days, and 1·8–4·4 V for animals 60–64 days old. For animals less than 4 days old the pretetanus population spike was usually smaller than 1 mV. Once the voltage was determined, the stimulus intensity was not changed until the field e.p.s.p. threshold was tested at about 30 min after tetanic stimulation. Ten pretetanus stimuli were given at 1/30 s or 1/15 s. Tetanus was then delivered at 100 Hz for 1 s, at the same stimulus intensity. Post-tetanic potentiation (p.t.p.) was monitored at 5, 10, 15, 20, 25, 30, 45 and 60 s after the tetanus, and then the stimulus rate was reduced to 1/30 s. At 20 min post-tetanus, ten post-tetanus responses were obtained at the same stimulus intensity and frequency used to obtain the pretetanus responses. Finally, the field e.p.s.p. threshold was remeasured to determine response stability of the slice, and the electrode position was checked. In several experiments the post-tetanus response monitoring was then continued for an hour or longer.

Low-frequency stimulation (l.f.s.) control experiments

Control experiments were performed to determine whether potentiation was produced by stimulation alone or whether high-frequency stimulation was required to produce l.t.p. in the developing animals. Slices from litter-mates of some of the experimental animals (two each at 5 and 8 days, three at 15 days, and one each at 60 and 64 days) were tested with l.f.s. In four experiments, one each at 6, 9, 10 and 60 days, adjacent slices from the experimental (l.t.p.) hippocampus were used for the l.f.s. test. In these l.f.s. experiments the slices were selected and electrodes positioned in area CA1 using the same procedures as described above for the l.t.p. experiments.

Field e.p.s.p. threshold was obtained and ten pre-l.f.s. stimuli were given at a stimulus intensity producing about a 1 mV population spike. The stimulus intensity ranged from an average of 1·5 V at 60 days to 26 V at 6 days. At the same stimulus intensity, 100 continuous stimuli were applied at 1/15 s. At the end of these 100 stimuli, ten post-l.f.s. responses to the same stimulus intensity were obtained, and relative and absolute changes in the population spike amplitude were calculated. If l.f.s. resulted in a decline in the amplitude of the population spike, the stimulus intensity was

increased to ensure that the slice was healthy and capable of responding with a population spike. Capacity for potentiation was also tested following l.f.s. In some experiments a paired-pulse stimulation was given to test for facilitation on the second response. In a few experiments the entire protocol for producing p.t.p. and l.t.p. described above was followed at the conclusion of l.f.s.

RESULTS

Time course of post-tetanic potentiation

P.t.p. was distinguished from l.t.p. on the basis of two characteristics observed in the 60-day-old animals. First, p.t.p. was seen as a maximal potentiation in the amplitude of the population spike observed immediately post-tetanus. Secondly, the

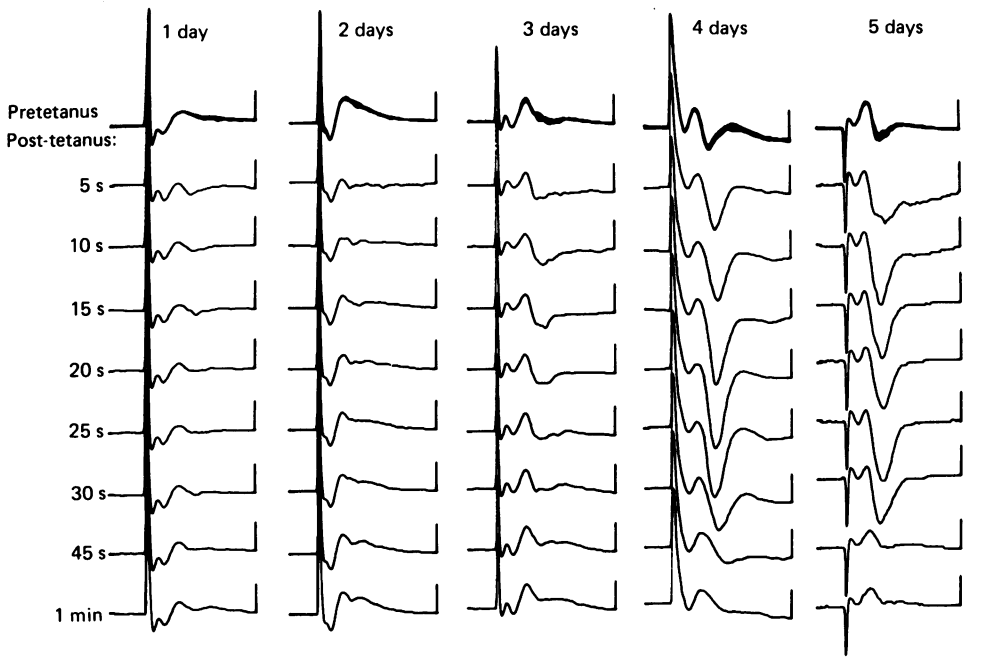


Fig. 1. Representative responses during the first minute after tetanus are plotted for 1- to 5-day-old animals. The ten pretetanus responses are superimposed in the first row, followed by single responses at 5 s to 1 min after tetanus. Calibration pulses are as in Pl. 1*F*.

time course of the maximal potentiation was less than 5 min. L.t.p. was a relatively stable response potentiation that lasted beyond the decline from maximal potentiation. Examples of post-tetanic responses from 1–5-day-old animals are shown in Fig. 1. Marked p.t.p. was first seen in two 4-day-old animals for 30 s after the tetanus. Frequently, post-tetanic stimulation produced a small negative-going potential ($< 0{\cdot}5$ mV in amplitude) in 1–3-day-old animals where a single pretetanus stimulus could not, even at high stimulus intensities. We interpret this negative potential as an immature population spike, and these slices were included as showing p.t.p. These examples illustrate that p.t.p. could occur without subsequent l.t.p. especially at post-natal day 4.

The time course of potentiation following tetanus is illustrated in Fig. 2. Values

of the population spike amplitude at each post-tetanus time were averaged from three animals per age. The change in the population spike amplitude was analysed statistically at the various post-tetanus times within and across the different ages. Multivariate analysis of variance was performed and followed by pair-wise comparisons

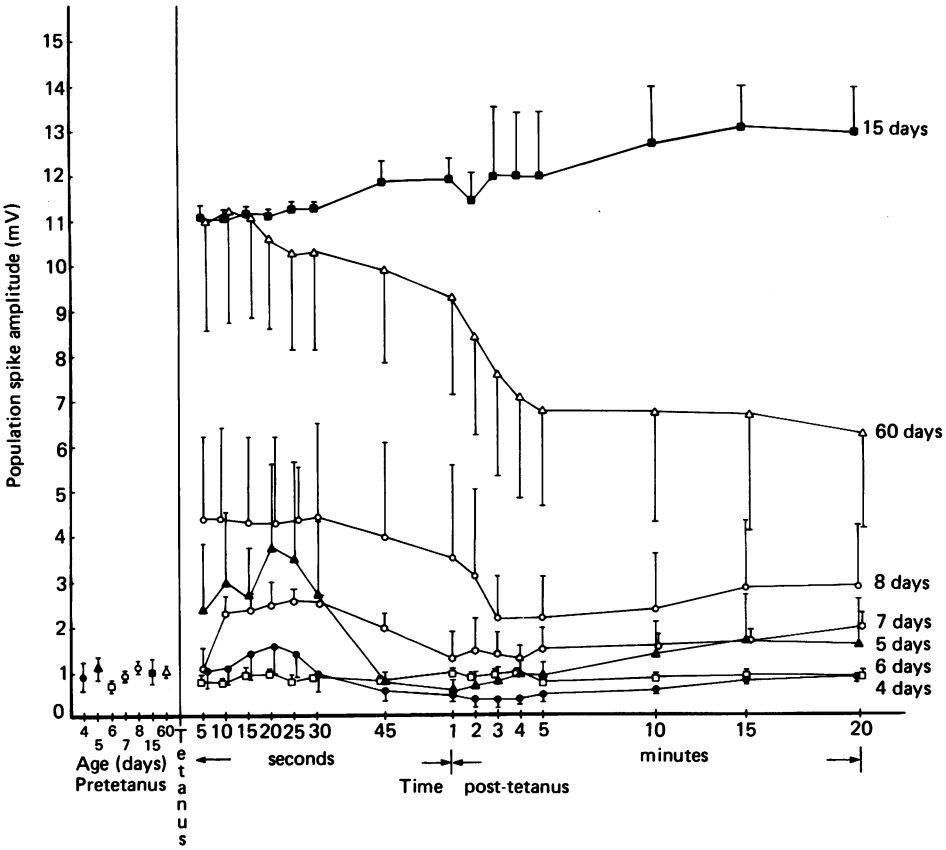


Fig. 2. Time course of p.t.p. Data from three animals having complete data sets for the entire post-tetanus time monitor were averaged to illustrate the magnitude of p.t.p. achieved by each age. The averages of ten pretetanus population spikes for three animals (thirty total response amplitudes, mean ±S.E. of mean) are plotted before the vertical tetanus line. The average post-tetanus response amplitudes are plotted after the vertical line at the indicated times. Note the expanded time scale (seconds) during the first minute after tetanus. For the sake of clarity, each mean is plotted with a standard error bar in only one direction. Results from 1-, 2- and 3-day-old animals were below the graph for 4-day-old animals. They are not plotted because fewer than three complete data sets were available. Data from one animal at each of ages 8, 15 and 60 days are also not included here, because the tetanic stimulation induced a brief seizure activity.

using the Neuman–Keuhls procedure (McNemar, 1970). The data from 5 s to 1 min and from 1 min to 20 min after tetanus were combined and treated as two separate groups of data with eight time variables in each group. In this way, the immediate post-tetanus effects could be analysed separately from the longer-lasting changes.

For the 5 s to 1 min time period these analyses revealed that the potentiation observed at 15 and 60 days was greater in magnitude than at all other ages ($P < 0{\cdot}05$).

At 45 s to 1 min more potentiation was seen at 15 than at 60 days ($P < 0{\cdot}05$). Within each age the post-tetanus response was stable at a maximum plateau for a limited time period immediately after tetanus. (A stable response was taken to be no significant change between the time points.) For 5-day-old animals the response was stable at a maximum from 5 to 30 s; for 7-, 8- and 15-day-old animals the response was stable at a maximum from 5 s to 1 min; and at 60 days the response was stable at a maximum from 5 to 20 s.

During the 1–20 min time period, potentiation at 15 days was greater in magnitude than at all other ages ($P < 0{\cdot}01$), and at 60 days it was greater than at 4–8 days ($P < 0{\cdot}05$). At all ages, except 15 days, the response declined during this time period to a lower stable level of potentiation. For 4–8-day-old animals that showed potentiation, this lower level was reached by 1 min after tetanus. For 60-day-old animals the response was still declining from 1 to 3 min ($P < 0{\cdot}05$) and then remained stable after 4 min. In contrast, the data from 15-day-old animals showed only a small dip in the response from 1 to 2 min ($P < 0{\cdot}05$). Then the response increased from 2 to 5 min and remained stable at a new maximum for the duration of the post-tetanus monitor.

In conclusion, the time course of p.t.p. showed a maximal response potentiation immediately after tetanus that lasted for longer time periods as the animals matured. Typically, the response then declined to a stable response potentiation. This time course at 15 days was different from that at other ages in that the response remained maximally potentiated for the duration of the post-tetanus monitor.

Magnitude of l.t.p.

A comparison of ten pretetanus wave forms and ten wave forms obtained at 20 min post-tetanus is presented in Fig. 3*A*. These data reveal a trend of increasing l.t.p. magnitude from post-natal day 5 to day 15. Surprisingly less l.t.p. was observed at post-natal day 60 than at post-natal day 15.

The absolute and relative magnitude of change in the post-tetanus response was averaged from at least three animals for each age older than 2 days (Fig. 3*B*). Since the amplitude of the pretetanus population spike was set at 1 mV, these two measures of l.t.p. are parallel. The average magnitude of l.t.p. was greater than zero after post-natal day 5, and significantly more l.t.p. was produced at post-natal day 15 than at any other age tested here ($P < 0{\cdot}001$, ANOVA, and *post hoc* Bonferroni t statistic: Myers, 1979).

Hippocampal slices from 8-, 15- and 60-day-old animals that showed seizure activity immediately post-tetanus, all recovered from postictal depression to show l.t.p. These were not included in this analysis, because the magnitude of l.t.p. was consistently less than that observed from other animals of the same age. However, the same pattern of l.t.p. magnitude was observed with the 15-day-old animals showing more recovery of potentiation than either 8- or 60-day-old animals. Hesse & Teyler (1976) have also noted that tetanus-induced seizure activity reduces the magnitude of l.t.p. measured in adult animals.

Longevity of l.t.p.

L.t.p. was monitored beyond the 20 min post-tetanus test in several experiments. Four examples of the longevity of l.t.p. are presented in Fig. 4. Of the four ages

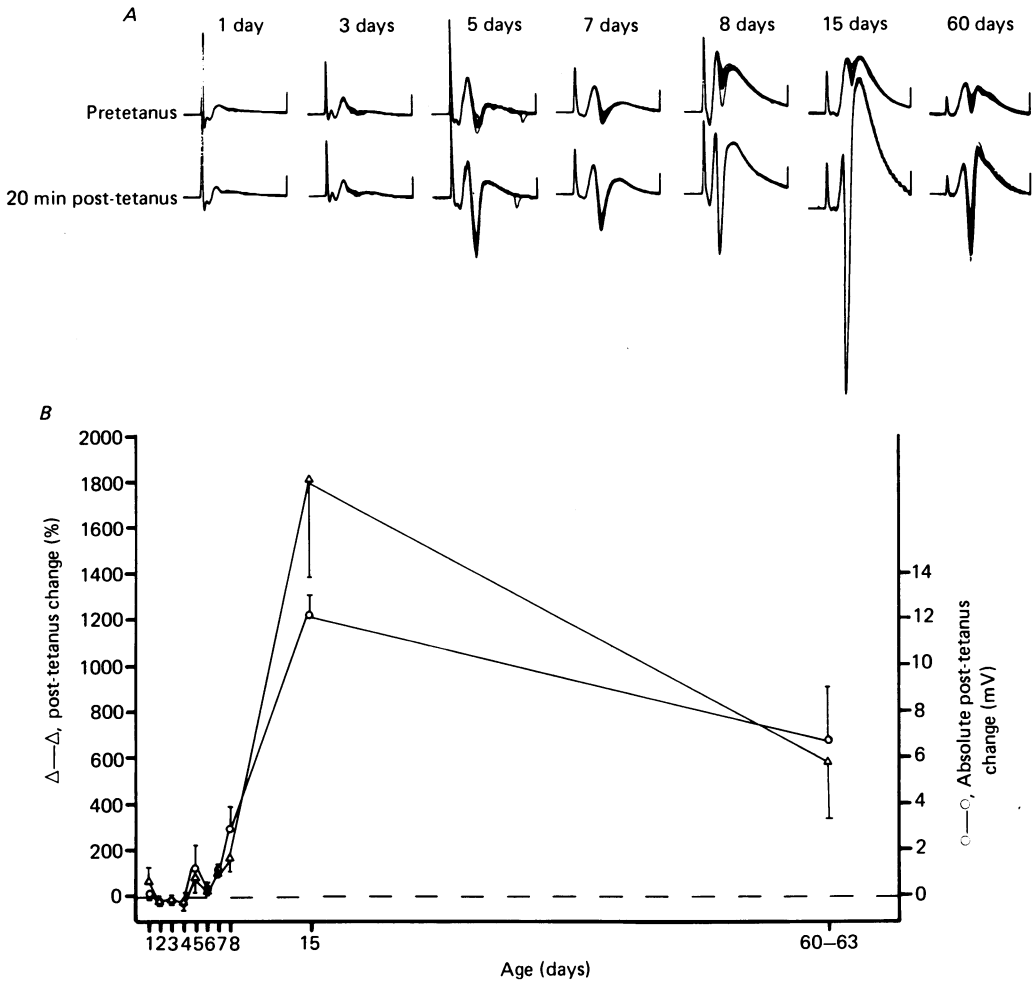


Fig. 3. Magnitude of l.t.p. *A*, pre- and post-tetanus wave forms from representative experiments. The wave forms are superimposed for each example. Calibration pulses are as in Pl. 1*F*. *B*, two estimates of the magnitude of l.t.p. produced at several ages. On the left, the amount of l.t.p. was calculated as a percentage change in the amplitude of the post-tetanus population spike relative to the pretetanus population spike ([post−pre/post] × 100 %). On the right, the amount of l.t.p. was calculated as the absolute increase in the amplitude of the population spike following tetanus (post−pre). The dashed line at zero indicates no change in the response. For the sake of clarity the means are plotted with standard error bars in one direction only. Data from at least three animals were averaged for each point.

studied, the responses from the 15-day-old animal remained stable throughout the testing period, while responses from the other ages decayed to base line by about 1–1½ h after tetanus. For all ages, the response magnitude was stable at 20 min when the l.t.p. comparisons were made.

An unusual response pattern is seen in the data from the 5-day-old animal. This pattern occurred at 24, 36 and 48 min post-tetanus (small arrows). At these times

the spontaneous background activity was elevated and the evoked response was further elevated. Following this enhanced activity the response declined below base line, and later recovered to the potentiated level. This response pattern has been described as 'spontaneous unison firing' and is discussed in more detail in an earlier paper (Harris & Teyler, 1983). This spontaneous unison firing was also seen in a litter-mate of the 5-day-old animal. Slices from the brother were used for an l.f.s.

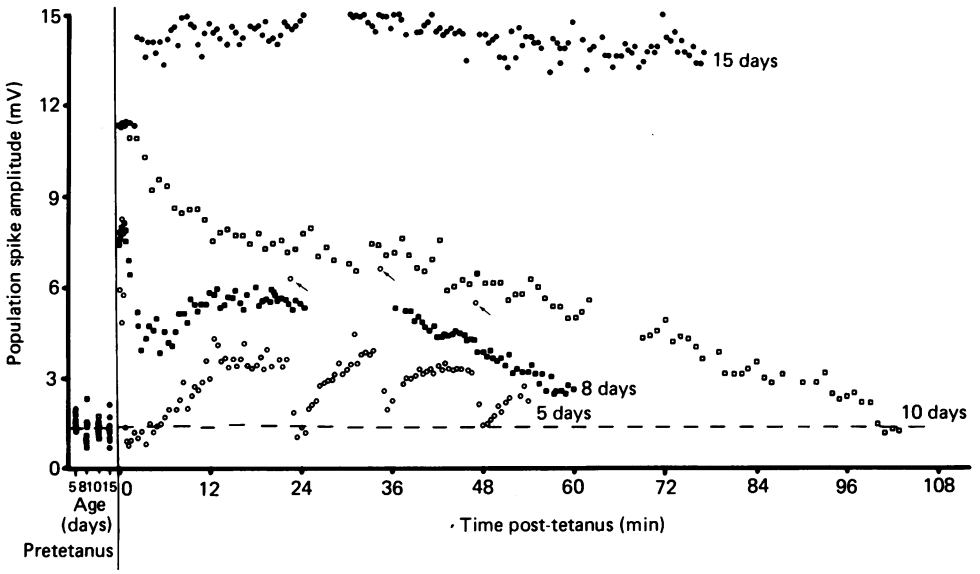


Fig. 4. The longevity of l.t.p. was monitored beyond the 20 min post-tetanus test in several experiments. Four examples are presented here. The ten pretetanus population spike amplitudes are plotted before the vertical line. The vertical line indicates the time at which tetanus was delivered, and then a scatter plot of the post-tetanus response amplitudes is shown for 1-, 5-, 8-, 10- and 15-day-old animals. The dashed line is at the 1 mV population spike amplitude to illustrate the magnitude of change following tetanus. Gaps in the records from the 8-day-old (at 25–30 min, ■), 10-day-old (at 63–68 min, □) and 15-day-old animals (at 22–27 min, ●) are where the field e.p.s.p. threshold was measured, and the monitor of l.t.p. was discontinued briefly. At 8 and 10 days the response declined back to base line at about 1 h and 1½ h respectively. By 15 days the response was stable at a potentiated level for at least 72 min, the duration of this monitor. An unusual response pattern is present at 5 days (○). Temporary increases in the response amplitude, followed by response depression, occurred at 24, 36 and 48 min after tetanus (arrows). This activity is referred to as 'spontaneous unison firing' (see text).

experiment, so that no tetanus was given. When spontaneous unison firing occurred in the l.f.s. slice, the population spike was only briefly increased. Following the decline, its amplitude returned to control base line, rather than to a potentiated level. Therefore, it seems likely that the spontaneous unison firing is superimposed upon potentiation for the l.t.p. experiment.

Together these results illustrate that stable l.t.p. can be observed at 20 min for all of these post-natal ages. However, the length of time that l.t.p. is retained increases with maturation of the animal.

Low-frequency stimulation

Results from the l.f.s. experiments, where the stimulation rate was 0·67 Hz instead of 100 Hz, reveal that l.f.s. did not produce an increase in response amplitude in 1–15-day-old animals, and usually the response was somewhat depressed following l.f.s. (Fig. 5). In contrast, this rate of stimulation eventually resulted in response potentiation for the 60–64-day-old animals. At least thirty stimuli at 0·67 Hz were

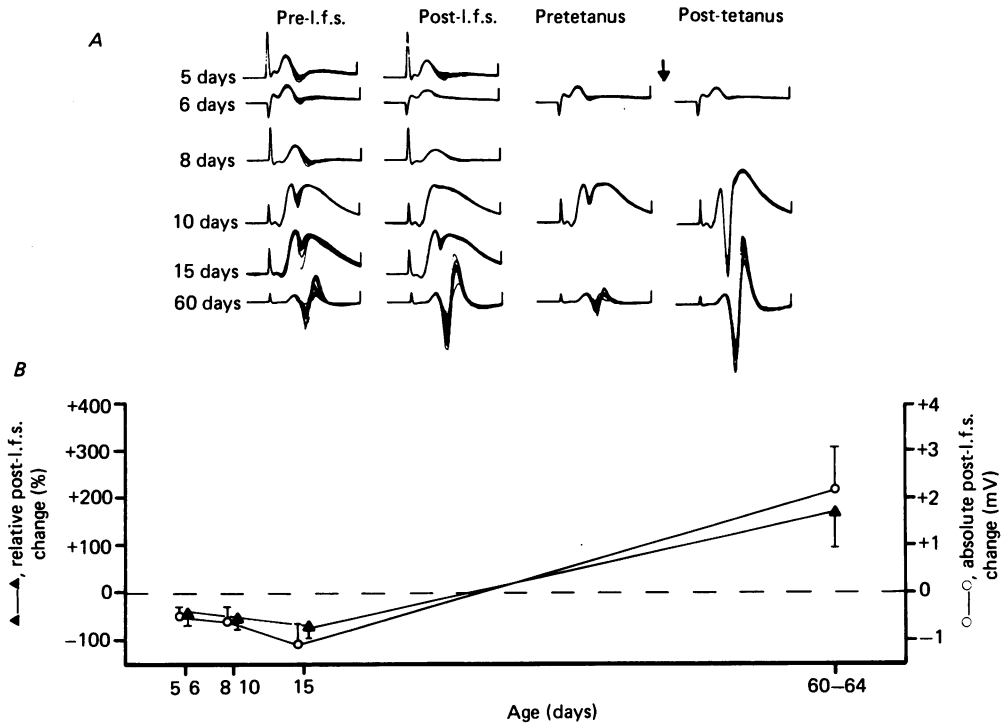


Fig. 5. Magnitude of potentiation from low-frequency stimulation (l.f.s.). *A*, ten wave forms, pre- and post-l.f.s. are superimposed in the first two columns. Examples are from the ages listed at the left. For three examples (6, 10 and 60 days) the stimulus intensity was adjusted to give about a 1 mV population spike at the end of the l.f.s. experiments, and then tetanus was applied at 100 Hz for 1 s (at the arrow). The post-tetanus responses were obtained at 20 min after tetanus. Calibration pulses are as in Pl. 1*F*. *B*, data from three animals per age were averaged, and are plotted as means with standard error bars (in one direction only, for the sake of clarity). Note that only the 60–64-day-old animals produced an increased response following l.f.s., whereas all the younger animals showed a response decrement. The dashed line at zero indicates no change in response.

required before the response increase was observed, and for two of the animals more than fifty stimuli were required. These results suggest that 60-day-old animals are more sensitive to lower rates of stimulation than the younger animals. When tetanus was given after l.f.s., l.t.p. was observed at all ages tested. At 60 days the magnitude of l.t.p. from tetanus was greater than the potentiation produced by l.f.s. (see columns 3 and 4 of Fig. 5*A*).

The next four results concerning the individual response characteristics illustrate that the magnitude of l.t.p. produced at each age is not strictly correlated with the pretetanus excitability of the cells in area CA1 at each age.

Field e.p.s.p. threshold.

Pre- and post-tetanus wave forms at four stimulus intensities near the field e.p.s.p. threshold are plotted in Fig. 6*A*. The amplitude of the field e.p.s.p. observed at

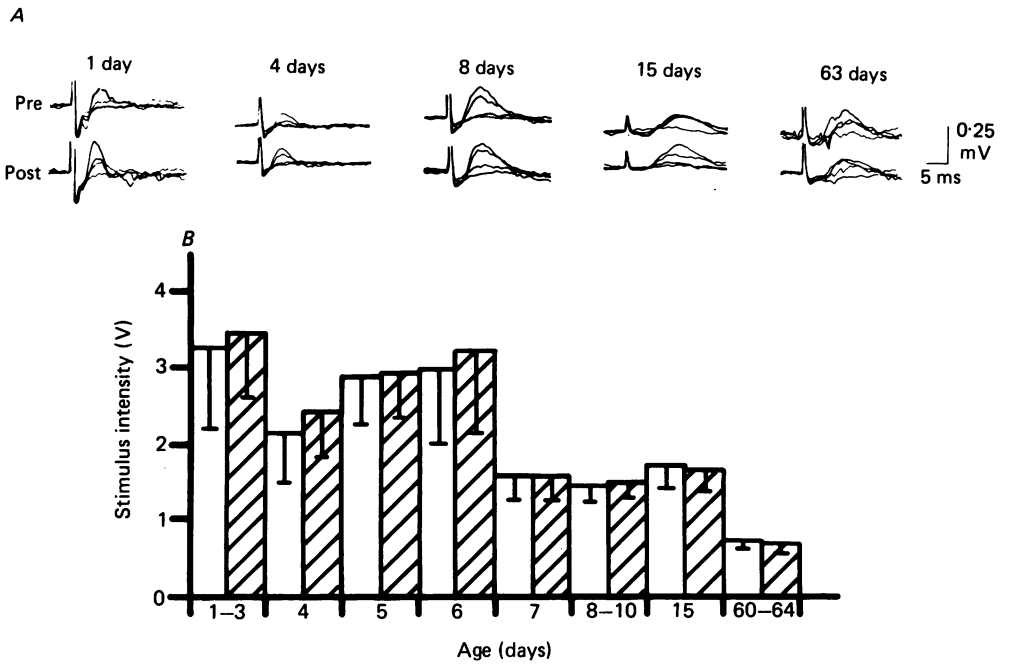


Fig. 6. Field e.p.s.p. threshold. *A*, representative field e.p.s.p. thresholds are presented for pre- and post-tetanus measurements at representative ages. These were hand-traced from the Houston *X*–*Y* plots. *B*, the average stimulus intensities required to produce the field e.p.s.p. at threshold are given for all the experiments across each age. The means for pre-l.f.s. or tetanus (open columns) and post-l.f.s. or tetanus (hatched columns) are shown with standard error bars.

threshold sometimes fluctuated (Fig. 6*A*), but the stimulus intensity at which a field e.p.s.p. was first evoked was usually the same both pre- and post-tetanus, and pre- and post-l.f.s., for all ages (Fig. 6*B*). It is evident from Fig. 6*B* that the stimulus intensity required to reach field e.p.s.p. threshold was greater at 1–6 days than that required at 7–15 days ($P < 0{\cdot}01$, *t* test for independent means), and that an even lower voltage was required at 60–64 days ($P < 0{\cdot}02$). These differences cannot be fully accounted for by differences in stimulating electrodes, since only three stimulating electrodes were used throughout the experiments, and these were used at all ages. Furthermore, the stability of this threshold throughout a single experiment suggests that properties of the electrode resistance or tissue resistance did not change significantly after tetanus or l.f.s. We conclude that the stimulus intensity required to first depolarize these cells decreased with maturation.

Population spike latency and synchrony of cell firing

Measures of population spike latency were made to determine whether the increase in population spike amplitude is due to the recruitment of new cells to fire or simply an increased synchrony of cells already firing. A reduction in population spike latency would suggest that the cells fire more readily after tetanus. A reduction in variability of the population spike latency would suggest that the cells fire more synchronously.

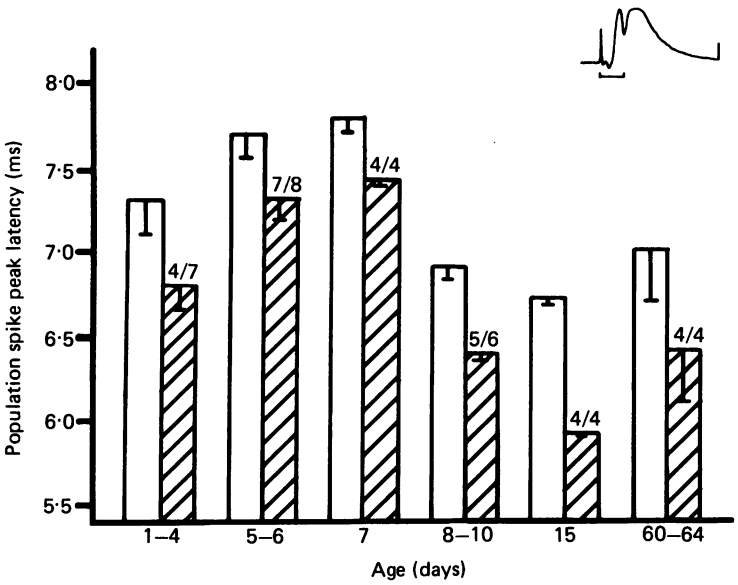


Fig. 7. Population spike latency. The latency to peak negativity of the population spike from the stimulus artifact was measured as indicated in the inset for both the ten pretetanus responses (open columns) and the ten post-tetanus responses (hatched columns). The means are graphed across ages, and the error bars (S.E. of mean) illustrate the average variability in spike latency for individual experiments at each age.

Measures of population spike latency were made on the ten pretetanus responses and the ten responses that were obtained at 20 min post-tetanus (Fig. 7). Population spike latency was decreased following tetanus for 5–6, 7, 15 and 60–64 day-old-animals ($P < 0{\cdot}05$, t test of correlated means). Only about half of the slices from 1–4-day-old animals showed a post-tetanus decrease in population spike latency. At 8 days one animal showed an increase in population spike latency as well as an increase in spike amplitude. In Fig. 7 the error bars show the average variability in population spike latency within each of the experiments for a given age. At all ages there was only a small decrease in the variability (statistically not significant) of spike latency following tetanus.

This measure of population spike latency from the stimulus artifact was not compared quantitatively across ages, because variations in the relative positions of the stimulus and recording electrodes can alter the latency. However, observations of the latency from the rising edge of the first positive-going peak to the population spike revealed a trend of decreasing latency as the animals matured. This trend can be seen in Figs. 1 and 3*A* in the pretetanus wave forms.

Pretetanus pyramidal cell excitability

Two direct estimates of pyramidal cell excitability have been obtained before tetanic stimulation at different ages. The first estimate of pyramidal cell excitability was a measure of the field e.p.s.p. slope at population spike threshold. This measure reflects the amount of synaptic depolarization that was required to fire the most

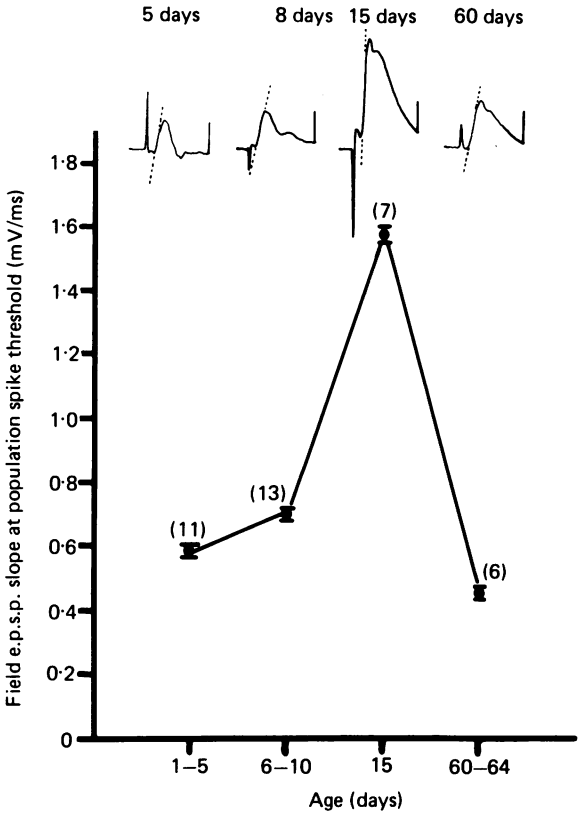


Fig. 8. The pretetanus slope of the field e.p.s.p. at population spike threshold was measured as indicated by the dashed lines. These data are from animals used for the l.t.p. and l.f.s. experiments. The means (±S.E. of mean) are shown for the total number of animals used to obtain these measures (given in parentheses). The example illustrated for 60 days had the largest slope that we observed for this age. For most of the 60-day-old animals the slope was less than that observed in animals less than 10 days old. Calibration lines are as in Pl. 1 *F*.

excitable cells. Population spike threshold was defined by two of the following three criteria: (1) at a constant stimulus intensity the population spike was observed intermittently; (2) at the threshold stimulus intensity no population spike was observed but a small increment in stimulus intensity produced a population spike greater than 0·5 mV; and/or (3) the size of the population spike was less than 0·5 mV. Establishing these criteria gave a measure which was easy to compare across ages, and was a measure of the field e.p.s.p. which was relatively uncontaminated by the

population spike. A larger field e.p.s.p. slope at spike threshold would suggest either that more synaptic drive was required to fire the most excitable cells, or that more dendrites were being depolarized in greater synchrony before cell firing.

The slope of the field e.p.s.p. was measured by fitting a tangent line to the steepest part of the curve and obtaining the slope of that line from enlarged axes (Fig. 8). This measure was obtained by an unbiased observer who was blind to experimental

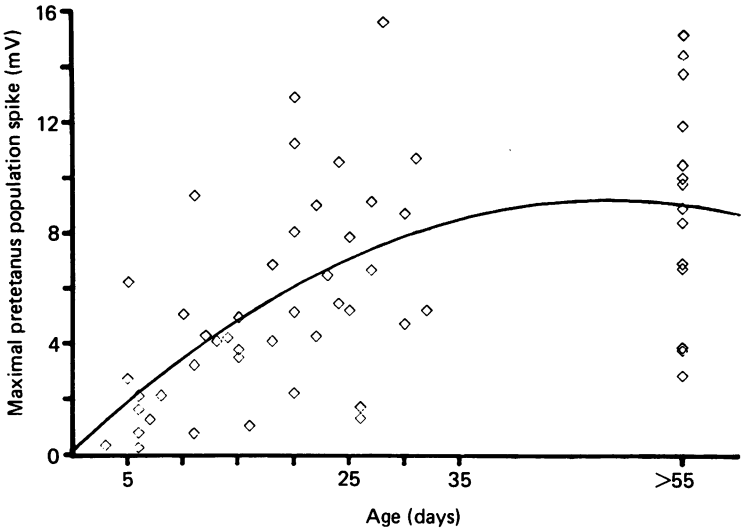


Fig. 9. The maximal population spike amplitude was measured (means ± S.E. of mean) in area CA1 of hippocampal slices from fifty-seven rats that were not used for the l.t.p. experiments, but were of the same strain and were raised in an identical fashion to those used for the l.t.p. experiments. This graph illustrates that as these animals mature, the size of the population spike to maximal stimulation increases. Each data point is the amplitude of the maximal pretetanus population spike in area CA1 of a hippocampal slice from one animal. The line is a best-fit polynomial of power 2 generated by a VAX (Digital Equipment Corportation) computer.

treatment or age of the animal. These data were available for the pretetanus or pre-l.f.s. responses from nearly all the animals of the experiments presented here, because in the effort to find a stimulus intensity which produced a 1 mV population spike, several responses at population spike threshold were also obtained. Unfortunately, these data were only available for a small number of post-tetanus responses, so that a direct comparison of field e.p.s.p. slope at population spike threshold could not be made before and after tetanus. The graph in Fig. 8 reveals that the field e.p.s.p. slope at population spike threshold is largest for the 15-day-old animals, with both the 1–10-day-old and 60–64-day-old animals having smaller values.

A second measure was that of the population spike amplitude at maximal stimulation, and was obtained from a separate group of animals (Harris *et al.* 1980). Stimulus–response curves were obtained from area CA1 for many ages. A maximal population spike was measured at the stimulus intensity where further increases in stimulus intensity produced no further increases in the population spike amplitude.

Results from these earlier experiments are graphed as the maximal population spike amplitude (Fig. 9). This graph illustrates that the magnitude of the maximal pretetanus population response in area CA1 increases with age, although at any one age a wide variability in maximal population response exists. When these data are compared with Fig. 2, they suggest that the population spike amplitude produced after tetanus can be greater than the maximal pretetanus population spike seen here.

DISCUSSION

In summary, l.t.p. first occurred at post-natal day 5, and was consistently seen by post-natal days 7 and 8. At 15 days substantially more l.t.p. was produced than that observed at either the younger ages or at 60 days. The magnitude of p.t.p. was equivalent at 15 and 60 days, indicating that some difference occurs in the retention of the potentiated response. These results cannot be explained by the level of pretetanus excitability in area CA1 because several measures including population spike latency, field e.p.s.p. threshold and maximal population spike amplitude all indicate an increase in excitability with age. In addition, l.f.s. resulted in response potentiation only at 60 days. It is clear from these experiments that the magnitude and pattern of l.t.p. produced by CA1 pyramidal cells changes with maturation of the rat hippocampus. However, there are several cautions that need to be considered when comparing the level of l.t.p. attained by animals of differing ages.

First, the cells of stratum pyramidale are more densely packed in older animals than in the younger animals, so that an extracellular recording electrode might obtain responses from more cells in the older animals (Dzidzishvili & Kvirkvelia, 1968; Schwartzkroin, 1982). This was controlled for, in part, by comparing relative changes in the evoked response after tetanus, so that individual animals could be used as their own base line controls.

Second, the relative intensity of the tetanic stimulation was different across ages with respect to its position on the stimulus–response curve for the age. We partially controlled for this by setting the tetanic stimulation level at less than 50% of the response capacity for animals older than 3 days. However, this control procedure does not prevent the influence of more subtle differences in stimulus intensity on the magnitude of l.t.p. produced at different ages. It has been shown that relatively higher stimulus intensities increase the probability of observing l.t.p. in area CA1 of adult rats (Schwartzkroin & Wester, 1975). Therefore, having the stimulus intensity for tetanus relatively higher in the younger animals should facilitate the possibility of observing l.t.p. The 1 mV population spike was above threshold for producing stable responses, and it provided a useful base line for comparison across ages.

A third caution concerns the effect of the tetanic stimulus frequency on the level of l.t.p. achieved. In adult animals, higher frequencies of stimulation produce more l.t.p. than lower frequencies (Douglas, 1977; Dunwiddie & Lynch, 1978), and it is likely that some optimum frequency of stimulation also exists for the developing animals.

Allowing for these cautions, our results raise several questions about the phenomena and mechanism(s) of l.t.p. in developing animals. One question is whether the pattern of l.t.p. development parallels the development in excitability of CA1 pyramidal cells.

Several measures were analysed in an attempt to answer this question. First, it was found that the voltage required to evoke a threshold field e.p.s.p. was greatest in the youngest animals and declined with maturation, to lower values in the adults. This finding suggests an increase in excitability of area CA1 with maturation. There are at least three possible mechanisms for this finding: (1) there might be a higher density of presynaptic axons in the older animals, with lower thresholds, or greater

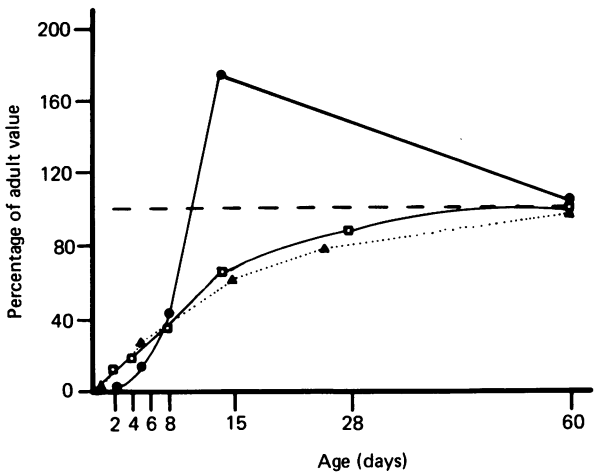


Fig. 10. Comparison of the development of l.t.p. production (circles) with the development of synapses (squares) and pretetanus maximal response (triangles). See text for discussion.

effectiveness in conducting an action potential; (2) more neurotransmitter might be released at the synapses of older animals in response to a single stimulation; or (3) synaptic effectiveness might be increased with maturation by the presence of more post-synaptic receptors as reported by Baudry *et al.* (1981). A second measure of excitability, the maximal population spike amplitude, also increased with maturation. A third measure of excitability, the population spike latency, showed a decrease as the animals aged. A fourth measure of excitability involved a comparison of the field e.p.s.p. slope at population spike threshold across ages. This measure reflects the amount of synaptic drive required to fire the most excitable cells in area CA1. Comparing this value at post-natal days 15 and 60 illustrated again that the CA1 cells are more readily fired at 60 days because they require less synaptic drive. It was curious that this measure is less for the 1–10-day-old animals. This result might indicate that the much smaller pyramidal cells of the 1–10-day-old animals (unpublished observations of Golgi impregnations) require less synaptic drive to depolarize their smaller dendritic fields; however, the longer latency to the population spike suggests a slower response mechanism. Further intracellular studies will be required to analyse this result.

Together these findings suggest that the magnitude of l.t.p. achieved by CA1 pyramidal cells is not strictly correlated with the excitability of the cells before

tetanus. If it were, one would expect the 60-day-old animals to show more l.t.p. than the 15-day-old animals.

Fig. 10 shows the development of l.t.p. along with the development of the number of synapses in stratum radiatum and the development of the pretetanus maximal response. In this graph, the magnitude of l.t.p., total number of synapses in stratum radiatum and pretetanus maximal response are expressed as a percentage of the adult

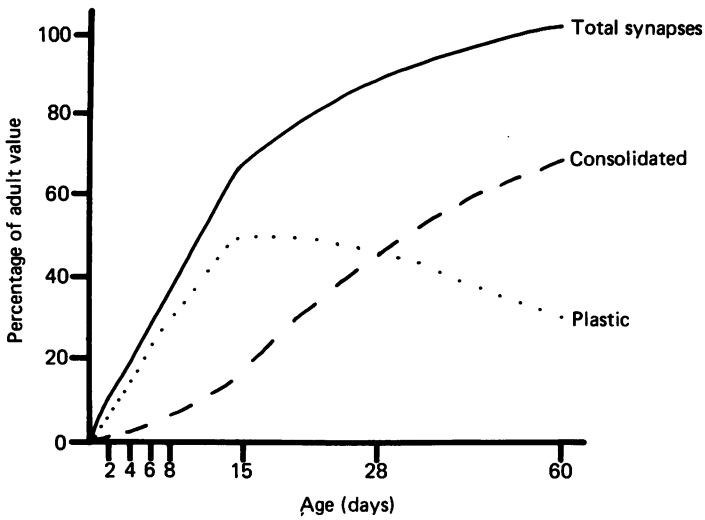


Fig. 11. A hypothetical graph illustrating how a peak and fall off in the production of l.t.p. might be mediated by the size of an available pool of plastic synapses. This hypothesis is discussed in detail in the text.

value. The l.t.p. graph was transformed from Fig. 3*B*. The graph for total synapses was transformed from data that have been reported elsewhere for the rabbit hippocampus (Schwartzkroin *et al.* 1982) because quantitative measures of synaptic number have not yet been made for rats during development. However, spine counts in stratum radiatum of the rat hippocampus reveal a similar increase with maturation (Minkwitz, 1976*a*, *b*, 1977). The maximal response curve was transformed from Fig. 9. It is clear from Fig. 10 that the magnitude of l.t.p. produced in the hippocampus is greatest during a time when many new synapses have just been formed. The behavioural repertoire of the developing animals is also rapidly increasing at this time: the eyes and ears are opening (Barnett, 1975), voluntary movements and exploratory behaviour are prominent (Campbell, Lytle & Fibiger, 1969; LeBlanc & Bland, 1979), and retention of at least some learned behaviours has reached adult values by post-natal day 15 (Campbell & Coulter, 1976).

In order to explain how a peak and decline in l.t.p. production might be mediated, we propose that as an animal matures, synapses are removed from a pool of synapses that are available for potentiation, by the process of l.t.p. (see also Changeaux & Danchin, 1976; Goddard, 1980). This hypothesis is illustrated in Fig. 11, where the total number of synapses in stratum radiatum is replotted as a percentage of the adult

value (Schwartzkroin *et al.* 1982). We have postulated that synapses are in at least one of two states: 'plastic' or 'consolidated'. Plastic synapses have not been maximally potentiated, and if they are activated by tetanic stimulation they produce l.t.p. They become consolidated by conditions producing maximal l.t.p. Consolidated synapses have been maximally potentiated and no longer produce l.t.p. in response to tetanic stimulation.

We propose that at the time of first synaptic contact, all synapses are of the plastic type and then become consolidated by potentiation experiences. As the animal matures, the number of consolidated synapses increases, but at a rate slower than the rate of over-all synaptogenesis because 'consolidation' requires experience. At each age, the number of available plastic synapses is then calculated as: (plastic = total − consolidated). The magnitude of l.t.p. produced during development would thus estimate the number of 'plastic' synapses available.

McNaughton, Douglas & Goddard (1978) have investigated properties of p.t.p. and l.t.p. in area dentata of the rat. They have shown that there is a threshold stimulus intensity for tetanic stimulation to produce l.t.p. If tetanus is given at a lower stimulus intensity, only p.t.p. is produced (lasting less than 5 min). This finding has been corroborated in areas CA3 and CA1 of the rat hippocampus (Yamamoto & Sawada, 1981; Lee, 1982). These results suggest that some minimal number of synapses must be co-activated to produce l.t.p. These researchers propose that co-activation of synaptic input increases the relative strength of those synapses involved in the tetanic stimulation. A corollary of their findings would be that co-activation produces more l.t.p. when more synapses are available for potentiation via tetanic stimulation.

If the magnitude of l.t.p. produced is proportional to the number of plastic synapses available for co-activation, then at the time of peak synaptogenesis, 15–20 days, one would expect to have maximal l.t.p., as we have seen. Examination of the plastic synapses curve suggests that most of the synapses in animals less than 5 days old should be plastic synapses. If the magnitude of l.t.p. production is proportional to the number of plastic synapses that are present, why was no l.t.p. produced before post-natal day 5? One answer to this question might be that a minimum number of synapses must be available for co-activation before l.t.p. can be produced, and 10% of the total synapses found in adults is a density less than that required for l.t.p. P.t.p. can be produced at lower stimulus intensities, suggesting that fewer synapses need to be co-activated (McNaughton *et al.* 1978; Yamamoto & Sawada, 1981). This could explain why p.t.p. was sometimes seen at 1–5 days in the absence of l.t.p. In addition, we have shown that the magnitude of p.t.p. is approximately equivalent for 15- and 60-day-old animals. If our hypothesis is correct, this finding suggests that the consolidated synapses of 60-day-old animals can produce p.t.p. but not l.t.p.

At this time we do not know what anatomical or biochemical properties would differentiate plastic and consolidated synapses; however, some of the results from other studies suggest some possible components.

First, a plastic synapse might be one that is located on a long thin dendritic spine. The vast majority of synapses in stratum radiatum are found on dendritic spines (Andersen, Silfvenius, Sundberg & Sveen, 1980*a*). Rall (1974) has proposed that synaptic current is greatly attenuated by the thin spine neck, and that a change in

a spine neck causing it to become shorter and fatter would increase the amount of current reaching the parent dendrite and depolarizing the cell. A plastic synapse might become consolidated by a contraction or swelling of the spine neck making it wider, and thereby reducing resistance to current flow (Crick, 1982). Recent experimental evidence has suggested that spine shapes change following tetanic stimulation in the hippocampus (Van Harreveld & Fifkova, 1975; Moshkov, Petrovskaia & Bragin, 1977, 1980; Lee, Schottler, Oliver & Lynch, 1980; Desmond & Levy, 1981; Fifkova & Anderson, 1981; Fifkova, Andersen, Young & Van Harreveld, 1982), indicating a possible mechanism of l.t.p. Before post-natal day 4, the number of dendritic spines on dendrites of CA1 pyramidal cells is minimal (Minkwitz, 1976*a*, *b*, 1977; Pokorny & Yamamoto, 1981*a*, *b*). Perhaps the relative deficiency in spines accounts for the absence of l.t.p. in these younger animals.

A second possibility is that some property of the synaptic contact is different for plastic and consolidated synapses. The ontogeny of l.t.p. in area CA1 has been studied by Baudry *et al.* (1981) in relation to the development of glutamate binding sites in area CA1 of the rat hippocampus. Their results parallel ours, in that p.t.p. was seen to occur earlier than l.t.p. In contrast, they did not observe l.t.p. before post-natal day 10. This difference in developmental onset of l.t.p. might be due to strain differences (Baudry *et al.* used Sprague–Dawley rats: personal communication), or to differences in experimental procedures.

While the time of developmental onset for l.t.p. production found by Baudry *et al.* is not exactly the same as in our studies, their results on glutamate binding might be used to describe some potential properties of plastic synapses. Baudry *et al.* show that specific binding of glutamate, the presumed neurotransmitter of afferents in stratum radiatum (Fonnum, Karlsen, Malthe-Sorenson, Skrede & Walaas, 1979), is virtually absent on post-natal day 4, rises to a peak around post-natal day 10 and then declines very gradually to adult levels. This curve parallels our hypothetical curve for the development and reduction of plastic synapses. Furthermore, the absence of glutamate binding sites before post-natal day 4 might have contributed to the absence of l.t.p. that we observed in the 1–4-day-old animals.

A question is also raised about the significance of l.t.p. in 60-day-old animals. If the magnitude of l.t.p. production declines with age, and if l.t.p. is involved in learning and memory, would one expect the 60-day-old animals to be less responsive to potentiating experiences than the 15-day-old animals? The results from the low-frequency stimulation (l.f.s.) experiments suggest that qualitative changes in potentiation capacity occur with maturation. While the 15-day-old animals showed a greater magnitude of l.t.p. from 100 Hz tetanic stimulation, the 60-day-old animals showed potentiated responses from lower rates of stimulation. This stimulation rate has been seen to produce potentiation in area CA1 of adult rats in other experiments as well (T. J. Teyler, unpublished). One possibility is that the hippocampus of the 60-day-old animals is sensitive to a wider range of potentiating experiences, but that each level of stimulation does not produce as much absolute potentiation. Barnes (1979) has shown that as the rat ages, the magnitude and duration of l.t.p. in area dentata decline. Her results suggest that as more of the synapses become maximally potentiated, i.e. 'consolidated', in the hippocampus of senescent rats, there is a further decline in the number of synapses available for l.t.p. (see also Goddard, 1980).

In as much as this research was primarily concerned with determining when l.t.p. is first produced in area CA1, it should only be considered a preliminary report on the development of l.t.p. Further experimentation will be required to determine whether other ages between post-natal days 15 and 60 also yield more l.t.p. than the adult animals. Other factors, such as changes in the stimulus intensity or frequency during tetanus, or prior experience of the animal, might also influence the developmental pattern of l.t.p. observed. The work presented here suggests that l.t.p. develops in parallel with the onset of exploratory behaviours in the rat. The magnitude of l.t.p. peaks during a time when the animal is learning a lot about its environment, i.e. shortly after eye and ear opening. Together with these observations, the results presented here suggest a developmental link between the processes of learning and memory, and long-term potentiation.

This work was submitted in partial fulfilment of the requirements for the doctoral dissertation to the Program in Neurobiology at Northeastern Ohio Universities College of Medicine and Kent State University. We are grateful to Dr D. Max Snodderly for his many helpful comments and discussion of the experimental design and results and for extensive comments on the manuscript. We thank Gordon Cantor and Bill Mayhew for their technical assistance on the data acquisition; Larry Cauller for several thoughtful discussions of the results; Jance Lentz-Hatch, Kim Straitiff and Larry Cherkas for their help on some of the figures; Dr Dennis Landis for the use of his word processor; and Darlene Jackson for expert typing. This work was supported in part by N.I.M.H. predoctoral fellowship no. 1 F31 MH08313-01 to K.M.H. and by N.S.F. Research Grant no. 78-23947 to T.J.T.

REFERENCES

ALGER, B. E., MEGELA, A. L. & TEYLER, T. J. (1978). Transient heterosynaptic depression in the hippocampal slice. *Brain Res. Bull.* **3**, 181–184.

ALGER, B. & TEYLER, T. (1976). Long-term and short-term plasticity in the CA1, CA3, and dentate regions of the rat hippocampal slice. *Brain Res.* **110**, 463–480.

ANDERSEN, P., BLISS, T. V. P. & SKREDE, K. K. (1971). Lamellar organization of hippocampal excitatory pathways. *Exp. Brain Res.* **13**, 222–238.

ANDERSEN, P., SILFVENIUS, H., SUNDBERG, S. H. & SVEEN, O. (1980*a*). A comparison of distal and proximal dendritic synapses on CA1 pyramids in guinea pig hippocampal slices *in vitro*. *J. Physiol.* **307**, 273–299.

ANDERSEN, P., SUNDBERG, S. H., SVEEN, O., SWANN, J. W. & WIGSTROM, H. (1980*b*). Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea-pigs. *J. Physiol.* **302**, 463–482.

ANDERSEN, P., SUNDBERG, S. H., SVEEN, O. & WIGSTROM, H. (1977). Specific long-lasting potentiation of synaptic transmission in hippocampal slices. *Nature, Lond.* **266**, 736–737.

BARNES, C. A. (1979). Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. comp. physiol. Psychol.* **93**, 74–104.

BARNETT, S. A. (1975). *The Rat: Study in Behavior*. Chicago: Chicago University Press.

BAUDRY, M., ARST, D., OLIVER, M. & LYNCH, G. (1981). Development of glutamate binding sites and their regulation by calcium in rat hippocampus. *Devl Brain Res.* **1**, 37–48.

BAYER, S. A. (1980*a*). Development of the hippocampal region in the rat. I. Neurogenesis examined with ^{3}H-thymidine autoradiography. *J. comp. Neurol.* **190**, 87–114.

BAYER, S. A. (1980*b*). Development of the hippocampal region in the rat. II. Morphogenesis during embryonic and early postnatal life. *J. comp. Neurol.* **190**, 115–134.

BLISS, T. V. P. & GARDNER-MEDWIN, A. R. (1971). Long-lasting increases of synaptic influence in the unanaesthetized hippocampus. *J. Physiol.* **216**, 32–33*P*.

BLISS, T. V. P. & GARDNER-MEDWIN, A. R. (1973). Long-lasting potentiation of synaptic transmissions in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232**, 357–374.

BLISS, T. V. P. & LOMO, T. (1970). Plasticity in a monosynaptic cortical pathway. *J. Physiol.* **207**, 61*P*.

BLISS, T. V. P. & LOMO, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232**, 331–356.

CAMPBELL, B. A. & COULTER, X. (1976). The ontogenesis of learning and memory. In *Neural Mechanisms of Memory and Learning* ed. ROSENSWEIG, M. R. & BENNETT, E. L. Cambridge, MA: M.I.T. Press.

CAMPBELL, B. A., LYTLE, L. D. & FIBIGER, H. C. (1969). Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science, N.Y.* **166**, 635–636.

CHANGEAUX, J. P. & DANCHIN, A. (1976). Selective stabilization of developing synapses as a mechanism for the specification of neuronal networks. *Nature, Lond.* **264**, 705–712.

CRICK, F. (1982). Do dendritic spines twitch? *Trends Neurosci.* **5**, 44–46.

DESMOND, N. L. & LEVY, W. B. (1981). Ultrastructural and numerical alterations in dendritic spines as a consequence of long-term potentiation. *Anat. Rec.* **199**, 68–69A.

DOUGLAS, R. M. (1977). Long-lasting synaptic potentiation in the rat dentate gyrus following brief high-frequency stimulation. *Brain Res.* **126**, 361–365.

DOUGLAS, R. M. & GODDARD, G. V. (1975). Long-term potentiation of the perforant path–granule cell synapse in the rat hippocampus. *Brain Res.* **86**, 205–215.

DUFFY, C. J. & TEYLER, T. J. (1975). A simple tissue slicer. *Physiol. Behav.* **14**, 525–526.

DUFFY, C. J. & TEYLER, T. J. (1978). Development of potentiation in the dentate gyrus of rat: physiology and anatomy. *Brain Res. Bull.* **3**, 425–430.

DUNWIDDIE, T. & LYNCH, G. (1978). Long-term potentiation and depression of synaptic responses in the rat hippocampus: localization and frequency dependency. *J. Physiol.* **276**, 353–367.

DZIDZISHVILI, N. N. & KVIRKVELIA, L. R. (1968). Electrophysiological signs of hippocampal development in ontogenesis. *Prog. Brain Res.* **22**, 414–426.

FIFKOVA, E. & ANDERSON, C. L. (1981). Stimulation-induced changes in dimensions of stalks of dendritic spines in the dentate molecular layer. *Expl Neurol.* **74**, 621–627.

FIFKOVA, E. & ANDERSON, C. L., YOUNG, S. J. & VAN HARREVELD, A. (1982). Effect of anisomycin on stimulation-induced changes in dendritic spines of the dentate granule cells. *J. Neurocytol.* **11**, 183–210.

FONNUM, F., KARLSEN, R. L., MALTHE-SORENSEN, D., SKREDE, K. K. & WALAAS, I. (1979). Localization of neurotransmitters, particularly glutamate, in hippocampus, septum, nucleus accumbens, and superior colliculus. *Prog. Brain Res.* **51**, 167–201.

GODDARD, G. V. (1980). Component properties of the memory machine: Hebb revisited. In *The Nature of Thought: Essays in Honour of D. O. Hebb*, ed. JSCZYK, P. N. & KLEIN, R. M., pp. 281–247. Hillsdale, N.J.: Lawrence Erlbaum Associates.

HARRIS, K. M. (1982). Development onset of physiological plasticity in area CA1 of the rat hippocampus. Doctoral dissertation, Kent State University and Northeastern Ohio Universities College of Medicine. Available from University Microfilms, Ann Arbor, MI.

HARRIS, K. M. & TEYLER, T. J. (1982). Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. *Neurosci. Abstr.* **8**, no. 89.10.

HARRIS, K. M. & TEYLER, T. J. (1983). Evidence for late development of inhibition in area CA1 of the rat hippocampus. *Brain Res.* (in the Press).

HARRIS, K. M., TEYLER, T. J. & CRUCE, W. L. R. (1980). A developmental comparison of LTP in area CA1 and fascia dentata of the rat hippocampal formation. *Neurosci. Abstr.* **6**, no. 31.7.

HESSE, G. W. & TEYLER, T. J. (1976). Reversible loss of hippocampal long-term potentiation following electroconvulsive seizures. *Nature, Lond.* **264**, 562–564.

LEBLANC, M. D. & BLAND, B. H. (1979). Developmental aspects of hippocampal electrical activity and motor behavior in the rat. *Expl Neurol.* **66**, 220–237.

LEE, K. S. (1982). Cooperation amongst afferents to the stratum radiatum of CA1 for long-term potentiation of evoked responses. *Neurosci. Abstr.* **8**, no. 215.8.

LEE, K. S., SCHOTTLER, F., OLIVER, M. & LYNCH, G. (1980). Brief bursts of high frequency stimulation produce two types of structural change in the rat hippocampus. *J. Neurophysiol.* **44**, 247–258.

LOY, R. (1980). Development of afferent lamination in Ammon's horn of the rat. *Anat. Embryol.* **159**, 257–275.

McNaughton, B. L., Douglas, R. M. & Goddard, G. V. (1978). Synaptic enhancement in fascia dentata: cooperativity among co-active afferents. *Brain Res.* **157**, 277–293.

McNemar, Q. (1970). *Physiology Statistics*. New York: Wiley.

Minkwitz, H. G. (1976*a*). Zur Entwicklung der Neuronenstruktur des Hippocampus während der pra- und postnatalen Ontogenese der Albinoratte. II. Neurohistologische Darstellung der Entwicklung von Interneuron und des Zusammenhanges lang- und kurzaxoniger Neurone. *J. Hirnforsch.* **17**, 233–253.

Minkwitz, H. G. (1976*b*). Zur Entwicklung der Neuronenstruktur des Hippocampus während der prä- und postnatalen Ontogenese der Albinoratte. III. Mittelung: Morphometrische Erfassung der Ontogenetischen Veranderungen in Dendritenstruktur und Spinebesatz an Pyramidenneuronen (CA1) des Hippocampus. *J. Hirnforsch.* **17**, 255–276.

Minkwitz, H. G. (1977). Quantitative Aspekte der ontogenetischen Entwicklung von Pyramidenneuronen (CA1) aus dem Hippocampus der Ratte. *Verh. anat. Ges., Jena* **71**, 149–156.

Moshkov, D. A., Petrovskaia, L. L. & Bragin, A. G. (1977). Posttetanic changes in the ultrastructure of the giant spinous synapses in hippocampal field CA3. *Dokl. Akad. Naud. SSSR* **237**, 1525–1528.

Moshkov, D. A., Petrovskaia, L. L. & Bragin, A. G. (1980). Ultrastructural study of the bases of postsynaptic potentiation in hippocampal sections by the freeze-substitution method. *Tsitologiia* **22**, 20–26.

Myers, J. L. (1979). *Fundamentals of Experimental Design*. Boston, MA: Allyn & Bacon.

Pokorny, J. & Yamamoto, T. (1981*a*). Postnatal ontogenesis of hippocampal CA1 area in rats. I. Development of dendritic arborization in pyramidal neurons. *Brain Res. Bull.* **7**, 113–120.

Pokorny, J. & Yamamoto, T. (1981*b*). Postnatal ontogenesis of hippocampal CA1 area in rats. II. Development of ultrastructure in stratum lacunosum and moleculare. *Brain Res. Bull.* **7**, 121–130.

Rall, W. (1974). Dendritic spines, synaptic potency and neuronal plasticity. In *Cellular Mechanisms Subserving Changes in Neuronal Activity*, ed. Woody, C., Brown, K., Crow, T. & Knipsel, J. U.C.L.A. CA: Brain Information Service Publication.

Rawlins, J. N. P. & Green, K. F. (1977). Lamellar organization in the rat hippocampus. *Exp. Brain Res.* **28**, 335–344.

Schwartzkroin, P. A. (1981). Development of rabbit hippocampus: anatomy. *Devl Brain Res.* **2**, 453–468.

Schwartzkroin, P. A., Kunkel, D. D. & Mathers, L. H. (1981). Development of rabbit hippocampus: physiology. *Devl Brain Res.* **2**, 469–486.

Schwartzkroin, P. A. & Wester, K. (1975). Long-lasting facilitation of a synaptic potential following tetanization in the *in vitro* hippocampal slice. *Brain Res.* **89**, 107–119.

Skrede, K. K. & Westgaard, R. N. (1971). The transverse hippocampal slice: a well-defined cortical structure maintained *in vitro*. *Brain Res.* **35**, 589–593.

Swanson, L. W., Teyler, T. J. & Thompson, R. F. (1982). Hippocampal LTP: mechanisms and functional implications for memory. *Neurosci. Res. Prog. Bull.* **20**, no. 4.

Teyler, T. J. (1980). The brain slice preparation: hippocampus. *Brain Res. Bull.* **5**, 391–403.

Teyler, T. J., Mahew, W., Chrin, C. & Kane, J. (1982). Neurophysiological, field potential analysis by microcomputer. *J. Neurosci. Methods* **5**, 291–303.

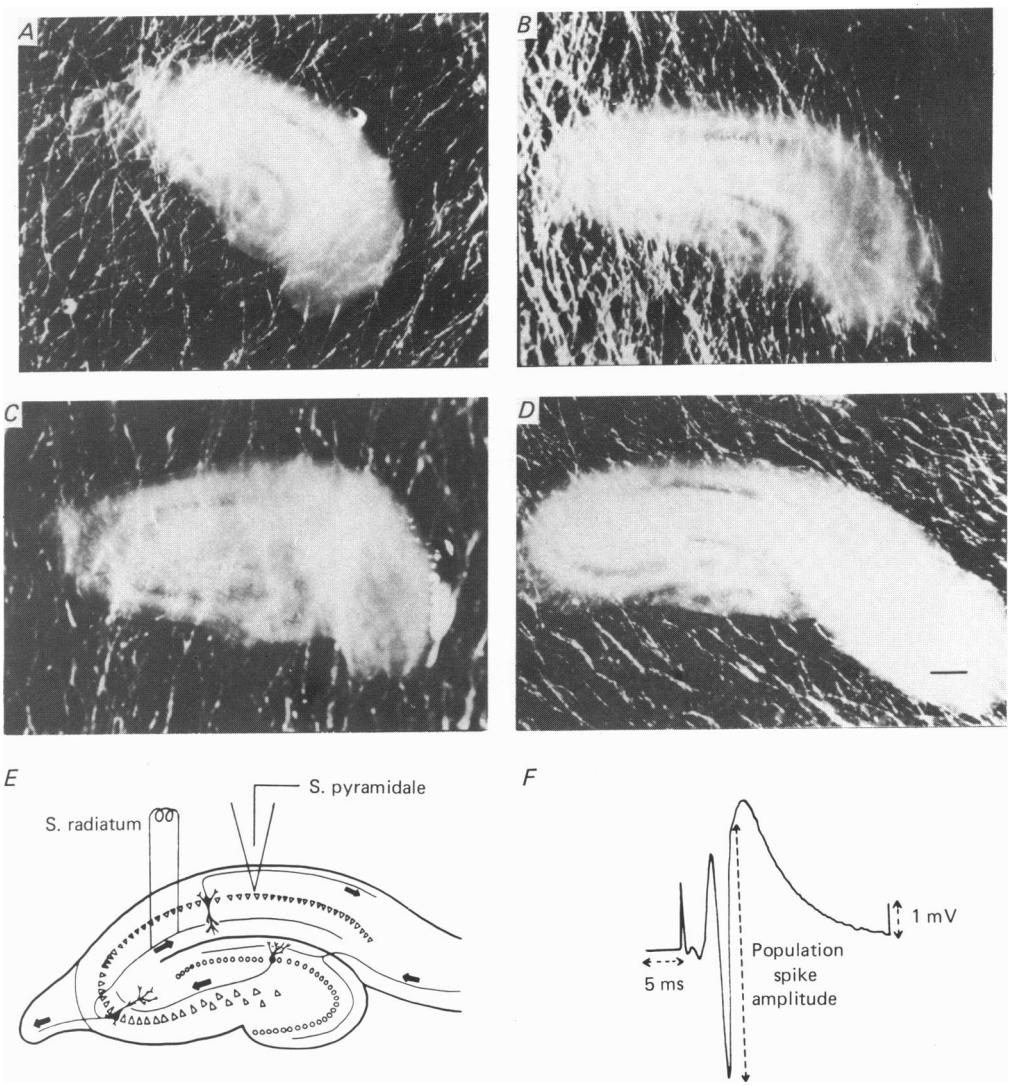
Van Harreveld, A. & Fifkova, E. (1975). Swelling of dendritic spines in the fascia dentata after stimulation of the perforant fibers as a mechanism of posttetanic potentiation. *Expl Neurol.* **49**, 736–749.

Yamamoto, C. & Chujo, T. (1978). Long-term potentiation in thin hippocampal sections studied by intracellular and extracellular recordings. *Expl Neurol.* **58**, 242–250.

Yamamoto, C. & Sawada, S. (1981). Important factors in induction of long-term potentiation in thin hippocampal sections. *Expl Neurol.* **74**, 122–130.

EXPLANATION OF PLATE

Lamellar organization of hippocampal slices from animals aged: *A*, 4 days; *B*, 5 days; *C*, 7 days; and *D*, 8 days. Calibration bar represents 0·1 mm. *E*, schematic diagram illustrating electrode positioning in area CA1 of the hippocampal slices. *F*, illustration of the amplitude of the population spike from CA1 cells that was measured by the Apple computer. For all the wave forms presented in the following Figures, the calibrations are as indicated, with a 5 ms time calibration pulse before the stimulus artifact and a 1 mV voltage calibration pulse at the end of the wave form. For all the Figures negative polarity is down.



(*Facing p.* 48)