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LONG-TERM CHANGES IN HIPPOCAMPAL PHYSIOLOGY AND LEARNING ABILITY OF RATS AFTER INTRAHIPPOCAMPAL TETANUS TOXIN

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SUMMARY

1. A chronic epileptic syndrome can be induced by injecting minute doses of tetanus toxin into rat hippocampi. This causes intermittent epileptic fits over a period of 2–4 weeks, after which the fits cease, and the electroencephalogram (e.e.g.) appears to return to normal over the following 2–3 weeks. However, once they have recovered from the seizures, the rats exhibit a remarkably persistent impairment of learning and memory, which is the subject of the present study.

2. Learning ability was assessed using a radial arm maze task, in which the rats had to visit each of eight arms for a food reward. The toxin-injected rats learnt this task more slowly than control-injected.

3. Evoked potentials from the CA3 pyramidal cells were recorded in terminal experiments under halothane anaesthesia. Long term potentiation of the post-synaptic response to the commissural pathway from the contralateral hippocampus appeared to be unaffected by the previous toxin treatment, at least over periods of up to 5 h.

4. The toxin-injected group differed from the control in having consistently smaller post-synaptic population spikes in their evoked responses, so that stimuli were less effective in exciting the post-synaptic neurones. This applied both to the contralateral commissural input, and to the ipsilateral mossy fibre input.

5. No differences were found between the toxin and control groups in the size of the antidromic population spike in the commissural response, or in the population excitatory post-synaptic potential (e.p.s.p.) for either input. Thus the depressed output from CA3 pyramidal cells cannot be explained either by a loss of these neurones (confirming earlier neuropathological observations), or by a loss of excitatory afferents.

6. While its precise cause remains unknown, the depressed output from the CA3 region was statistically correlated with the learning impairment, and we believe provides a reasonable explanation of this behavioural deficit.

INTRODUCTION

A substantial body of experimental evidence exists concerning the role of the hippocampus in learning and memory (Weiskrantz, 1982). Much of this evidence relates to what experimental animals with large hippocampal lesions can and cannot learn and remember (Rawlins, 1985), and while detailed interpretations differ, there is no doubt that profound deficits in learning and memory can be demonstrated in such animals using appropriate tasks. The physiological basis of learning and memory in vertebrates remains unknown, but the hippocampus provides a model, at least, of memory processes in the phenomenon of long-term potentiation. This is a very long-lasting enhancement of synaptic transmission produced by repetitive stimulation of many pathways in the hippocampus, first described by Bliss & Gardner-Medwin (1973) and Bliss & Lømo (1973). More recently, Barnes (1979) has reported experiments which suggest a correlation between reduced ability of the hippocampus to support long-term potentiation and the decay of learning ability in senescent rats. This work provides circumstantial evidence for a role of long-term potentiation in memory.

Patients with temporal lobe epilepsy, which frequently originates from a focus in the hippocampus, often have problems with learning and memory (Glaser, 1975). Comparable deficits are seen in rats after apparent recovery from an experimental epilepsy induced by injecting minute doses of tetanus toxin into the hippocampus (Mellanby & George, 1979; Mellanby, Strawbridge, Collingridge, George, Rands, Stroud & Thompson, 1981; George & Mellanby, 1982; Mellanby, Hawkins & Wilks, 1984; Hawkins, Mellanby & Brown, 1985). Such animals exhibit a chronic, but eventually reversible, epileptiform syndrome. Intermittent fits occur for 2–4 weeks and then during the next 2–3 weeks the electroencephalogram (e.e.g.) (recorded from the skull surface or from the depths of the hippocampus) returns to normal. However, the apparently recovered animals still show deficits in learning tasks which are believed to depend on hippocampal function (Mellanby, Renshaw, Cracknell, Rands & Thompson, 1982). It was therefore of interest to see whether lasting physiological changes could be found in such animals and furthermore, if such changes occurred, whether they could be correlated with the behavioural deficits. In the present paper animals which had recovered from the tetanus toxin-induced epileptiform syndrome were trained on a radial arm maze and two months or more later the physiology of the CA3 area of the hippocampus was studied.

METHODS

Behavioural studies

Fifteen male Sprague–Dawley rats (350–450 g) were made epileptic by the bilateral injection of tetanus toxin into their hippocampi (and eight animals were injected with the toxin vehicle only). The incidence of fits in the toxin-injected rats was recorded over the next five weeks by continuous time-lapse video-recording. Eight weeks after the injection of tetanus toxin, training of the animals in a radial arm maze was started. This was continued for the next 25 days. Two or three months after the end of the radial arm maze training (that is four to five months after injection of toxin) electrophysiological investigation of hippocampal function was performed.

Tetanus toxin containing 2×10^6 mouse LD_{50}/mg was kindly provided by Dr R. O. Thomson of the Wellcome Research Laboratories, Beckenham, Kent. The toxin was dissolved and diluted in

0·05 M-phosphate buffer, pH 7·0, containing 0·2% gelatin (gelatin buffer). It was assayed by the method of Mellanby, Mellanby, Pope & van Heyningen (1968).

Operative procedure. The rats were anaesthetized with equithesin (81 ml sodium pentobarbitone, 198 ml propylene glycol, 50 ml ethanol, 21 g chloral hydrate, 10·6 mg $MgSO_4$ made up to 500 ml with distilled water), 3·0 ml/kg, I.P. The toxin (or gelatin buffer in the controls) was injected using a Hamilton microsyringe fitted onto a Kopf stereotaxic instrument. 0·2 μl–0·5 μl of a toxin solution containing about 40000 mouse LD_{50}/ml was injected bilaterally into the ventral hippocampi of the rats at coordinates anterior–posterior +0·30; lateral ±0·48; vertical −0·20, according to the atlas of De Groot (1967).

Recording fits. The toxin-injected rats were marked distinctively with black waterproof mascara, caged in groups of four and placed under the camera. A Link electronics camera with a Canon TV zoom lens was used with a vidicon infra-red sensitive tube. During 12 h of 'daylight', 09.00–21.00 h, overhead neon striplighting was used; at night an infra-red lamp was used, (Thorn reflector, 200/200V, 275W). The camera was connected to a National Panasonic time-lapse video-recorder using Scotch 455 high density videotape. The rats were filmed continuously. The films were played back at 20 × speed and the incidence and time of occurrence of fits was recorded. With practice (and validation by a second experimenter) fits could be reliably identified at this speed (where a fit was suspected the tape could be stopped and played back frame by frame if necessary). Characteristically the fits started with the rats' ears flattening, giving the head a streamlined shape and the rat would usually then rear up. A fit was counted if myoclonic jerks of the paws occurred. It was also noted whether the animal fell over and then reared up again and continued fitting – in this case the fit was classified as 'major'.

Training on the radial arm maze. At the end of filming the rats were handled daily for 27 days. As handling continued the rats gradually became less jumpy. On day 15 of handling, food deprivation began. The animals were fed for progressively shorter periods until they were being fed for only 1½ h a day. During this time the animals were weighed regularly and by the latter stages of deprivation they had fallen to a steady 85% of pre-operative weight. The animals were fed between 17.00 and 18.30 h.

The maze was made of wood and had a Perspex guillotine door attached to a series of pulleys at the entrance to each of its eight arms (Olton, Collinson & Werz, 1977). At the end of each arm was a small food container which was baited with four Abels nutrient tablets. The orientation of the maze was kept constant in relation to external cues in the testing room. On the first two days of pre-training the rats were placed on the end of each arm for 2 min and encouraged to eat the food pellets. On the third day each rat was placed in the centre of the maze and after 10 s the doors of four arms were opened; the animal would choose an arm (four paws entering the arm was classified as a choice) and the other three doors were shut. When the rat left the arm the door was closed behind it and it was kept in the inner area for 10 s. After this time the same four doors were opened and the procedure repeated. The animal was allowed eight free choices and after this time it was placed at the end of any unvisited arms and left there for 2 min before being returned to its cage. Day 4 consisted of a repetition of the previous day except that the four other arms were opened.

Training then took place for 25 days. It took the same form as pre-training on days 3 and 4 except that all eight doors were opened. The number of the arm chosen was noted, as was the time to make eight choices. The animal was allowed to run the maze until it had visited all eight arms. The time taken to complete the task was noted. Seven out of eight (or eight out of eight) correct choices counted as being successful and the criterion for learning the maze was 5 successive correct days.

Physiological studies

Four to six months after the tetanus toxin injection into the rats, terminal experiments were performed to investigate pathways to the CA3 pyramidal cells. Throughout these experiments, anaesthesia was maintained with 1% halothane in air at atmospheric pressure, exchanged at 400 ml/min across the mouth of a tracheal cannula. Rectal temperature throughout the experiment was maintained at 36–38 °C with the aid of a d.c. heating lamp. The neocortex was exposed above the right hippocampus 2·5–4·0 mm lateral to the mid line, and above the left fimbria/fornix and rostral parts of the hippocampus 0·7–2·0 mm lateral; silicone oil was used to prevent drying of the exposed brain tissue. Tungsten micro-electrodes (Digitimer Ltd., NL05) for recording were placed at 2·6 mm caudal to bregma, 3·5 mm lateral and about 2·7 mm below the cortical surface, for

stimulating (cut back to 20–50 μm tip diameter), at 3·5 mm caudal, 3·5 mm lateral and 35 mm deep on the ipsilateral side and 1·0 mm caudal, 1·3 mm lateral, 4·0 mm deep on the contralateral side. The location of the recording electrode in CA3 was confirmed by recording a large antidromic population spike in response to the contralateral stimulation. The vertical positions of the electrodes were adjusted to maximize the post-synaptic field potentials in CA3; only rarely was it necessary to move the electrodes in the horizontal plane to obtain suitable responses.

The following data were obtained from each rat: stimulus–response curves for ipsi- and contra-lateral stimulation; the time course of inhibition of the ipsilateral (mossy fibre) response following contralateral (commissural) stimulation; and long-term potentiation (l.t.p.) of the commissural pathway, using tip-negative stimuli of 8–12 V, 0·1 ms, which usually gave a small post-synaptic population spike. During each l.t.p. experiment, control records were taken at 60 s intervals for 1 h before the conditioning train of 50 pulses at 4 ms; responses recorded at 1/min for a further hour, the stimulus–response curves were then repeated and finally every 4th response to commissural stimulation at 1/min was recorded from 80–360 min after the conditioning train. Recordings and measurements were made using a Research Machines Ltd. (Oxford) 380Z microcomputer equipped with a Digitimer Ltd. (Welwyn) D201 ADC. Two types of measurement were made from the recorded evoked potentials. First, the (population) excitatory post-synaptic potential (e.p.s.p.) was estimated as the rate of rise (V/s) of the positive component of the post-synaptic response, for a limited range of stimuli (with large stimuli other components encroached, making this measurement unreliable or impossible). Second, population spikes, generated by the synchronous discharge of the pyramidal cells following antidromic or orthodromic activation (▲ and ●, Fig. 3*A*), were measured as the amplitude of the negative peak from a base-line interpolated between the adjacent positive maxima.

The protocol for the physiological measurements was kept constant so that quantitative and statistical comparisons could be made between experimental groups. The second half of the study was performed blind to avoid biasing the results from the tentative conclusions drawn from the first half, and produced essentially the same results.

Statistical analysis of results

For analysis, the learning data for each rat, as correct choices in the first eight trials each day, were summed over blocks of five days. This procedure, by increasing the range of possible values, removed discontinuity in the data and made the analysis of variance valid.

The physiological data (spike size and the slope of the e.p.s.p.) was transformed to give homogeneity of variance and a normal distribution by adding a constant (usually the most negative value) to all the values and then taking $\log_{10}$. Since the threshold for a population spike varied between animals, only the responses to stimuli at 6–30 V were used in the analysis of the population spikes. Analysis of variance was carried out using the GENSTAT library and the factors analysed were treatment (toxin or control) and stimulus strength. A correlation matrix was run for the values from just the toxin-injected rats for all data on the occurrence of fits and all the physiological and behavioural measures taken. A further correlation matrix was run for all the rats (controls as well as toxin-injected) for just the physiological and behavioural measures.

RESULTS

Fits

The number of fits which each rat experienced within a 24 h period (midnight–midnight) was calculated and an average 'fit-rate' per hour obtained for each toxin-injected rat for each day for the 28 days of filming (Fig. 1). The data on the occurrence of fits are summarized in Table 1.

Learning the radial arm maze

It can be seen from Fig. 2 that both groups improved their performance over the 20 days of training above the level expected by chance (28·5 correct choices out of 40). The toxin-injected group, however, performed significantly worse throughout.

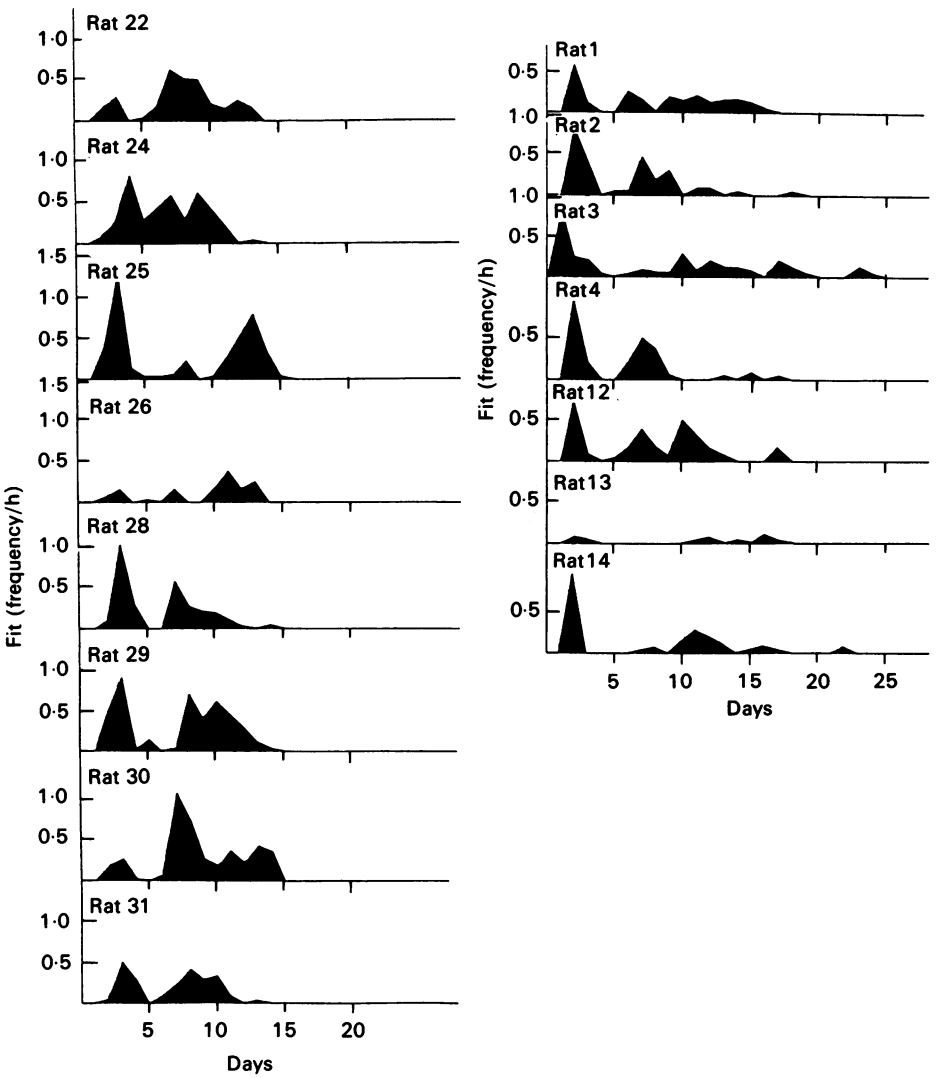

Fig. 1. Life histories of epilepsy in toxin-injected rats. The rats had received 8–20 LD_{50} tetanus toxin bilaterally into their hippocampi on day 0. Fits were recorded using time-lapse videofilming over the next 28 days (for details of criteria for identification of fits, see Methods). The number of fits each rat experienced each day has been expressed as mean frequency/h and plotted against the days after operation.

Analysis of variance showed a significant main effect of the epilepsy (d.f.: 1,24; $F = 24{\cdot}186$; $P < 0{\cdot}001$), and a significant effect of blocks × epilepsy (d.f.: 4,96; $F = 3{\cdot}453$; $P < 0{\cdot}05$) with a significant quadratic component (d.f: 1,96; $F = 7{\cdot}834$; $P < 0{\cdot}01$). Examination of the curve in Fig. 2 shows that this quadratic component is due to the control animals improving their performance at a faster rate during the early part of the experiment. However, despite the fact that the toxin-injected rats continued to improve their performance throughout the experiment, the scores over the last block of 5 days are still significantly different (d.f.:1,24; $F = 24{\cdot}186$;

$P < 0{\cdot}001$). By the last day of training, all but three rats (all toxin-injected) had reached the criterion of 5 consecutive days with at least seven correct choices out of the first eight. In contrast to the learning impairment found in the toxin group, there was no significant difference in the time taken to make eight choices between the groups.

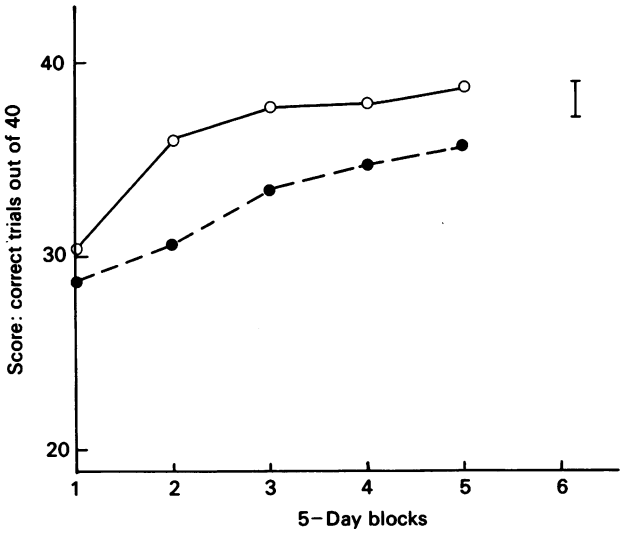

Fig. 2. Learning curves for control and toxin-injected rats on the radial arm maze. Controls, ○; toxin, ●; vertical bar = S.E. of mean. The toxin-injected rats ($n = 15$) had received the tetanus toxin (and the controls, $n = 8$, gelatin buffer) bilaterally into their hippocampi eight weeks before training was started. (For details of training, see Methods.) The rats were trained on the radial arm maze for 25 consecutive days. On each day they were trained until they had visited every arm once. The number of correct choices out of the first eight has been summed for each rat over blocks of 5 days and the means of these scores for the control and toxin groups are plotted.

TABLE 1. Summary of mean values (± standard deviation) for various features of the epileptic syndrome. (A 'major' fit was defined as one in which the rate fell over and then resumed fitting.)

n	Total fits	*Maximum no. of fits occurring in a 24 h period	Day on which* occurred	Total major fits	Maximum no. of major fits occurring in a 24 h period
15	66·4 (±27·0)	18·5 (±7·2)	4·5 (±4·1)	19·2 (±7·6)	7·5 (±3·9)

A correlation matrix was run using GENSTAT between learning scores (trials to criterion and errors on days 21–25) for each rat and all the data for the occurrence of fits shown in Table 1. No significant correlations were found between the data on learning and on fits.

Physiological responses

The functional states of two inputs to the CA3 pyramidal cell population were examined in terminal experiments under 1 % halothane, four to six months after toxin injection (at least two months after the animals had stopped having fits). The

pathways used were the commissural projection from the contralateral CA3 pyramidal cells, and the ipsilateral mossy fibre projection from the granule cells of the dentate area. (One of the toxin rats died between the testing on the radial arm maze and the physiological experiments.)

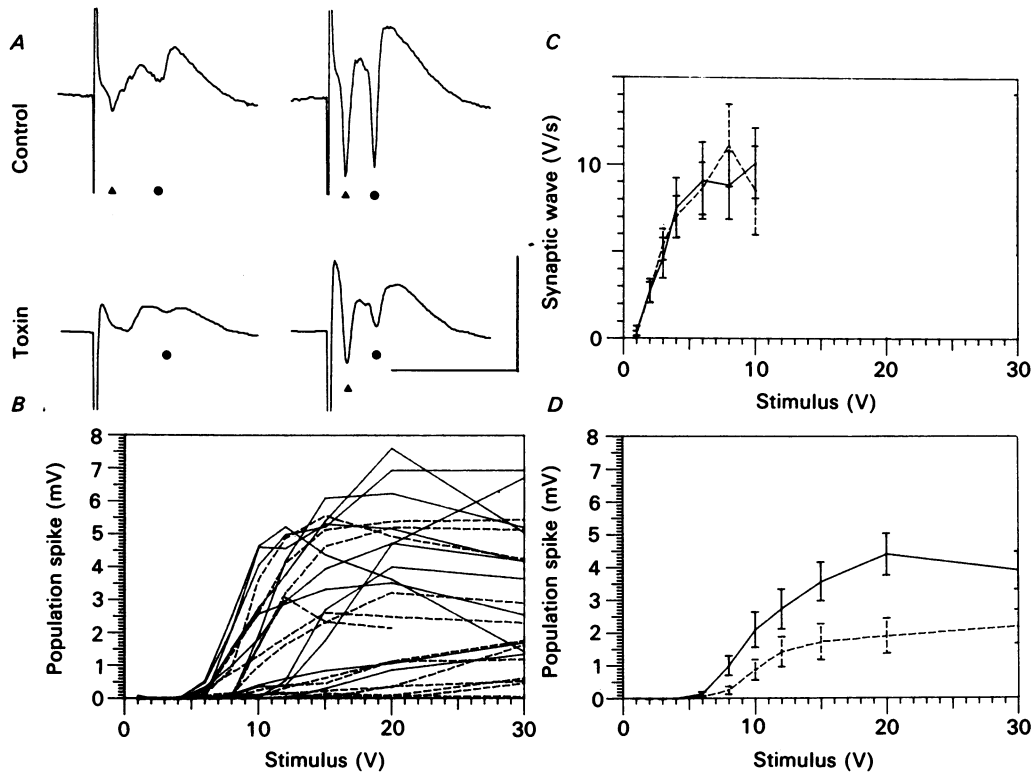

Fig. 3. Commissural responses in CA3. *A*, evoked potentials from a control rat (upper pair of traces) and a toxin rat (lower pair) were selected as the closest match to the respective group mean population spike amplitudes for 8 and 20 V stimuli (left and right traces respectively). (The apparent difference in the duration of the stimulus artifact is a consequence of the digitization and does not reflect any difference in the stimulus variables between the toxin and control rats.) *B*, post-synaptic population spikes have been plotted against stimulus strength for all animals, the dashed curves being from toxin animals. *C*, *D*, measurements at each stimulus strength have been averaged to provide group stimulus response curves for the slope of the population e.p.s.p. (*C*) and for the population spike amplitude (*D*); (error bars ± 1 standard error; calibration: 5 mV and 10 ms; all stimuli are 0·1 ms in duration).

Commissural input to CA3

Because of the reciprocal commissural connexions between the left and right CA3 regions, it is difficult to avoid antidromic population spikes in the CA3 recordings, especially with large stimuli (examples are marked ▲ in Fig. 3*A*). There was no statistically significant difference in the size of the antidromic population spikes recorded in the two groups, although the mean antidromic population spike was marginally larger in the toxin-injected group (d.f: 1,22; $F = 3{\cdot}972$).

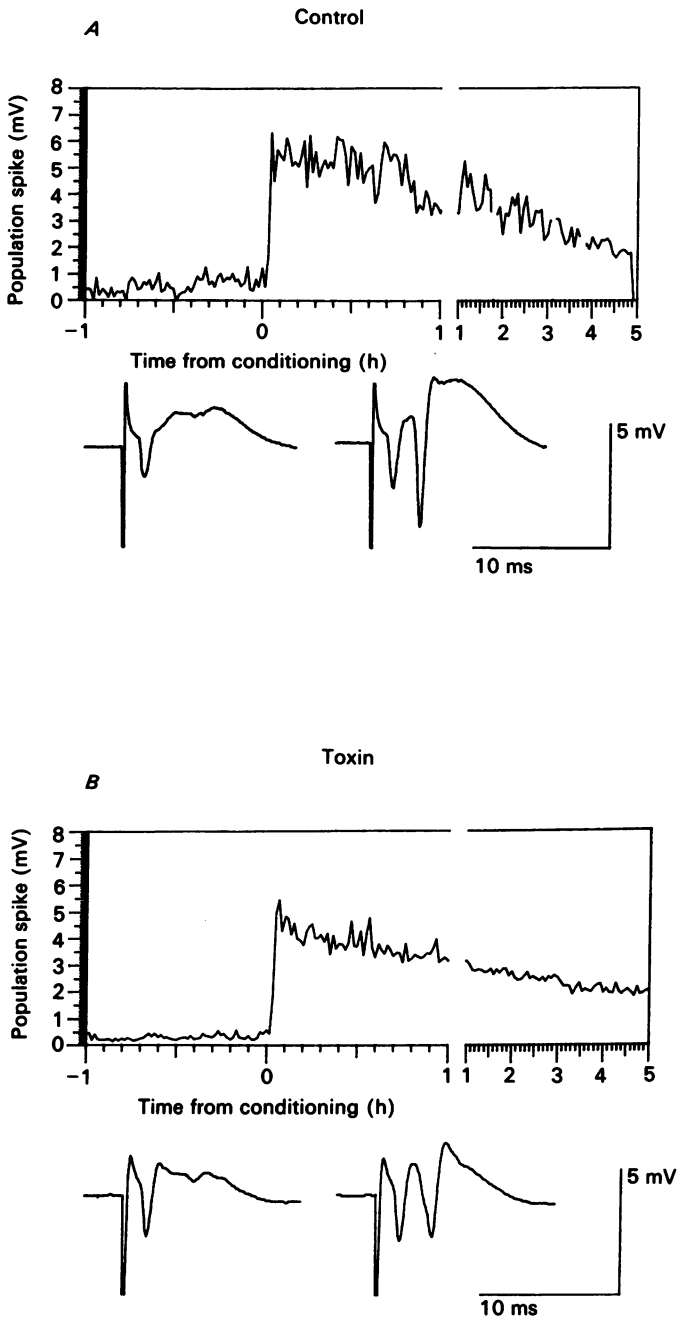

Fig. 4. Long-term potentiation: responses to a weak commissural stimulus were recorded at 1/min for 1 h before, and 1 h after a conditioning train of 50 stimuli at 250/s, and every 4th min thereafter. Below each time course are illustrated evoked potentials taken 30 min before and 1 h after the conditioning train. Toxin animal (*B*) was selected because it had one of the lowest behavioural scores. (Calibration: 5 mV and 10 ms.)

The post-synaptic component of the commissural response recorded at the cell body layer consists of a positive wave on which is superimposed a negative population spike (marked ● in Fig. 3*A*). The form of the response was the same in the toxin and control groups (Fig. 3*A*). The amplitude of the post-synaptic population spike was measured for a series of stimulus strengths in each rat, and all the stimulus–response curves have been plotted in Fig. 3*B*. While there is clearly much variability between rats, the toxin results (dashed lines) tend to cluster below the controls (continuous lines). Six of the toxin animals produced stimulus–response curves for the population spike which did not differ from the controls while the remaining eight were much smaller. The mean data (Fig. 3*D*) show clearly that the responses of the toxin group are lower than those of the controls.

A 2-way analysis of variance on the data showed that the difference between groups was highly significant. Using transformed data (see Methods) the analysis showed a significant main effect of toxin injection (d.f: 1,20; $F = 4{\cdot}64$; $P < 0{\cdot}05$), and of stimulus × toxin (d.f: 5,100; $F = 2{\cdot}711$; $P < 0{\cdot}05$) and this was due to a highly significant linear component (d.f: 1,100; $F = 9{\cdot}165$; $P < 0{\cdot}01$). Since it was conceivable that the toxin-induced reduction in the size of the orthodromic spike might be somehow related to the increase (non-significant) in the antidromic spike, an analysis of covariance was carried out, and this showed that no such interaction occurred since the pattern of results was the same.

The population e.p.s.p. could only be measured reliably up to 6 or 8 V stimuli because of encroaching population spikes (as is clear from the 20 V traces illustrated in Fig. 3*A*); however, up to this point the two mean curves are closely superimposed (Fig. 3*C*). This was confirmed by the 2-way analysis of variance which showed no differences between the slope of the stimulus–response curves of the two groups.

The nature of the differences between the responses from toxin and control rats is illustrated by the sample traces in Fig. 3*A*, which were taken from the rat in each group that most closely approximated the respective group means at 8 and 20 V. The only significant difference between the groups was the markedly smaller post-synaptic population spike (less than one-half) in the toxin group; there was no corresponding change in the spike's latency, in the e.p.s.p. slope, or in the antidromic population spike.

Long-term potentiation

The prolonged time course of l.t.p. gives it a special interest in a study on impaired learning. Here we have followed l.t.p. in the commissural projection to CA3 for up to 5 h after the single conditioning train of 50 stimuli at 250/s. The marked increase in population spike from the 1 h base-line recording is obvious in both the control and toxin examples (Fig. 4*A* and *B*, about 7- and 10-fold increases respectively). The decay of l.t.p. was similar in both cases, reaching half of its maximum in 3–4 h. However, the toxin rat illustrated had never reached criterion on the behavioural task, and thus had one of the worst scores of the group. The finding (see above) that the population spike, but not the e.p.s.p. was reduced in the toxin group makes it difficult to standardize conditions for precise measurements of l.t.p., but all the toxin animals produced apparently normal l.t.p. (i.e. similar to those illustrated), and we conclude that l.t.p. has not been disrupted, over the time scale of a few hours.

A systematic comparison of l.t.p. over its full time course could not be made in the two groups because of difficulties in choosing suitable test stimuli which provide a measurable spike before l.t.p. and which did not 'saturate' once potentiated.

Stimulus–response curves were repeated 1 h after conditioning and did permit systematic comparison. The synaptic wave and the population spike curves have been plotted for the control and toxin groups (Fig. 5*A*, *B* and *C*, *D* respectively). The post-conditioning curves (○) have been plotted with their respective pre-conditioning

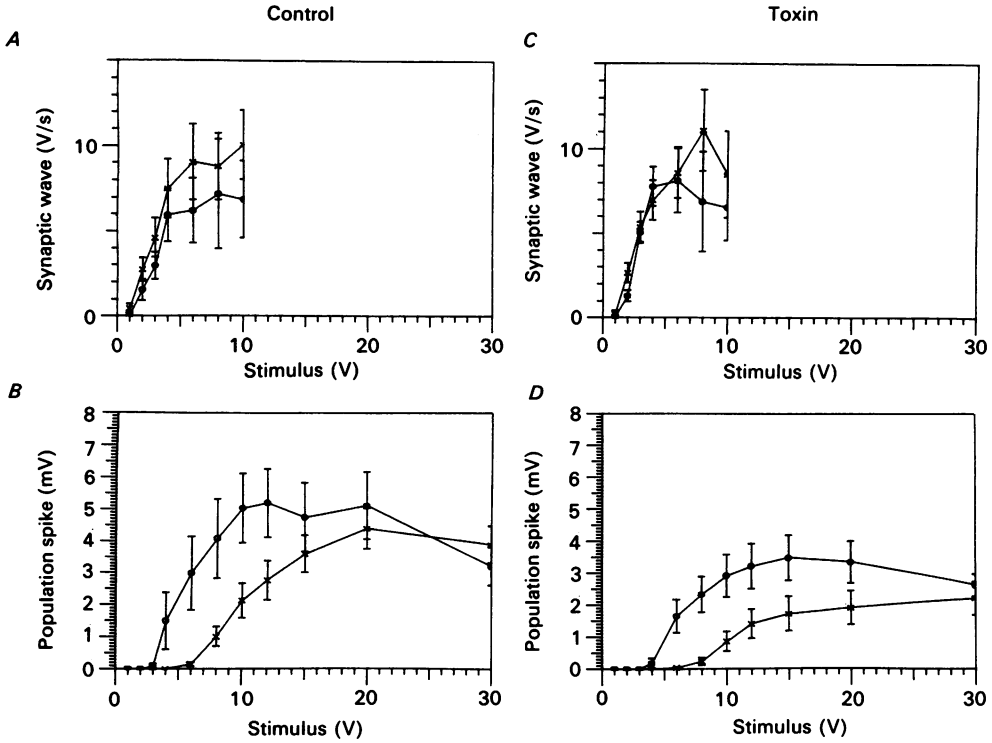

Fig. 5. Stimulus–response curves for the synaptic wave (*A*, *C*) and population spike (*B*, *D*) elicited by the commissural input to CA3, taken 1 h before (×) and 1 h after (○) the conditioning train of 50 stimuli at 250/s. Mean values ± 1 S.E. for the control group are plotted in *A* and *B*, and for the toxin group in *C* and *D*.

curves (×). The population spikes are clearly potentiated in both groups 1 h after conditioning (Fig. 5*B* and *D*). The amount of potentiation reaches a maximum for stimuli for 12–15 V (the range used for the conditioning stimuli) and progressively declines with larger stimuli. Unexpectedly, the synaptic wave was not potentiated in either group of animals 1 h after conditioning (Fig. 5*A* and *C*). Whatever the reason for the apparent lack of potentiation of the synaptic wave in these experiments, it occurred in both the toxin and the control groups, and thus does not reflect an action of the toxin. The synaptic potentials in the control group actually decreased after conditioning (Fig. 5*A*), probably due to increased contamination by the population spike after potentiation. Comparision of the population spikes after potentiation revealed that the difference between the control and the toxin groups was less marked,

and indeed was no longer statistically significant; however this observation should not be given great biological significance in view of the difficulty in standardizing the stimuli used for conditioning, given the results illustrated in Fig. 3.

Mossy fibre input

The mean population spike stimulus–response curves for the mossy fibre stimulation showed that the responses were smaller in the toxin rats (0·3–0·5 of controls, Fig. 6*D*).

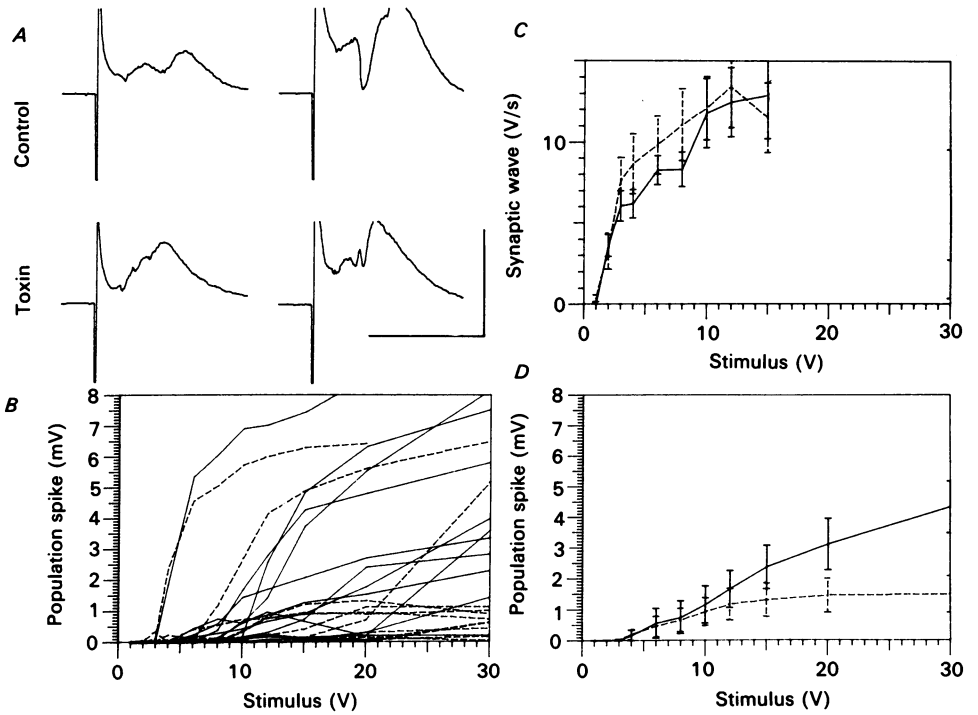

Fig. 6. Stimulus–response curves for the ipsilateral, mossy fibre input to CA3, format and scales as Fig. 3.

Analysis of variance showed that the difference is significant (effect of stimulus × toxin; d.f.: 5,110; $F = 2{\cdot}376$; $P < 0{\cdot}05$; due to a significant linear component, d.f.: 1,110; $F = 10{\cdot}388$; $P < 0{\cdot}01$). (Two stimulus-response curves, one toxin and one control, appear to be outliers (see Fig. 6*B*), perhaps due to a contamination from commissural or other afferents, but were not excluded.) The substantial difference in the population spikes in the two groups is illustrated by the evoked potentials (Fig. 6*A*), chosen as the closest to their respective mean stimulus–response curves. The mossy fibre responses generally had little or no antidromic component so that the e.p.s.p. could be measured over a greater range than in the commissural responses. There appeared to be no differences (Fig. 6*C*) and this was confirmed by the statistical analysis. The evidence available here suggest that mossy fibre responses have been affected by the toxin treatment in much the same manner as the commissural.

Correlation of behavioural and physiological results

One of the aims of this study was to try to correlate physiological changes with the learning impairment associated with the tetanus toxin epileptic syndrome. While both measures were subject to substantial variability, there was evidence of an inverse correlation between the most reliable physiological deficit, the reduction of the size of the post-synaptic commissural population spike, and the learning scores in the radial arm maze task. Thus the rats with the most errors on days 21–25, in either toxin or control groups, had the smaller commissural population spikes. A correlation matrix was run between two measures of learning – the number of trials to criterion and the number of errors in the last 5 days – and the physiological measures (all expressed as the slopes of the stimulus–response curves), using transformed data. Significant negative correlations were found between both criterion and error scores with the commissural population spike (d.f: 21, $r = -0{\cdot}5914$; $P < 0{\cdot}01$; $r = -0{\cdot}5104$; $P < 0{\cdot}02$) and between the mossy fibre spike and the criterion score ($r = -0{\cdot}4393$; $P < 0{\cdot}05$).

DISCUSSION

The animals which had recovered from the epileptic effect of tetanus toxin injection into the hippocampus were significantly slower in learning the radial arm maze task than the control animals. They did, however, learn the task, which contrasts with animals with surgical lesions to the hippocampus (Olton *et al.* 1977), which never scored higher than chance value. Thus the treatment with tetanus toxin does produce an impairment, but a less severe one than that brought about by surgical lesions. Furthermore, treatment with the toxin also impaired the physiological responses of the hippocampus – specifically, smaller responses were elicited in CA3 pyramidal cells by afferent stimulation in these animals. These physiological and behavioural deficits were statistically correlated, and we believe it is a reasonable hypothesis that they are causally related.

The physiological deficit in the toxin-injected animals, that is the smaller post-synaptic population spikes, appears to be due to the CA3 pyramidal cells being less excitable. No reduction in the antidromic population spike in CA3 was recorded, suggesting that there was no loss of pyramidal cells, or at least none sufficient to explain the depression of the post-synaptic population spikes; this is in agreement with a previous histopathological report (Mellanby, George, Robinson & Thompson, 1977). There appeared to be no change in the afferent input to CA3 since synaptic potentials were unchanged in the responses to the two distinct inputs used. These physiological changes make an interesting contrast with those reported in studies of senescence in rats for another hippocampal pathway (perforant path – granule cell) where the population spike size was maintained in the face of a loss of afferents and a concomitant reduction in the recorded synaptic potential (Barnes & McNaughton, 1980, 1983).

We had originally proposed (Mellanby *et al.* 1982; George & Mellanby, 1982) that the persistent learning impairments seen in rats which had apparently recovered from the tetanus toxin-induced hippocampal epilepsy might have resulted from interference with mechanisms of the l.t.p. which is such a remarkable feature of hippocampal

pathways (Bliss & Lømo, 1973; Bliss & Gardner-Medwin, 1973). Contrary to our expectations, we found no evidence of any abnormality in l.t.p. over periods of up to 5 h following single conditioning trains. Stimulus–response curves revealed that the population spikes of both the toxin and the control groups were still potentiated 1 h after the conditioning. In both groups the amount of potentiation (the difference between the pre- and post-l.t.p. stimulus–response curves) increases with stimulus strength up to the values used for the conditioning trains, beyond which the stimulus–response curves converge; this observation is consistent with the proposal that l.t.p. is restricted to the synapses activated during the conditioning train (McNaughton, Douglas & Goddard, 1978). Unexpectedly, in neither experimental group was the population e.p.s.p. potentiated at 1 h. This is perhaps surprising as a high frequency train of stimuli, as was used here, should have its major effect on the synapses of the stimulated afferents. The l.t.p. produced here was specific to the commissural pathways; mossy fibre stimulus–response curves revealed no potentiation, so that a generalized excitability change can be ruled out. It appears that the conditions used here favour the 'E–S' form of potentiation (Andersen, Sundberg, Sveen, Swann & Wigstrøm, 1980). However, the essential point is that no differences in the properties of the l.t.p. could be detected between the toxin and control groups.

The l.t.p. used in this study may not be the most relevant to learning. The correlation between the scores on a circular platform task and l.t.p. which Barnes (1979) had reported in senescent rats, involved l.t.p. with a time course of several days following repeated conditioning trains. (Racine, Milgram & Hafner (1983) demonstrated a similar impairment of longer time-course components they extracted from l.t.p., recorded at several hippocampal and other limbic sites, following kindling-induced seizures.) However, while this more persistent l.t.p. should be examined in the tetanus toxin model, we feel it is more likely that the explanation of the behavioural impairment is to be found in the, conceptually rather simpler, depression of CA3 responses demonstrated here.

The present data do not allow us to conclude much about the cellular basis of the remarkably persistent depression of CA3 responsiveness. Possible causes are increased inhibition (particularly tonic or feed-forward) or intrinsic changes in the CA3 pyramidal cells themselves. The protocol of the physiological experiments used in this study included an attempt to assess recurrent inhibition in CA3, by investigating inhibition of the CA3 population spike in response to mossy fibre stimulation by preceding commissural volleys. However, these experiments were confounded by the finding that the toxin group had a reduced population spike which would be expected to activate fewer recurrent inhibitory inputs.

At this stage it is not clear whether the depression of CA3 could be a direct, very long-lasting effect of the toxin or whether it arises indirectly from the epileptic focus it produces. Certainly the toxin can block synaptic transmission peripherally for several weeks and it has been shown at the skeletal neuromuscular junction that recovery of synaptic function depends on the growth of new terminals rather than the repair of old ones (Duchen, 1973; Duchen & Tonge, 1973). In the central nervous system, the direct effect of the toxin appears to be generally excitatory, through a presynaptic block of inhibitory transmission (see Mellanby & Green, 1980; Collingridge

& Davies, 1980; Collingridge, Thompson, Davies & Mellanby, 1981; Wellhöner, 1982), although it can also block synaptic excitation (Kanda & Takano, 1983). Even if the block of inhibition were sufficiently long-lasting it would be in the wrong sense to account directly for the depression of CA3 excitability reported here. Conceivably it might account for it secondarily if there were supersensitivity at recovering synapses. It is, however, perhaps more likely that the depression of CA3 responses results indirectly from the intermittent seizure activity which the toxin had produced in the hippocampus over a period of several weeks. If this were the case, the depression of the population spikes might be expected to be correlated with the number of fits that an animal had experienced, which it was not. However, visible seizures are not a wholly reliable guide to abnormal electrical activity in the brains of epileptic rats (C. A. Hawkins & J. Mellanby, unpublished observation), and it may be necessary to investigate other aspects of seizure activity than their ability to spread and produce motor fits in order to find correlations between epileptic events and physiological impairment.

It would be most interesting if hypo-excitability were a general consequence of, and perhaps an adaptive response to, repeated seizures. It was previously found with the tetanus toxin model that 18 days after toxin injection there was a considerably increased latency to convulsions in response to pentylenetetrazol (Mellanby *et al.* 1981), but by three months after injection this was no longer apparent. It may be relevant that in the hippocampal kindling model (Tuff, Racine & Adamec, 1983; Maru & Goddard, 1984) hypo-excitability of granule cells is present after a few weeks. Other experimental epilepsies should be examined in the light of these observations.

The essential findings of the present study are that the learning impairment following the tetanus toxin epileptic syndrome has been demonstrated in a further 'hippocampal' task, that electrophysiological responses from the CA3 pyramidal cells were depressed, and that the physiological and behavioural impairments were correlated.

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