Pergamon

www.elsevier.com/locate/neuroscience

PII: S0306-4522(00)00225-6

Neuroscience Vol. 99, No. 3, pp. 413–422, 2000
© 2000 IBRO. Published by Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0306-4522/00 $20.00+0.00

LAMINAR DIFFERENCES IN RECURRENT EXCITATORY TRANSMISSION IN THE RAT ENTORHINAL CORTEX *IN VITRO*

A. DHILLON*†‡; and R. S. G. JONES*†‡§

*University Department of Pharmacology, Mansfield Road, Oxford OX1 3QT, UK

†Department of Physiology, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK

Abstract—Paired intracellular recordings were used to investigate recurrent excitatory transmission in layers II, III and V of the rat entorhinal cortex *in vitro*. There was a relatively high probability of finding a recurrent connection between pairs of pyramidal neurons in both layer V (around 12%) and layer III (around 9%). In complete contrast, we have failed to find any recurrent synaptic connections between principal neurons in layer II, and this may be an important factor in the relative resistance of this layer in generating synchronized epileptiform activity. In general, recurrent excitatory postsynaptic potentials in layers III and V of the entorhinal cortex had similar properties to those recorded in other cortical areas, although the probabilities of connection are among the highest reported. Recurrent excitatory postsynaptic potentials recorded in layer V were smaller with faster rise times than those recorded in layer III. In both layers, the recurrent potentials were mediated by glutamate primarily acting at α-amino-3-hydroxy-5-methyl-4-isoxazole receptors, although there appeared to be a slow component mediated by *N*-methyl-D-aspartate receptors. In layer III, recurrent transmission failed on about 30% of presynaptic action potentials evoked at 0.2 Hz. This failure rate increased markedly with increasing (2, 3 Hz) frequency of activation. In layer V the failure rate at low frequency was less (19%), and although it increased at higher frequencies this effect was less pronounced than in layer III. Finally, in layer III, there was evidence for a relatively high probability of electrical coupling between pyramidal neurons.

We have previously suggested that layers IV/V of the entorhinal cortex readily generate synchronized epileptiform discharges, whereas layer II is relatively resistant to seizure generation. The present demonstration that recurrent excitatory connections are widespread in layer V but not layer II could support this proposal. The relatively high degree of recurrent connections and electrical coupling between layer III cells may be a factor in it's susceptibility to neurodegeneration during chronic epileptic conditions.
© 2000 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: entorhinal cortex, recurrent excitatory synapses, epilepsy.

There is increasing evidence that the entorhinal cortex (EC) may be a site of seizure generation in temporal lobe epilepsy. Temporal lobe resection to control refractory epilepsy is usually aimed at removing hippocampal tissue, but entorhinal tissue is invariably removed as well,[56] and the outcome of such surgery is positively related to the degree of removal of parahippocampal tissue.[51] In humans, seizures may occur simultaneously in EC and other limbic areas, but can also arise independently or preferentially in the EC.[55] Similar results have been reported in an animal model of limbic status epilepticus produced by electrical stimulation of the hippocampus.[5,36] In both human temporal lobe epilepsy and animal models of the condition there is a characteristic loss of cells in layer III of the EC.[14–16,32]

In vitro experiments in brain slices have demonstrated a pronounced susceptibility of the EC to acutely provoked epileptogenesis.[3,24,25,29–31,45,48,68,70] Jones and Lambert[30,31] have provided evidence that pharmacologically elicited epileptiform discharges were likely to be initiated in the deep layers (IV–VI) of the EC and to propagate from there into the superficial layers (II–III) and into the hippocampus. Differences between the discharges in the two layers lead to the suggestion that the deep layers may be 'seizure sensitive' and the superficial layers 'seizure-resistant'. We have identified a number of factors that may contribute to such a difference in seizure susceptibility (see discussion). In the present study we have examined whether differences in recurrent excitatory transmission in different layers could be a factor in predisposition to seizure generation.

Recurrent excitatory connections between excitatory neurons are probably the physiological and anatomical substrate for neuronal synchronization in cortical networks. It seems likely that such connections serve the fundamental functions of cross-correlation and integration of afferent synaptic information between subsets of cortical neurons, and the importance of recurrent excitation in cortical function has often been stressed.[1,13,18,44,52,54,69] The other side of the coin is that unrestrained recruitment of these connections between pyramidal neurons could lead to abnormal levels of synchronized activity between neurons and the generation of epileptic discharges. The role of recurrent excitation in the generation of epileptic activity has often been stressed.[6,12,34,40–42,50,57,58,65,66] In the present study, we have used dual intracellular recordings to determine the probability of occurrence of single axon excitatory connections between pairs of principal neurons in layers II, III and V of the EC. The major finding of this study was that recurrent connections occur with a high probability in layer V and (to a lesser extent) layer III, but that such connections were undetectable in layer II. Some of the results have been presented in Abstract form.[11]

‡Present address: Department of Physiology, University of Bristol.
§To whom correspondence should be addressed. Tel.: + 44-0117-928-7821; fax: + 44-0117-928-8923.
E-mail address: roland.jones@bristol.ac.uk (R. S. G. Jones).

Abbreviations: ABC, avidin-biotinylated horseradish peroxidase complex; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole; 2-AP5, D,L-2-amino-5-phosphonovalerate ; ACSF, artificial cerebrospinal fluid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EC, entorhinal cortex; rEPSP, recurrent excitatory postsynaptic potential; NBQX, 6-nitro-7-sulphamoylbenzo[f]quinoxalone-2,3-dione disodium; NMDA, *N*-methyl-D-aspartate; PB, phosphate buffer; TBS, Tris-buffered saline.

EXPERIMENTAL PROCEDURES

Slice preparation

Experiments were performed on slices containing EC and hippocampus prepared from male Wistar rats (120–150g, $n=49$). All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986, European Communities Council Directive 1986 (86/609/EEC) and the University of Bristol ethical review document. All efforts were made to minimize the number of animals utilized in these experiments and to eliminate any suffering. Animals were decapitated under anaesthesia induced with ketamine (120 mg/kg) and xylazine (8 mg/kg) administered by intra-muscular injection. The brain was rapidly removed and submerged in chilled (4–5 ºC) artificial cerebrospinal fluid (ACSF) during dissection. Slices (450 μm) were cut using a Campden Vibroslice. They were transferred directly to the recording chamber where they were maintained at the interface between a continuous stream (1.2 ml/min) of ACSF and warm, moist carbogen gas (95% O_2: 5% CO_2). The ACSF had the following composition (in mM): NaCl (126); KCl (3.0) $CaCl_2$ (2.0); $MgSO_4$ (2.0); NaH_2PO_4 (1.25); $NaHCO_3$ (24.0); D-glucose (10) and had a pH of 7.4. The temperature of the recording chamber was maintained at 33 ± 0.3 ºC. Slices were allowed to equilibrate for at least 1-h before recording began.

Intracellular recording

Somatic intracellular recordings were made from pairs of neurons in layer II, layer III or layer V of the medial division of the EC using an Axoprobe-1A amplifier (Axon Instruments) in conventional bridge mode. Electrodes were filled with K-acetate (3M), and had resistances of 70–120 MΩ.

For recording, separate electrodes were connected to the two channels of the amplifier and advanced into the slices using separate manipulators. A major difficulty of using two intracellular microelectrodes in close proximity is the capacitative coupling that occurs between them. Separating the tips and shanks of the electrodes as far as possible could considerably reduce the size of this artefact. This was achieved by inserting both electrodes at angles of 30º from vertical. The remaining coupling artefact was greatly reduced by means of the cross capacitance compensation facility on the Axoprobe 1A amplifier. This circuitry operates by injecting an adjustable, compensating current into the driven shield of one channel that is derived from the voltage in the other channel. The circuit was modified according to a design of Dr A. Mason (unpublished data) to allow it to operate on either channel, at an appropriate gain, permitting precise control over artefact cancellation.

Electrodes were placed onto the surface of the slice within 50–300 μm of each other. One electrode was advanced until a stable impalement was obtained. This neuron was required to have a stable resting potential more negative than −65 mV (without holding current) and to show overshooting (by >15 mV) action potentials in response to depolarizing current pulses. When an impalement meeting these criteria was obtained, the other microelectrode was advanced into the slice until a second impalement was obtained. The criterion initially imposed upon this impalement was that action potentials of 50–60 mV could be evoked reliably by depolarizing current pulses before testing a neuron as being presynaptic to the original impalement. In practice, the vast majority of recordings were made from two “healthy” neurons meeting the criteria of $E_m > -65$ mV and action potentials overshooting zero by > 15 mV. It should be stressed that all the postsynaptic potentials presented in this paper were examined in healthy neurons.

When a pair of recordings was achieved, continuous depolarizing current was applied to one neuron of the pair to evoke action potentials. On-line averaging was often required to determine if a single-axon postsynaptic potential was present in the second (non-firing) neuron. Action potentials in the (potential) presynaptic cell were used to trigger an oscilloscope (Gould DRO1604), and initially, 32–64 sweeps were averaged. If no recurrent excitatory postsynaptic potentials (rEPSPs) were detected in the second neuron, then the procedure was reversed and the presence of rEPSPs in the second cell as a result of action potentials evoked in the first was tested for. All data was recorded on tape using a Sony DAT recorder modified for use as a data recorder. Offline analysis was performed using the WCP programme of the Strathclyde Electrophysiological Software (Courtesy of Dr John Dempster). Action potentials in the presynaptic cell were used to trigger sweeps in the ‘event detect’ mode. For any given situation, as many sweeps as possible were accumulated into a single data file. This varied greatly from neuron to neuron and with the different situations, but generally we considered about 200 sweeps was sufficient to obtain a decent average. Data files were then visually inspected trace by trace and manually edited to reject sweeps where transmission failed, where it was not possible to determine whether a postsynaptic response occurred or not, or whether the potential was clearly contaminated by baseline fluctuation or spontaneous synaptic potentials. The remaining sweeps in a file were then used to produce an averaged rEPSP. The analysis function of the WCP programme was used to calculate rise time (10–90%), peak amplitude and time to 50% decay ($T_{50\%}$) of the averaged rEPSPs. Where mean values are given in the text for rEPSP characteristics these represent the mean of the average values from individual neurons. Errors represent S.E.Ms. Statistical comparisons were made with Students *t*-test.

Biocytin fills

In experiments, where we attempted to study the morphology of connected neurons the electrode tips were backfilled with a 2% solution of biocytin dissolved in 1.5 M $KMeSO_4$. Biocytin was injected using negative current pulses (500 ms, 0.5–0.8 nA, 1 Hz) for 5–15 min. Both electrodes were withdrawn and the slice immediately removed from the baths sandwiched between 2 Millipore filters and immersed overnight in 0.1M phosphate buffer (PB 0.1M containing 3% paraformaldehyde, 0.1% glutaraldehyde). The following day the slices were embedded in gelatine and immersed in 0.1 M PB for 1–2 h before being resectioned at 60–80 μM. They were washed in PB, rinsed in tris (hydroxymethyl) amino methane (0.05M; pH 7.4) Tris-buffered saline (TBS) and incubated for 30 min in 0.3% Triton X-100. After further rinsing in PB and TBS the sections were incubated overnight in 1% avidin–biotinylated horseradish peroxidase complex (ABC; Vector Laboratories). Unbound ABC was removed by washing in TBS, and the sections were reacted in TBS with 3,3-diaminobenzidine tetra hydrochloride (0.05%). After rinsing they were mounted on gelatinized slides, dried overnight and subsequently dehydrated in ethanol. They were rinsed in xylene and infiltrated with DPX mountant for light microscopy examination. Labelled cells were located and reconstructed from the serial sections using a drawing tube.

Materials

Salts used in the preparation of ACSF were obtained from BDH and were Analar grade. All drugs were applied by bath perfusion at concentrations stated in the text. The drugs used were D,L-2-amino-5-phosphonovalerate (2-AP5, Tocris); 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX, Tocris); 6-nitro-7-sulphamoylbenzo[f]quinoxalone-2,3-dione disodium (NBQX, Tocris).

RESULTS

Probability of connection

The cells recorded in layer V had the electrophysiological characteristics of the regular-spiking neurons described by Jones and Heinemann.[29] No morphological examination of recorded cells was made in the present studies but previous Lucifer Yellow fills (Jones R.S.G., unpublished data) have shown that these neurons are medium-sized pyramidal cells. Recordings were made from 89 pairs of neurons. In four of these, loss of one impalement meant that connections were only tested for in one direction, making a total of 174 possible connections. rEPSPs were detected in 20 of these, giving a high connection probability of 0.115. In each case, the connection was one way, no reciprocally connected pairs of cells having been found as yet.

The cells in which we recorded rEPSPs in layer III had regular firing characteristics similar to those recorded in layer V. Three pairs of cells which were synaptically connected were successfully filled with biocytin, and subsequently recovered by histological analysis. Both pre and

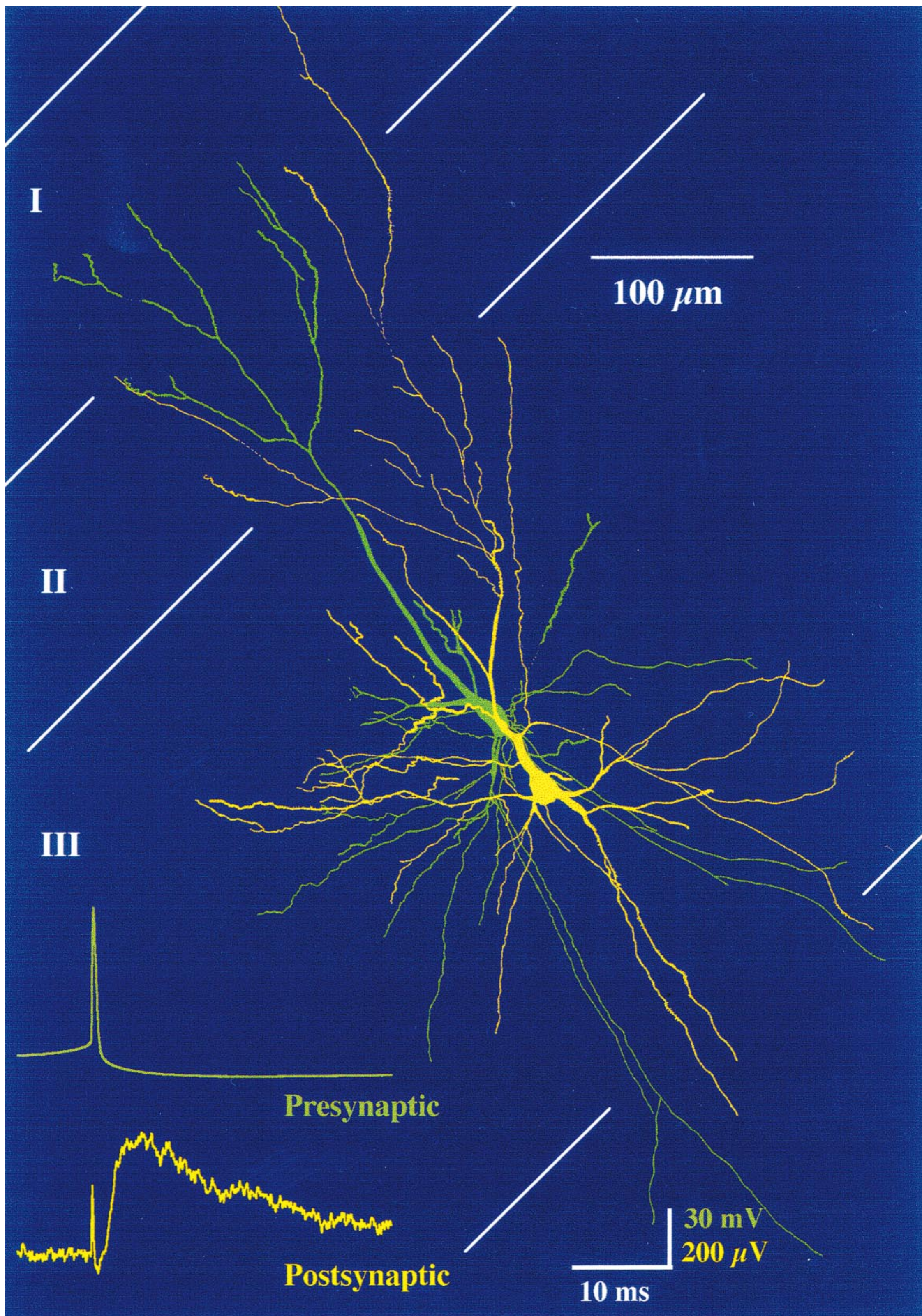

Fig. 1. Camera lucida reconstructions of synaptically connected neurons in layer III. Both neurons had a pyramidal morphology. The green neuron was presynaptic to the yellow. The traces in the lower left corner show a single action potential in the presynaptic neuron and the averaged EPSP elicited by 132 action potentials in the postsynaptic cell.

postsynaptic cells in each pair had a pyramidal morphology. One of these pairs is illustrated in Fig. 1 which shows camera lucida reconstructions of the two neurons and their dendritic arbors. The inset shows an action potential in the presynaptic neuron and the averaged rEPSP recorded in the cell postsynaptic to it. The pyramidal nature of both neurons is clear. The axons of both could only be followed for a short distance from the soma towards the white matter, through

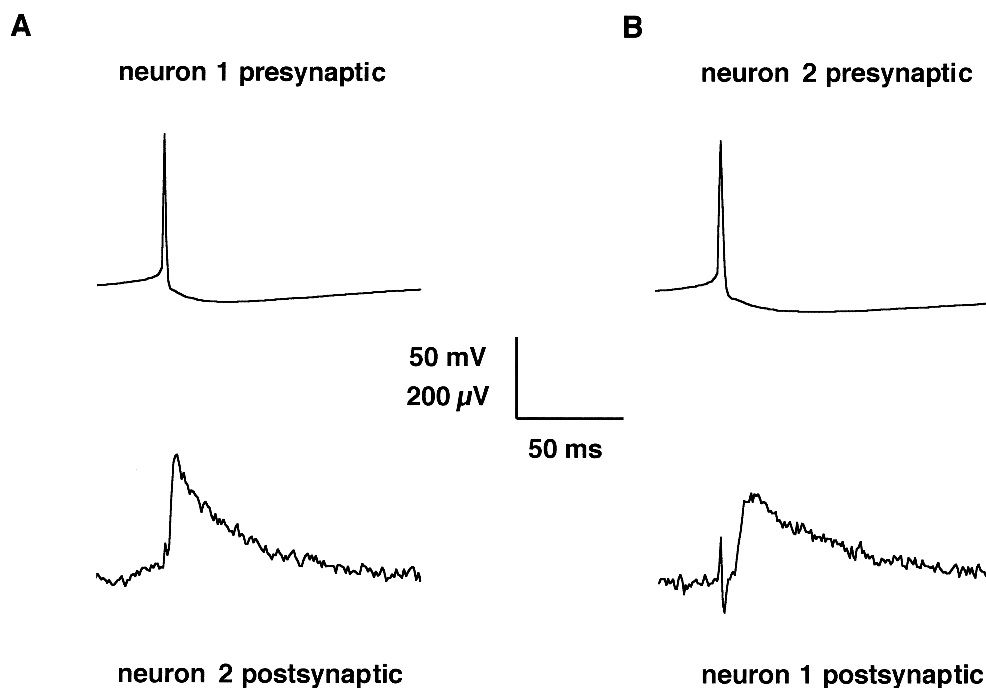

Fig. 2. Recurrent EPSPs recorded in a pair of layer III neurons. (A) Spikes in neuron 1 elicited a clear EPSP (average of 220 sweeps) in neuron 2. (B) The connection was reciprocal since spikes in neuron 2 also elicited an EPSP (average of 261 sweeps) in neuron 1.

layer IV/V. Unfortunately this was the case in all three pairs of filled neurons, so we have no information as to likely sites of synaptic contact of recurrent collaterals. Glovelli *et al* have described four morphologically and physiologically distinct groups of pyramidal neurons in layer III.[20] It would be interesting to determine the relative preponderance of recurrent connections between these different groups, or if connections between cells of different groups had different characteristics, but at present we do not have enough morphological or electrophysiological data to confidently assign our connected pairs of neurons to specific subgroups.

The probability of connection between layer III cells was somewhat less than in layer V, although still quite high at 0.084. A total of 393 possible connections have been tested for in 202 pairs in layer III, with the discrepancy of 11 possible connections not tested because one neuron of the pair was lost before reciprocal connectivity could be examined. In 33 of these possible connections rEPSPs were clearly present. Two of these connections arose in a single pair of reciprocally connected neurons. This is illustrated in Fig. 2. Initiation of action potential firing in either neuron elicited a clear rEPSP in the other.

The neurons we have recorded from in layer II were electrophysiologically characterized as type I or type II, as previously described.[27] To date, we have tested for a total of 268 single axon connections in 135 cell pairs in layer II. Surprisingly, we have found no evidence for the existence of rEPSPs at resting membrane potential in any of these, nor did recording the postsynaptic neuron at depolarized or hyperpolarized potentials reveal any synaptic responses. We tried altering the orientation of cutting the slices but this made no difference. Thus, we have to conclude, on the basis of our studies to date, that the probability of recurrent excitatory connections in layer II in our slices remains at zero, and that recurrent excitatory connections between stellate cells in layer II are sparse or non-existent.

Characteristics of recurrent excitatory postsynaptic potentials

Layer V. rEPSPs in layer V neurons were generally small with fast rise times. The mean (±S.E.M.) peak amplitude was 149 ± 14 μV with a range of 71–201 μV when recorded at a mean resting potential of −73.4 ± 3.5 mV. They had a mean 10–90% rise time of 1.2 ± 0.2 ms, which was significantly ($P < 0.05$) faster than their counterparts in layer III (see below). The mean $T_{50\%}$ was 41.3 ± 15.4 ms. Examination of individual sweeps also revealed transmission failures (see below).

Layer III. rEPSPs in layer III neurons were significantly ($P < 0.05$) larger and had slower rise times than those in layer V. The mean peak amplitude was 341 ± 47 μV at a mean resting potential of −74 ± 5 mV. The largest rEPSP was 637 μV and the smallest 105 μV. 10–90% rise times varied between 2.1 ms and 7.6 ms with a mean of 4.1 ± 0.4 ms. $T_{50\%}$ varied from 15.0 to 94.7 ms with a mean of 42.3 ± 7.8 ms. In some neurons in which the largest amplitude rEPSPs were recorded, stepwise fluctuations in amplitudes of rEPSPs could be detected. Figure 3B shows our best example of this. We have not analysed such results further, given the rarity with which we could record large EPSPs for the prolonged periods of time required for such analysis.

Voltage dependence of recurrent excitatory postsynaptic potentials

The effect of changing the postsynaptic membrane potential on rEPSPs was determined in 11 pairs of neurons in layer III. rEPSPs evoked at resting membrane potential had a mean amplitude of 343.5 ± 50.7 μV, rise time of 4.6 ± 0.4 and $T_{50\%}$ of 38.2 ± 8.2 ms. Steady sub-threshold depolarization (+7–8 mV) of the postsynaptic neuron ($n = 10$), did not significantly alter these parameters (289 ± 53.2 μV, 5.1 ± 0.8 ms and 55.2 ± 13.9 ms) although there was a clear trend towards

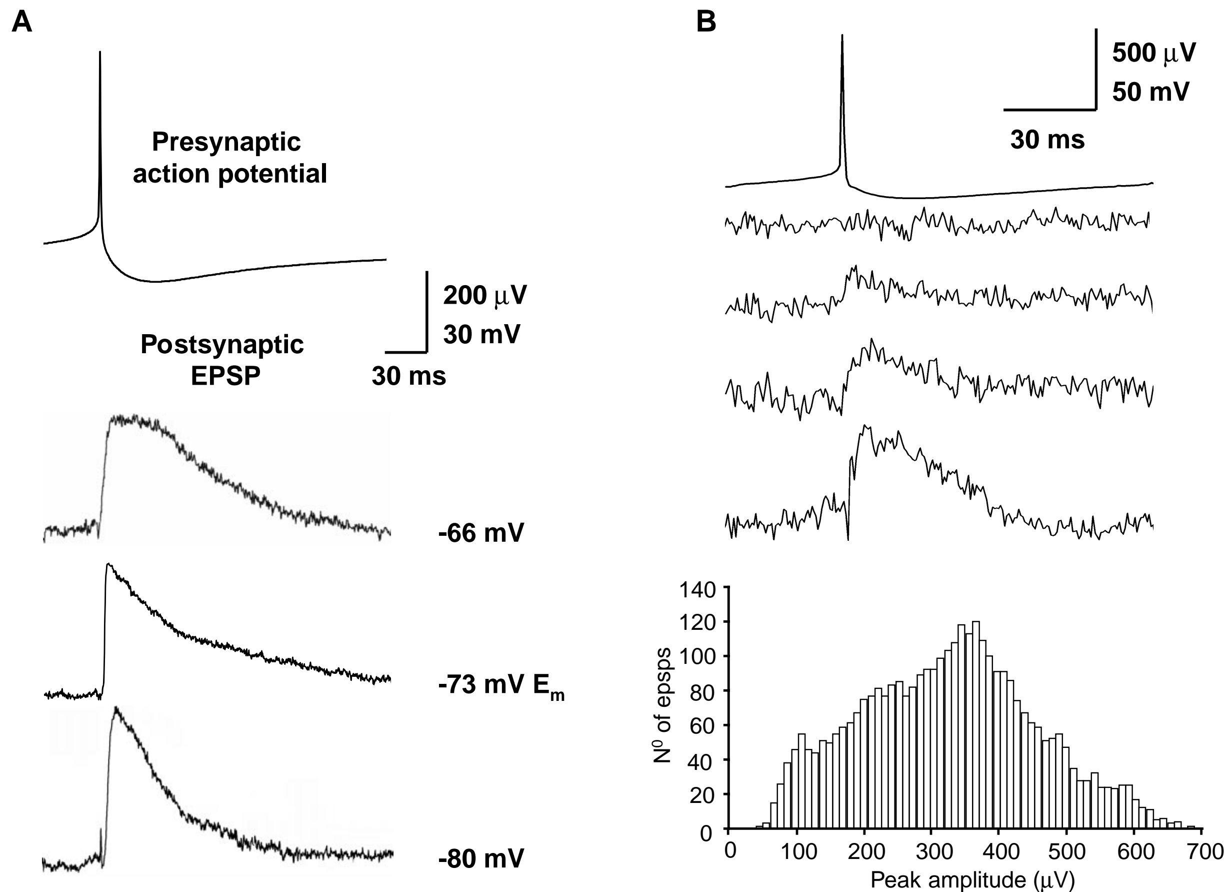

Fig. 3. (A) Recurrent EPSPs elicited in a layer III cell at different membrane potentials. At resting potential, the fast EPSP (average of 530 sweeps) gave way to a slower phase of depolarization. With the cell membrane hyperpolarized, the fast phase was increased in amplitude and the slow phase was less pronounced (average of 420 sweeps). The reverse was seen when the membrane was steadily depolarized (average of 390 sweeps). (B) Fluctuations in EPSP amplitude in a pair of layer III neurons. The traces show individual sweeps. Spikes in the presynaptic neuron evoked EPSPs of different amplitudes in the postsynaptic neuron, and failures of transmission could also occur (upper trace). The histogram of EPSP amplitudes (3434 responses, failures excluded) may show some evidence of separate peaks at around 100-μV intervals.

a decrease in amplitude (−16%) and an increase in decay time (+45%). The large variations in these parameters meant that they failed to reach significance.

With steady hyperpolarization (8–10 mV, $n = 11$) there was an increase in mean amplitude (396 ± 34.2 μV, +15%), with little change in rise time (4.3 ± 0.3 ms, −7%). Again, neither change was significant. However, the shortening (−46%) of the $T_{50\%}$ (17.8 ± 2.1ms) was significant ($P < 0.05$). One example of the voltage dependence of rEPSPs is shown in Fig. 3A. In this case, there were fast and slow phases in the decay of the rEPSP at resting potential. With hyperpolarization the amplitude of the fast phase of the EPSP increased and the slower phase was less evident. Depolarization elicited the reverse, the fast phase declining in amplitude, with a concurrent increased contribution of the slow phase of the EPSP.

Frequency-dependent changes in recurrent excitatory postsynaptic potentials

The effect of varying the presynaptic firing rate on the postsynaptic rEPSPs was determined in seven cells in layer III. In these experiments, single action potentials were evoked in the presynaptic neuron by injecting short (3–4 ms) positive current pulses at the desired frequency. The mean amplitude, rise times and decay times of rEPSPs evoked at 0.2 Hz were not significantly different to those evoked at either 2 Hz or 3Hz (Table 1). However, although there was no clear change in shape or amplitude of the averaged rEPSPs recorded at different frequencies, there was a change in the rate of success of transmission at the recurrent synapses. Thus, when the presynaptic firing rate was 0.2 Hz, transmission failures were recorded to 28% of spikes in the presynaptic neuron. This failure rate increased to 36% when the presynaptic firing rate was 2 Hz, and to 48%, at 3Hz.

Frequency dependent changes in rEPSPs have also been examined in five layer-V neurons. Again, there was little difference in amplitude, rise or decay times between 0.2, 2 and 3 Hz (Table 1), but this time there was also less change in failure rates. At 0.2 Hz, transmission failures were noted with only 19% of action potentials and this was only increased to 26% at 2 Hz, and 29% at 3 Hz.

Pharmacology of recurrent excitatory postsynaptic potentials

N-methyl-D-aspartate receptor blockade. The effects of the NMDA receptor antagonist, 2-AP5 (50 μM) was tested on rEPSPs in five layer-III neurons. The antagonist had little effect on amplitude or rise time of rEPSPs but significantly ($P < 0.05$) reduced the decay phase of the responses (Table

Table 1. Frequency dependent alterations in recurrent excitatory postsynaptic potentials

Hz	Amplitude (μV)	Rise time (ms)	Decay time (ms)	Failures (%)
Layer III				
0.2	342 ± 73	4.7 ± 0.8	39.7 ± 5.2	28
2	293 ± 60	4.1 ± 0.7	44.5 ± 11.0	36
3	377 ± 57	5.0 ± 0.8	34.3 ± 3.9	48
Layer V				
0.2	121 ± 23	1.4 ± 0.4	41.2 ± 7.7	19
2	131 ± 19	1.6 ± 0.3	37.6 ± 6.4	26
3	127 ± 31	1.4 ± 0.7	35.7 ± 5.6	29

2). An example of the effect of 2-AP5 is shown in Fig. 4B. After 20 min perfusion there was little detectable change in rEPSP amplitude, but there was a noticeable change in shape, with a distinct reduction in the falling phase.

We have also tested the effects of 2-AP5 on rEPSPs in three layer-V neurons. Again, there appeared to be a reduction in the $T_{50\%}$ (from 34.7 ± 12.8 ms to 27.8 ± 13.1 ms) with little consistent alteration in amplitude or rise time, although the data were too variable to draw any firm conclusions.

AMPA/kainate receptor blockade. The effects of the AMPA/kainate receptor blockers, NBQX or CNQX (5 μM) were tested on rEPSPs in five neurons in layer III and in two neurons in layer V. In all seven studies the rEPSPs were virtually abolished within 15–20 min of perfusion with the antagonist. One example, in a layer-V neuron, is shown in Fig. 4A. In three of the studies in layer III the AMPA antagonist was tested after perfusion with 2-AP5. The neuron in Fig. 4B is one example of this. 2-AP5 reduced the decay phase of the EPSP, and the remaining EPSP was abolished by NBQX.

Electrical coupling. Four pairs of cells in layer III, which were probably not synaptically connected, showed clear evidence of electrical coupling. Thus, either depolarizing or hyperpolarizing current-pulses injected into one neuron changed the membrane potential in both, and action potentials generated in one cell were almost always accompanied by action potentials in the other (Fig. 5A). However, by injecting steady depolarizing current into one neuron and hyperpolarizing current into the other, it was possible to generate action potentials in one neuron only. In this situation, the action potentials were coincident with coupled depolarizing events (5–15 mV) which did not reach threshold for spike generation in the other cell (Fig. 5B). However, the potential was only just sub-threshold for firing in the coupled cell (Fig. 5B), and when it did fire it prolonged the decay phase of the action potential in the other cell. The delay between the spike and the coupled events varied from about 50 μs up to around 500 μs in the four pairs recorded. The impalements illustrated in Fig. 5 were estimated to be around 200 μm apart. Assuming that both impalements were somatic, then this would indicate that the site of electrical coupling was likely to be dendro–dendritic or somato–dendritic. If the two-way electrical coupling in layer III is included with the synaptic connections ($n = 33$) noted above, this would raise the probability of finding a connection of either electrical or synaptic between layer III neurons to 0.104.

Table 2. Effect of 2-AP5 on recurrent excitatory postsynaptic potentials in layer III

	Amplitude (μV)	Rise time (ms)	Decay time (ms)
Control	299 ± 48.5	4.1 ± 0.6	37.6 ± 3.5
2-AP5	323 ± 73.4	4.2 ± 1.6	26.8 ± 4.5*

DISCUSSION

In comparison to most studies of recurrent excitation, the probability of detecting such connections was relatively high in both layer V (0.115) and layer III (0.084) of the EC. In the cingulate and sensorimotor cortex, probabilities of 0.04[60] and 0.01[63] have been reported in layers II/III and V/VI, respectively. In visual cortex, connection probability in layer V was 0.015,[43] but in layer II/III was comparable with the values we obtained at 0.087.[39] In the hippocampus, Miles and Wong[42] found a connection probability of 0.02 in CA3, whereas Deuchars and Thomson[10] reported a value of only 0.005 for CA1 pyramid–pyramid connections. A much higher probability of connections was found between CA3 and CA1 (0.063; Ref. 49). Very much higher probabilities were found in hippocampal slice cultures (0.56 in CA3; 0.76 in CA3–CA1; 0.8 in CA1–CA3; Ref. 9), but how these relate to "normal" slices is a matter for debate. It is clear that the values we have obtained for layer V and layer III are among the highest so far reported, indicating that these layers exhibit a high degree of recurrent excitatory connections.

These probability ratios should underestimate the degree of recurrent excitatory connections since many would be cut in a 400-μM-thick slice. Markram *et al.* [37,38] have recently made visually guided, whole-cell patch clamp recordings, specifically from pairs of thick-tufted layer V pyramidal cells in somatosensory cortical slices. This approach provided greater electrical resolution of small single axon responses, and allowed selection of neurons in close apposition to each other (<50 μM apart), whose axon pathways could be followed for some distance from the soma with a defined anatomical orientation to each other. These studies yielded a connection probability of 0.1, which is virtually the same as that we obtained in layer IV/V of the EC. However, previous studies in layer V of the neocortex have yielded connection probabilities of only 0.015[43] and 0.01,[63] and Markram *et al.*[37] have suggested that the higher probability in their studies could result from selection of a uniform population of neurons with defined anatomical relationships. They have also indicated, for various reasons, that the actual probability of

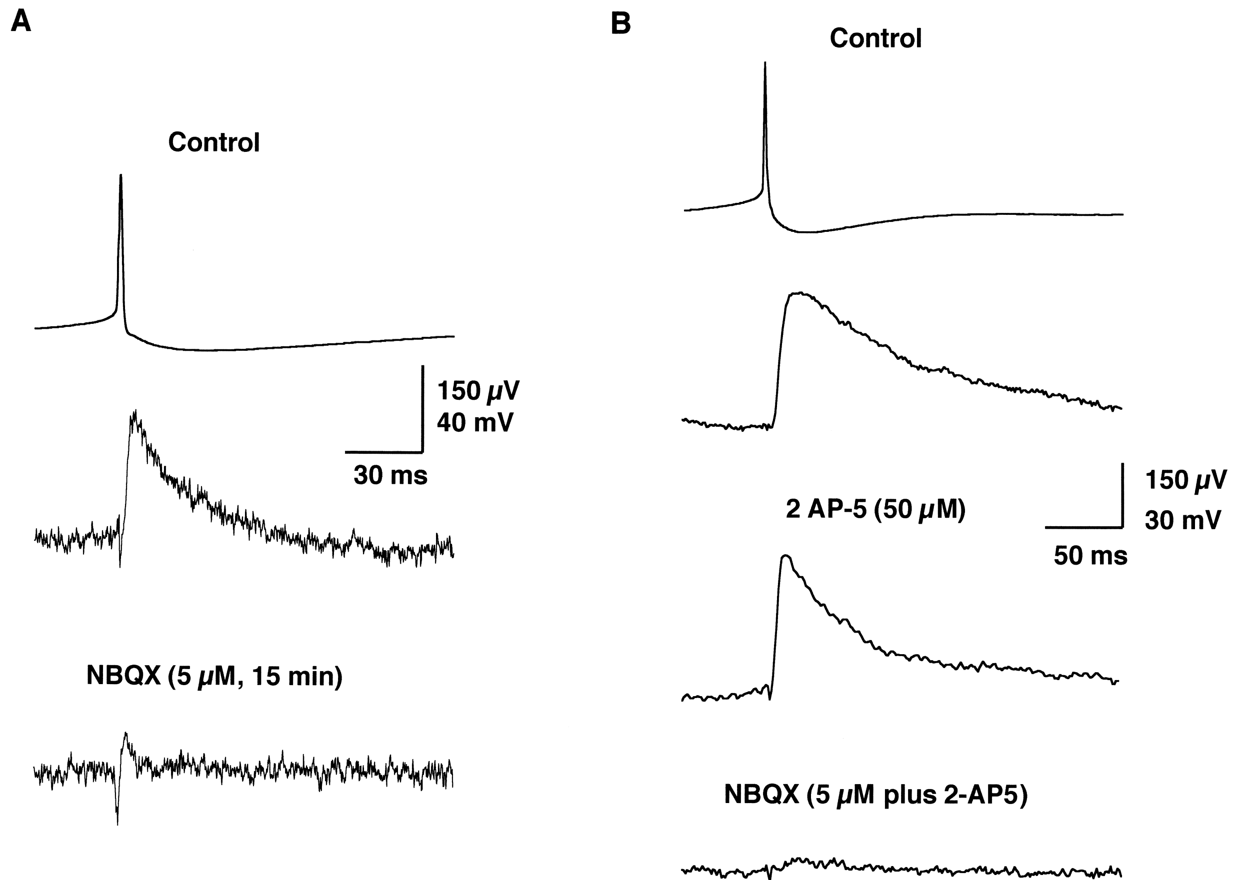

Fig. 4. (A) Blockade of a rEPSP (250 sweeps) in a layer-V neuron during perfusion (243 sweeps) with the AMPA-receptor antagonist, NBQX. (B) The control response (277 sweeps) in this layer-III neuron was relatively prolonged. Perfusion with the NMDA-receptor antagonist reduced the decay phase with little alteration in amplitude (177 sweeps). Addition of NBQX (103 sweeps) all but abolished the remaining EPSP.

connection may well be substantially higher than the one in 10 that they found. If we assume that the same reasoning applies to our situation, then it is possible that the probability of finding recurrent connections in the EC would be much higher if we were to study a visually identified, homogeneous population of neurons.

Perhaps the most startling observation in the present study was the failure to detect any recurrent excitatory connections between neurons in layer II. It seems unlikely that this was a result of the experimental approach since such connections were readily detectable in other layers. Layer-II principal neurons are a heterogeneous population,[2,27,33,67] and it is conceivable that recurrent connections only exist between certain classes of neurons. However, our sample size should have been large enough for us to detect at least some degree of connectivity, and we have to conclude that recurrent excitation is very low or non-existent. Morphological studies have shown that the axons of many layer-II cells arborize extensively within the layer[27,33,35,59] so the lack of recurrent excitatory connections seems surprising. Evoked synaptic responses in layer II are dominated by GABAergic inhibition[19,27] and it may be that intralaminar recurrent axon collaterals in layer II make exclusive contact with inhibitory interneurons, rather than acting as a substrate for integration of excitatory information between principal neurons.

rEPSPs recorded in layer III and layer V displayed both differences and similarities. In both layers, evoked excitatory responses exhibit a fast component mediated by AMPA/kainate receptors and a slow component mediated by NMDA receptors.[20,23,29,47] rEPSPs in both layers appeared to have a similar dual receptor mediation. They were virtually abolished by CNQX or NBQX, demonstrating that they were primarily mediated by AMPA/kainate receptors. However, there was also some contribution from NMDA receptors since 2-AP5 reduced the duration of rEPSPs. This dual receptor mediation of rEPSPs is similar to that reported in hippocampus[9,10] and neocortex.[37,61] The contribution of NMDA receptors during the latter part of the rEPSP was also suggested by a shortening and lengthening at hyperpolarized and depolarized membrane potentials, respectively. However, it has been suggested that such alterations may reflect a role of voltage activated conductances (e.g. persistent Na^{+} or Ca^{2+} currents) in shaping rEPSPs[7,42,61] and this cannot be ruled out.

Generally, the mean amplitudes of rEPSPs in both layer V and layer III were small compared to those reported for other cortical areas.[37,39,42,43,40,63] However, those in layer V were particularly small, although similar to those seen at Schaffer collateral synapses on to CA1 pyramidal cells.[49] rEPSPs in layer III were clearly larger than in layer V, but on average smaller than those recorded at other recurrent synapses. The largest rEPSP we have recorded was around 700 μV, whereas others have reported rEPSPs up to 4 or 5 mV.[37,39,42,43,40,63] Resting membrane potentials in our neurons were about the same as in other studies, so this is unlikely to be a factor. Other than the study of Markram *et al*[37] the technical

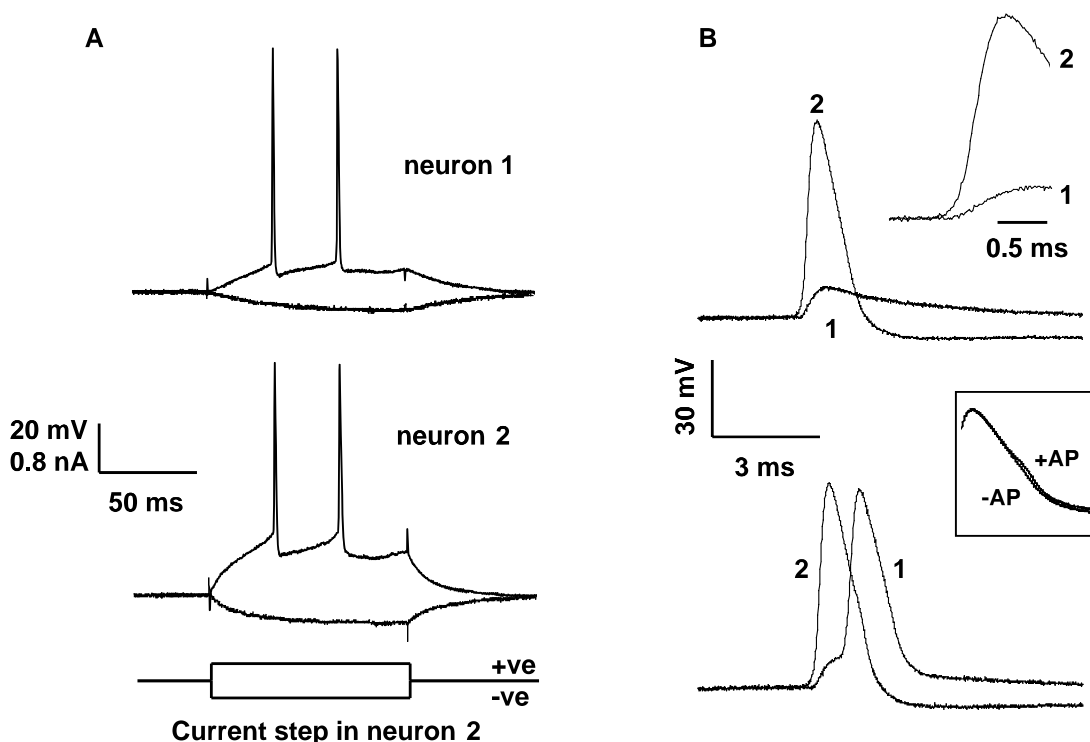

Fig. 5. Electrical coupling between a pair of layer III-cells. (A) Injection of a 100-ms positive current-pulse in neuron 2 elicited a membrane depolarization and the generation of two action potentials. This was mirrored by a similar response in neuron 1. Likewise, negative current injected into neuron 2 elicited a hyperpolarizing response in both neurons. (B) In the upper panel, neuron 2 was driven to fire action potentials by injection of continuous depolarizing current, whilst neuron 1 was just prevented from firing by a continuous hyperpolarization (by 2–3 mV). This revealed a coupled sub-threshold depolarizing potential in neuron 1 that arose at a very short latency and decayed slowly compared with the action potential in neuron 2. The inset shows the temporal relationships between events in the two cells on a faster time-base. The lower panel shows that the coupled in event in neuron 1 could readily give rise to an action potential. The boxed inset shows the falling phase of the action potentials in neuron 2 from the upper and lower panels superimposed. When an action potential is generated on the coupled potential in neuron 1 (+AP) this induces a reciprocal coupled potential in neuron 2 which is seen as a deflection on, and prolongation of, the falling phase of the action potential.

approaches were virtually identical so we assume that the small rEPSPs in EC reflect a true difference between EC and neocortex.

At present, we can only speculate on what may underlie differences in rEPSP amplitude. One possibility would be that recurrent synapses on layer-V neurons are located at more distal dendritic locations, and suffer greater electrotonic attenuation than those in layer III, which could be located closer to the cell soma. We have estimated (see Refs 22 and 53) electrotonic length (L_M) of neurons during whole cell voltage clamp recordings (Ref. 4 and Jones, unpublished). L_M in layer V and III neurons were similar (1.57 ± 0.15 vs 1.63 ± 0.18) whereas layer II neurons were less electrotonically compact (2.46 ± 0.29). If recurrent synapses on layer III neurons were closer to the soma, it might be expected that rEPSPs would have a faster rise time in layer III than in layer V. The reverse was true, so dendritic location may not play a major role in the difference in amplitude. Other factors which could account for the difference could be a lower quantal content in layer V, a smaller number of release sites, a decreased probability of release, or even differences in post-synaptic receptor activation.

Failure rates at layer-V recurrent synapses also differed from layer III. At 0.2 Hz, transmission failures were noted on around 29% of trials in layer III compared with only 19% in layer V. This may be surprising, since, at other cortical synapses,[37,62] failure rate was inversely proportional to rEPSP amplitude. With the small amplitude EPSPs seen in layer V it might be expected that a higher failure rate might have been encountered there. Although the neurons selected for this analysis were those with relatively large rEPSPs, this applied to both layers, so it does not explain the discrepancy. What was clear was that failure rate increased with increasing frequency of presynaptic firing. Again, there was a difference in that layer III rEPSPs failed on around 50% of trials at 3 Hz, whereas those in layer V were more resilient and only failed on around 30%. Thomson's group have reported increasing failure rates related to increasing frequency in CA1[64] and somatosensory cortex.[62] This was accompanied by a decrease in amplitude of the rEPSPs, but it is not clear whether they excluded failures from the averaged potentials at the different frequencies. We did, and found that there was little alteration in amplitude, or rise and decay kinetics at higher frequencies. The most likely explanation for the increase in failures is a decline in release probability.[62,64] Assuming this to be the case in the EC, it would seem that recurrent excitatory transmission in layer V has a greater safety factor when presynaptic frequency starts to increase.

Evoked excitatory responses in all layers of the EC exhibit a frequency-dependent facilitation at 1–3 Hz.[8,21,26,28] We considered the possibility that this facilitation may be due, in part, to progressive recruitment of recurrent excitation with increasing frequency of activation of afferent pathways. However, the increasing failure of rEPSPs at higher frequencies, combined with the demonstrable lack of recurrent excitation in layer II would make this unlikely.

Do the present results have implications for the generation of epileptiform activity in the EC? We have previously suggested that layer V may be a relatively seizure-susceptible site, in contrast to layer II which is relatively seizure-resistant.[26,30] A number of factors could contribute to this. Evoked synaptic inhibition is much less pronounced in the deep than the superficial layers.[27,29] Conversely, frequency-dependent facilitation of evoked excitation is more powerful in layer V.[26] Whole-cell voltage-clamp studies of glutamate release have shown that spontaneous excitatory postsynaptic currents are larger in layer V, have a greater contribution from NMDA-receptors than in layer II, and that there is a higher frequency of pure NMDA-receptor mediated events in the deep neurons.[4] In contrast, the frequency of spontaneous GABA-mediated inhibitory postsynaptic currents in layer-II neurons is double that recorded in layer V.[71] Presynaptic group 4 metabotropic receptors inhibit glutamate release in layer II, but facilitate it in layer V.[17] The laminar specific differences in recurrent excitatory connections are a further factor that could contribute to layer V being a primary site where temporal lobe seizures may originate and reverberate.

Finally, in both chronic human, and animal models of temporal lobe epilepsy, layer III suffers marked neurodegeneration.[14–16,32] Whilst the reasons for this are still unclear, we could speculate that the high degree of recurrent excitation also found in this layer could combine with other factors to contribute to hyperexcitability and excitotoxicity leading to cell death during prolonged seizure activity. It is very interesting that we found evidence for a relatively high degree of electrical coupling between cells in layer III. It has been suggested that electrical junctions could be strongly involved in high frequency oscillations and synchronized epileptiform activity in cortical networks.[46] This, together with strong recurrent excitation could rapidly distribute synchronized excitation amongst the neuronal population of layer III and contribute to excitotoxicity during prolonged seizure activity.

CONCLUSION

Recurrent excitation occurs with a high degree of probability between pyramidal neurons in layer V and in layer III of the EC. This could contribute to the susceptibility of these layers to participation in epileptic discharges, and in the case of layer III to a propensity to degenerate during chronic epilepsy. In contrast, recurrent excitatory connections between principal neurons in layer II appear to be very sparse or absent, and this could be a factor in the resistance of this area to seizure generation.

Acknowledgements—We thank the Wellcome Trust, the MRC and the Taberner Trust for financial support and Dr John Dempster for the Strathclyde Software.

REFERENCES

1. Adorjan P., Levitt J. B., Lund J. S. and Obermayer K. (1999) A model for the intracortical origin of orientation preference and tuning in macaque striate cortex. *Visual Neurosci.* **16,** 303–318.
2. Alonso A. and Klink R. (1993) Differential electroresponsiveness of stellate and pyramidal-like cells of medial entorhinal cortex layer II. *J. Neurophysiol.* **70,** 128–143.
3. Bear J. and Lothman E. W. (1993) An *in vitro* study of focal epileptogenesis in combined hippocampal–parahippocampal slices. *Epilepsy Res.* **14,** 183–193.
4. Berretta N. and Jones R. S. G. (1996) A comparison of spontaneous synaptic EPSCs in layer V and layer II neurones in the rat entorhinal cortex *in vitro*. *J. Neurophysiol.* **76,** 1089–1100.
5. Bertram E. H., Lothman E. W. and Lenn N. J. (1990) The hippocampus in experimental chronic epilepsy: a morphometric study. *Ann. Neurol.* **27,** 43–48.
6. Chagnac-Amitai Y. and Connors B. W. (1989) Synchronized excitation and inhibition driven by intrinsically bursting neurones in neocortex. *J. Neurophysiol.* **62,** 1149–1162.
7. Clements J. D., Nelson P. G. and Redman S. J. (1986) Intracellular tetraethylammonium ions enhance group Ia excitatory post-synaptic potentials evoked in cat motoneurones. *J. Physiol.* **377,** 267–282.
8. Cunningham M. O., Dhillon A, Wood S. J. and Jones R. S. G. (1999) Reciprocal modulation of glutamate and GABA release may underlie the anticonvulsant effect of phenytoin. *Neuroscience* **95,** 343–351.
9. Debanne D., Guerineau N. C., Gahwiler B. H. and Thomson S. M. (1995) Physiology and pharmacology of unitary synaptic connections between pairs of cells in areas CA3 and CAl of rat hippocampal slice cultures. *J. Neurophysiol.* **73,** 1282–1294.
10. Deuchars J. and Thomson A. M. (1996) CA1 pyramid–pyramid connections in rat hippocampus *in vitro*: Dual intracellular recordings with biocytin filling. *Neuroscience* **74,** 1009–1018.
11. Dhillon A. and Jones R. S. G. (1995) Characteristics of single axon recurrent excitatory connections between neurones in layer III of the rat entorhinal cortex *in vitro*. *J. Physiol.* **489,** 20P.
12. Dichter M. and Spencer W. A. (1969) Penicillin-induced discharges from the cat hippocampus. I. Mechanisms underlying origin and restriction. *J. Neurophysiol.* **32,** 649–662.
13. Douglas R. J., Koch C., Mahowald M., Martin K. A. C. and Suarez H. H. (1995) Recurrent excitation in neocortical circuits. *Science* **269,** 981–985.
14. Du F. and Schwarz R. (1992) Amino-oxyacetic acid causes selective neuronal loss in layer III of the rat medial entorhinal cortex. *Neurosci. Lett.* **147,** 185–188.
15. Du F., Whetsell W. O., Abu-Khalil B., Blumenkopf B., Lothman E. W. and Schwarz R. (1993) Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. *Epilepsy Res.* **16,** 223–233.
16. Du F., Eid T., Lothman E. W., Kohler C. and Schwarz R. (1995) Preferential neuronal loss in layer III of the medial entorhinal cortex in rat models of temporal lobe epilepsy. *J. Neurosci.* **15,** 6301–6313.
17. Evans D. I. P., Jones R. S. G. and Woodhall G. L. (2000) Activation of presynaptic group III metabotropic receptors enhances glutamate release in layer V of the entorhinal cortex. *J. Neurophysiol.* **83,** 2519–2525.
18. Freeman W. J. (1995) The Hebbian paradigm reintegrated-local reverberations as internal representations. *Behav. Brain Sci.* **18,** 631.
19. Funahashi M. and Stewart M. (1995) GABA receptor-mediated post-synaptic potentials in the retrohippocampal cortices: regional, laminar and cellular comparisons. *Brain Res.* **787,** 19–33.
20. Glovelli T., Schmitz D., Empson R. M., Dugladze T. and Heinemann U. (1997) Morphological and electrophysiological characterization of layer III cells of the medial entorhinal cortex of the rat. *Neuroscience* **77,** 629–648.
21. Glovelli T., Schmitz D., Empson R. M. and Heinemann U. (1997) Frequency-dependent information flow from the entorhinal cortex to the hippocampus. *J. Neurophysiol.* **78,** 3444–3449.
22. Jackson M. B. (1992) Cable analysis with the whole-cell patch clamp. Theory and experiment. *Biophys. J.* **61,** 756–766.
23. Jones R. S. G. (1987) Complex synaptic responses of entorhinal cortical cells to subicular stimulation *in vitro*: demonstration of an NMDA-receptor mediated component. *Neurosci. Lett.* **81,** 209–214.
24. Jones R. S. G. (1988) Epileptiform events induced in entorhinal cortical cells by GABA-antagonists *in vitro* are partly mediated by *N*-methyl-D-aspartate receptors. *Brain Res.* **457,** 113–121.

25. Jones R. S. G. (1989) Ictal epileptiform events induced by removal of extracellular magnesium in slices of entorhinal cortex are blocked by baclofen. *Expl Neurol.* **104,** 154–161.
26. Jones R. S. G. (1993) Entorhinal–hippocampal connections: a speculative view of their function. *Trends Neurosci.* **16,** 58–64.
27. Jones R. S. G. (1994) Synaptic and intrinsic responses of cells of origin of the perforant path in layer II of the rat entorhinal cortex *in vitro*. *Hippocampus* **4,** 335–353.
28. Jones R. S. G. (1995) Frequency-dependent alterations in synaptic transmission in entorhinal–hippocampal pathways. *Hippocampus* **5,** 125–128.
29. Jones R. S. G. and Heinemann U. (1988) Synaptic and intrinsic responses of medial entorhinal cortical cells in normal and magnesium free medium *in vitro*. *J. Neurophysiol.* **59,** 1476–1497.
30. Jones R. S. G. and Lambert J. D. C. (1990) Synchronous discharges in the rat entorhinal cortex *in vitro*: Site of initiation and the role of excitatory amino acid receptors. *Neuroscience* **34,** 657–670.
31. Jones R. S. G. and Lambert J. D. C. (1990) The role of excitatory amino acid receptors in the propagation of epileptiform discharges from the entorhinal cortex to the dentate gyrus *in vitro*. *Expl Brain Res.* **80,** 310–322.
32. Kim J. H., Guimaraes P. O., Shen M. Y., Masukawa L. M. and Spencer D. D. (1990) Hippocampal neuronal density in temporal lobe epilepsy with and without gliomas. *Acta neuropathol.* **80,** 41–45.
33. Klink R. and Alonso A. (1997) Morphological characteristics of layer II projection neurons in the rat medial entorhinal cortex. *Hippocampus* **7,** 571–583.
34. Knowles W. D., Traub R. D., Wong R. K. S. and Miles R. (1985) Properties of neuronal networks—experimentation and modelling of the epileptic hippocampal slice. *Trends Neurosci.* **8,** 73–79.
35. Lingenhoh1 K and Finch D. M. (1991) Morphological characterization of rat entorhinal neurons *in vivo*: soma-dendritic structure and axonal domains. *Expl Brain Res.* **84,** 57–74.
36. Lothman E. W., Bertram E. H., Kapur J. and Stringer J. L. (1990) Recurrent spontaneous hippocampal seizures in the rat as a chronic sequala to limbic status epilepticus. *Epilepsy Res.* **6,** 110–119.
37. Markram H., Lubke J., Frotscher M., Roth A. and Sakmann B. (1997) Physiology and anatomy of synaptic connections between thick tufted pyramidal neurones in the developing rat neocortex. *J. Physiol.* **500,** 409–444.
38. Markram H., Wang Y. and Tsodyks M. (1998) *Differential signalling via the same axon of neocortical pyramidal neurones., Proc. natn. Acad. Sci. USA* **95,** 5323–5328.
39. Mason A., Nicoll A. and Stratford K. (1991) Synaptic transmission between individual pyramidal neurons of the rat visual cortex *in vitro*. *J. Neurosci.* **11,** 72–84.
40. McBain C. and Dingledine R (1992) Dual component excitatory synaptic currents in rat hippocampal pyramidal neurones. *J. Neurophysiol.* **68,** 16–27.
41. Miles R. and Wong R. K. S. (1983) Single neurones can initiate synchronized population discharges in the hippocampus. *Nature* **306,** 371–373.
42. Miles R. and Wong R. K. S. (1986) Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. *J. Physiol.* **373,** 397–418.
43. Nicoll A. and Blakemore C. (1993) Single-fibre EPSPs in layer 5 of rat visual cortex *in vitro*. *NeuroReport* **4,** 167–170.
44. Pakdaman K., Alvarez F., DiezMartinez O. and Vibert J. F. (1997) Single neurone model with recurrent excitation: Response to slow periodic modulation. *Biosystems* **40,** 133–140.
45. Pare D., De Curtis M. and Llinas R. (1992) Role of the hippocampus-entorhinal loop in temporal lobe epilepsy: extra-and intracellular study in the isolated guinea pig brain *in vitro*. *J. Neurosci.* **12,** 1867–1888.
46. PerezVelazquez J. L. and Carlen P. L. (2000) Gap junctions, synchrony and seizures. *Trends Neurosci.* **23,** 68–74.
47. Pralong E. and Jones R. S. G. (1993) Interactions of dopamine with glutamate and GABA mediated synaptic transmission in the rat entorhinal cortex *in vitro*. *Eur. J. Neurosci.* **5,** 760–767.
48. Rafiq A., Delorenzo R. J. and Coulter D. A. (1993) Generation and propagation of epileptiform discharges in a combined entorhinal cortex/hippocampal slice. *J. Neurophysiol.* **70,** 1962–1974.
49. Sayer R. J., Friedlander M. J. and Redman S. J. (1990) The time course and amplitude of EPSPs evoked at synapses between pairs of CA3/CA1 neurons in the hippocampal slice. *J. Neurosci.* **10,** 826–836.
50. Schneiderman J. H. and Sterling C. A. (1994) The role of excitatory amino acids in the generation of spontaneous hippocampal oscillations. *Brain Res.* **633,** 105–111.
51. Siegel A. N., Wieser H. G., Wichnann W. and Yasargil G. M. (1990) Relationships between MR imaged total amount of tissue removed, resection scores of specific mediobasal limbic sub-compartments and clinical outcome following selective amygdala-hippocampaectomy. *Epilepsy Res.* **63,** 56–65.
52. Simons D. J. and Carvell G. E. (1989) Thalamo-cortical response transformation in the rat vibrissa barrel systems. *J. Neurophysiol.* **61,** 311–330.
53. Soltez I. and Mody I. (1994) Patch-clamp recordings reveal powerful GABAergic inhibition in dentate hilar neurons. *J. Neurosci.* **14,** 2365–2376.
54. Somers D. C., Nelson S. B. and Sur M. (1995) An emergent model of orientation selectivity in cat visual cortical simple cells. *J. Neurosci.* **15,** 5448–5465.
55. Spencer S. S. and Spencer D. D. (1994) Entorhinal–hippocampal interactions in medial temporal lobe epilepsy syndrome. *Epilepsia* **35,** 721–727.
56. Sperling M. R., O'Connor M. F., Saykin A. J. and Plummer C. (1996) Temporal lobectomy for refractory epilepsy. *J. Am. med. Ass.* **276,** 470–475.
57. Stringer J. and Lothmann E. W. (1992) Reverberatory seizure discharges in hippocampal–parahippocampal circuits. *Expl Neurol.* **116,** 198–203.
58. Swann J. W., Smith K. L. and Brady R. J. (1993) Localized excitatory synaptic interactions mediate the sustained depolarization of electrographic seizures in developing hippocampus. *J. Neurosci.* **13,** 4680–4689.
59. Tamamaki N. and Noyjo Y. (1993) Projection of the entorhinal layer II neurones in the rat as revealed by intracellular pressure injection of neurobiotin. *Hippocampus* **3,** 471–480.
60. Thomson A. M., Girdlestone D. and West D. C. (1988) Voltage-dependent currents prolong single-axon postsynaptic potentials in layer III pyramidal neurons in rat neocortical slices. *J. Neurophysiol.* **60,** 1896–1907.
61. Thomson A. M., Girdlestone D. and West D. C. (1989) A local circuit neocortical synapse that operates via both NMDA and non-NMDA receptors. *Br. J. Pharmac.* **96,** 406–408.
62. Thomson A. M. and West D. C. (1993) Fluctuations in pyramid–pyramid excitatory postsynaptic potentials modified by presynaptic firing pattern and postsynaptic membrane potential using paired intracellular recordings in rat neocortex. *Neuroscience* **54,** 329–346.
63. Thomson A. M., Deuchars J. and West D. C. (1993) Large, deep layer pyramid–pyramid single axon EPSPs in slices of rat motor cortex display paired pulse and frequency-dependent depression, mediated presynaptically and self-facilitation, mediated postsynaptically. *J. Neurophysiol* **70,** 2354–2369.
64. Thomson A. M. and Radpour S. (1991) Excitatory connections between CAl pyramidal cells revealed by spike triggered averaging in slices of rat hippocampus are partially NMDA receptor mediated. *Eur. J. Neurosci.* **3,** 587–601.
65. Traub R. D. and Wong R. S. (1982) Cellular mechanisms of neuronal synchronization in epilepsy. *Science* **216,** 745–747.
66. Traub R. D., Miles R., Wong R. K. S. and Knowles W. D. (1985) Neuronal interactions during epileptic events *in vitro*. *Fed. Proc.* **44,** 2953–2955.
67. Van der Linden S. and Lopes da Silva F. H. (1998) Comparison of the electrophysiology and morphology of layers Ill and II neurones of the rat medial entorhinal cortex *in vitro*. *Eur. J. Neurosci.* **10,** 1479–1489.
68. Walther H., Lambert J. D. C., Jones R. S. G., Heinemann U. and Hamon B. (1986) Epileptiform activity in combined slices of hippocampus, subiculum and entorhinal cortex during perfusion with low magnesium medium. *Neurosci. Lett.* **69,** 156–161.
69. Wang X. J. (1999) Synaptic basis of cortical persistent activity: the importance of NMDA receptors to working memory. *J. Neurosci.* **19,** 9587–9603.
70. Wilson W. A., Swartzwelder H. S., Anderson W. W. and Lewis D. V. (1988) Seizure activity *in vitro*: a dual focus model. *Epilepsy Res.* **2,** 289–293.
71. Wood S. J. and Jones R. S. G. (1999) A comparison of characteristics of spontaneous IPSCs in layers II and V of the rat entorhinal cortex *in vitro*. *J.Physiol.* **515,** 120.

(*Accepted* 3 *May* 2000)