Research report

Responses of rat subicular neurons to convergent stimulation of lateral entorhinal cortex and CA1 in vivo

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Abstract

There has been little electrophysiological examination of the afferent projection from lateral entorhinal cortex to dorsal subiculum. Here we provide evidence that synaptic inputs from lateral entorhinal cortex and CA1 converge onto single dorsal subicular neurons in vivo. Subicular responses to CA1 stimulation consisted of excitation and/or long-duration inhibition. Neurons excited by CA1 activation usually showed inhibition to entorhinal stimulation. The latter inhibition was usually of short duration, however, long duration inhibition was seen in a significant proportion of responses. Entorhinal stimulation produced excitatory responses in four bursting cells and it was these cells that also tended to show the longest inhibition. Only bursting cells could be driven antidromically by entorhinal stimulation. Biocytin-filled multipolar and pyramidal cells displayed excitation–inhibition sequences to CA1 and inhibition to entorhinal stimulation. These data strongly suggest that subicular inhibitory neurons receive excitatory input from CA1 and display mutual inhibition. The source of entorhinal-evoked inhibition is less clear. The relative sparseness of observed entorhinal-evoked responses suggests that the input to dorsal subiculum from any one part of lateral entorhinal cortex is spatially restricted. These data show that excitation–inhibition sequences can be seen in subicular pyramidal and multipolar cells and that single subicular neurons receive convergent inputs from CA1 and entorhinal cortex. We show for the first time that bursting cells can be driven both orthodromically and antidromically by direct entorhinal stimulation. These data support the existence of a reciprocal excitatory connection between lateral entorhinal cortex and dorsal subiculum and suggest further that this connection may involve only bursting subicular neurons. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The hippocampal formation of the mammalian brain is composed of the dentate gyrus, hippocampus proper (areas CA1–CA4), the subicular complex and the entorhinal cortex (EC) [45]. The EC and subicular complex have been described as retrohippocampal structures whose function is to process and transmit information between the neocortex and hippocampus. Of the two, the subiculum is the major output structure of the hippocampus proper [34,45,46,55]. It is the principal target of CA1 pyramidal cell axons and is the final relay in a polysynaptic loop between the EC and hippocampus [3,13,32,45–47]. EC inputs to hippocampus may also bypass the dentate gyrus, terminating directly in the subiculum, CA1 or CA3, thereby shortening the EC-hippocampal loop by two to four synapses (for review see Amaral and Witter [4] and Lopes da Silva et al. [28]).

In contrast to hippocampal areas, the electrophysiology of retrohippocampal structures has received very little attention to date, despite their acknowledged importance in memory formation [2,42,58] and as foci for pathophysiological changes (e.g., [21,53]). Previous in vivo recordings performed in the freely moving rat have established that subicular neuronal firing has a spatial
correlate, that is, subicular neurons have ‘place fields’ [33,40]. Subicular neurons recorded in vivo can be subdivided into four classes based upon their firing characteristics [40]: (1) burster (whose spontaneous spiking activity largely consists of spike bursts); (2) non-burster (cells that fire more tonically); (3) depolarized burster (see below); and (4) theta (presumed inhibitory interneurons). In vitro studies have added further weight to the division of subicular principal neurons into bursters and non-bursters (and, to some extent, depolarized bursters); these two primary firing patterns are produced by distinct neuronal classes, that is, non-bursting neurons cannot be made to burst [5,29,44,49]. These two classes can also be distinguished neurochemically; only non-bursting neurons express nicotinamide adenine dinucleotide phosphate-diaphorase activity [19]. Bursting in subicular neurons appears to be a function of their membrane characteristics rather than simply the type of synaptic input that the cell receives [29,43,44,49]. This suggests that subicular bursting cells may act to amplify the input that they receive, converting suprathreshold single pulse synaptic inputs into burst outputs. This burst output may be a particularly effective means of transmitting neuronal information [26].

The hippocampal output to subiculum from CA1 is excitatory [11,12,14,49] involving the postsynaptic activation of AMPA and, to perhaps a lesser degree, NMDA receptors [30,49]. We have shown recently that this CA1 input to subiculum expresses long-term potentiation of synaptic transmission in vivo [7–9]. Far less is known regarding the EC input to subiculum. Jones [22] reported that in vitro stimulation of the medial EC produced biphasic inhibition in rat subicular principal neurons. The only major in vivo study to examine the nature of subicular EC afferents is from the cat [50,51]. These authors found that connections between the EC and subiculum are reciprocal. Anatomical evidence from the rat also supports the reciprocity of this connection [23,54].

As described above, previous studies have examined the individual inputs to the subiculum from CA1 and, to a lesser degree, EC. There has, however, until now been no in vivo analysis regarding whether single subicular neurons receive convergent inputs from multiple extra-subicular sites. Anatomical studies show that projections from CA1 and EC are evenly distributed within the subiculum, suggesting the presence of synaptic convergence from these sites onto single subicular neurons [48]. Indirect physiological evidence suggests that entorhinal inputs may excite bursting cells only [44]. It is important to investigate the inputs to subicular neurons as there is an apparently strong parcellation of subicular output, with most subicular neurons projecting to perhaps only a single site [34]. This parcellation may also exist at the level of different neuronal subtypes. Subicular bursting and non-bursting neurons may project to different areas; for example, non-bursting cells to entorhinal cortex and bursting cells to presubiculum [43]. As there appears to be sparse connec-

2. Materials and methods

2.1. Animal preparation

Experiments were performed on adult male Sprague–Dawley rats. The anaesthetic, surgical and recording procedures were carried out as described previously [14]. Briefly, rats were anaesthetized (chloral hydrate, 400 mg/kg i.p.) and placed in a stereotaxic frame. Supplementary injections of chloral hydrate were given as required (0.2–0.4 ml i.p.). Small craniotomies (about 2 mm diameter) were then performed to allow insertion of recording and stimulating electrodes.

2.2. Stimulation and recording

Recording electrodes were advanced with a microdrive. A low-impedance (5–10 MΩ) glass pipette recording electrode was first advanced into the dorsal subiculum. Recording coordinates for the subiculum were anterior (A) 2.2 mm, lateral (L) 4.0 mm with respect to the interaural line [37]. Two twisted bipolar stimulating electrodes (150 μm diameter stainless steel wire, insulated to the tips) were then lowered towards dorsal CA1 and lateral EC. Stimulating coordinates were: A 2.8, L 6.1, H 1.8 for the lateral EC (12° from vertical); and A 4.5, L 3.0, H 7.0 for dorsal CA1 (22° from vertical). Subicular field potentials were recorded during the movement of stimulating electrodes to obtain optimal placements. Electrical stimuli were photically isolated 0.2 ms pulses of 100–500 μA intensity (usually 500 μA) at 0.2 Hz The low-impedance recording
electrode was replaced with a high-impedance pipette once placement of the stimulating electrodes was complete. High-impedance electrodes (25–80 MΩ) contained either (a) 1 M NaCl saturated with Fast Green, or (b) 0.5 M KCl containing 2% biocytin (Sigma).

Test shocks were applied during the search for cells in order to maximize the number of cells encountered and minimize the effects of any sampling bias. This meant that ‘silent’ neurons (i.e., those cells that showed no spontaneous firing but did display an excitatory response) were also included in the sample population. However, because these cells fired only a single spike in response to excitation and had no spontaneous discharge, they could not be unambiguously categorized as either ‘bursters’ or ‘non-bursters.’ This is because most excitatory responses in classified bursting and non-bursting cells were indistinguishable as they each consisted of only a single evoked spike.

Orthodromic excitatory responses were characterized by evoked spikes showing significant latency jitter. Antidromic responses were characterized by constant-latency action potentials. Putative antidromic potentials were examined whenever possible for the presence of collision (e.g., see Fig. 2). Peri-stimulus time histograms (PSTH) were computed for all cells both on- and off-line (LabView 2.0). Hand-counting spikes ensured accuracy of PSTH bins in the vicinity of the stimulus artifact.

2.3. Histology

At the end of the recording session stimulating loci were marked by producing small lesions at the electrode tips (50 µA, 5 s of each polarity). During the recording session the locations of particularly responsive cells were marked either with a Fast Green dye spot (5 µA negative current for 20 min) or by injecting the cell with biocytin following the juxtacellular method of Pinault [38]. Animals were then subjected to terminal anaesthesia, perfused transcardially with 10% formalin and the brains removed. Prior to sectioning, brains were placed in a 30% sucrose solution. Frozen 100 µm sections were then cut in the frontal plane and collected in cold, buffered PBS (Zymed). Sections thought to contain filled cells were subjected to histochemical processing. Sections were incubated for 1 h in 0.5% Triton-X, then rinsed in PBS before being incubated free-floating in a solution of streptavidin-peroxidase (LABSA; Zymed) diluted 1:500 in PBS. Incubation lasted either 8 h at room temperature or overnight at 4°C. Cells were then visualized using a standard DAB reaction (Zymed). Sections were examined using a light microscope and recovered cells were reproduced using a drawing tube. Using this method cells were recovered in most animals. In successful cases the number of visualized cells was always the same as the number of cells injected during the experiment (usually one cell per animal). This suggests that there were no cases of ‘false positive’ labeling. The results of cell filling were indistinguishable from those using more traditional intracellular injection methods using fine-tipped pipettes in the same laboratory (e.g., [13]). In any one animal, the locations for cells that were not marked during the experiment were determined by comparing the microdrive depth readings for these cells to that recorded for either a Fast Green mark or a filled cell in the same animal. In some rats either a Fast Green mark was absent or there was a failure to recover filled cells. In these rats it was possible that some of the recorded cells were in the presubiculum rather than the subiculum. This is, however, extremely unlikely as electrode tracks could be seen penetrating only the subiculum in all cases where indications for cell location were absent. The present analyses are based on all cells lying in the subiculum.

2.4. Statistical analyses

Results were analyzed using Students’ t, Mann–Whitney, ANOVA and K-means clustering tests (using SPSS 9.0 software for PC). Differences were considered significant if the value for P was less than 0.05.

3. Results

3.1. Electrode placements

Recordings were made from 114 cells in 20 animals. After histological verification, stimulating electrodes aimed at CA1 were located in either dorsal alveus, dorsal CA1, superficial CA1 adjacent to the dorsal alveus or dentate gyrus (one rat only). Stimulating electrodes aimed at lateral EC were located in either lateral EC or ventral angular bundle. Locations of subicular neurons (given by Fast Green mark or biocytin fill) that were activated by stimulation of these sites are presented in Fig. 1.

3.2. Bursting versus non-bursting cells

The majority of subicular cells (60%) fired spontaneous bursts of spikes in the periods between stimuli. Non-bursting cells accounted for 32% of recorded cells and ten cells (8%) were unclassified as they only showed a single evoked spike with no spontaneous discharge. For the purposes of this study a burst was defined as a group of 2 or more spikes with an inter-spike interval of more than 1 ms but less than 8 ms. Using this cut-off value spontaneous subicular bursts had a mean inter-spike interval of 4.45±0.14 ms (mean±S.E.M.). This value is close to that quoted for bursting cells previously [40,43]. There were no differences in either latency or duration of excitation or inhibition between bursting and non-bursting cells after stimulation of CA1 or EC. Therefore, in the following analyses the data from bursting and non-bursting cells have been grouped together where appropriate. Antidromic
responses were obtained for both bursting and non-bursting cells after stimulation of the alveus, indicating that both types of cell are projection neurons whose axons exit the hippocampal formation through the alveus.

3.3. Response classification

Responses were categorized as one of the following: (1) excitation (usually a single spike; see below) then long-duration inhibition (usually c.300 ms); (2) antidromic activation followed by long-duration inhibition; (3) inhibition alone; (4) excitation or antidromic activation alone (these cells had a low or zero spontaneous firing rate so we had no indication of either the presence of inhibition or the cell type; see above); (5) no excitation (again where the spontaneous firing rate was too low to indicate any presence of inhibition); or (6) no response (where the spontaneous firing rate was high enough to indicate the absence of inhibition). In one case there appeared to be both evoked excitatory and antidromic spikes. There were four cases in which bursting cells displayed burst responses to either CA1 or alveus stimulation. This represents 16.7% of the excitatory responses seen in bursting cells. The number of evoked spikes in these bursts was either 2 \( (n=3) \) or 3 \( (n=1) \). The interspike interval of these evoked bursts was 2.56±0.58 ms (mean±S.E.M.). Note that this figure may not be based solely upon the responses of pyramidal neurons as it includes a neuron with an evoked interspike interval of 1.4 ms; this value is similar to that found for a multipolar neuron (see below). Of the non-bursting cells analyzed only one showed a response that occasionally included two spikes to CA1 stimulation (7% of all excitatory responses in non-bursting cells). There were five cases in which cells responded to EC stimulation with excitation. Four of these cases were bursting cells yet the evoked response in all four was almost always a single spike.

Latency values for pure inhibition were calculated for those cases where the spontaneous activity in the PSTH extended throughout the whole pre-stimulus period and clearly reached the stimulus onset. Inhibition duration could then be calculated as the period from where spiking ceased (usually slightly after the stimulus and, where present, evoked activity) to where spiking occurred again.
post-stimulus. Excitatory latency was calculated in those cases where the onset of the excitatory peak in the PSTH clearly started to rise above the background firing rate. Excitatory duration was calculated as the width of the excitatory peak in the PSTH.

3.4. Subicular responses to stimulation of CA1, EC and alveus

Following CA1 stimulation 29 cells (39%) showed excitation, 33 cells (33%) showed only inhibition, 11 cells (15%) were activated antidromically and 10 cells (13%) showed no obvious response. One cell showed an equivocal response and is not included in the analysis. Excitatory responses to CA1 were equally likely from bursting and non-bursting cells. Responses to EC were markedly different from those evoked by CA1 stimulation. Following EC stimulation five cells (5%) showed excitation, 41 cells (41%) showed only inhibition, three cells (3%) antidromic activation and 51 cells (51%) showed no obvious response. Of the cells showing excitation to EC stimulation four were bursting neurons and one cell could not be classified as it had a zero spontaneous rate. The mean excitatory latency to EC stimulation was 12.38±1.54 ms (mean±S.E.M.). All three cells driven antidromically by EC stimulation were bursting cells. These cells had a mean burst interspike interval of 4.15±0.14 ms (mean±S.E.M.).

Antidromic response latencies to EC stimulation were 11.40, 11.70 and 13.00 ms (12.03±0.49 ms; mean±S.E.M.). These values were significantly longer than those to CA1 or alvear stimulation (ANOVA, $F = 30.38, df=2,15, P<0.001$; CA1 vs. EC, $P<0.001$; alveus vs. EC, $P<0.001$; CA1 vs. alveus n.s.). Mean values for CA1 and alveus antidromic latencies were 2.87±0.71 and 3.80±0.34 (mean±S.E.M.) respectively. An example of antidromic activation following alvear stimulation is shown in Fig. 2.

As expected, excitatory and inhibitory latencies to CA1 stimulation were significantly shorter when compared to those from EC stimulation (Fig. 3). This probably reflects the longer conduction time for the EC-subiculum pathway. Excitatory duration was significantly longer to EC stimulation compared to CA1 stimulation and inhibitory duration was significantly shorter to EC stimulation (Fig. 3). On first inspection these results may be related in that the weaker inhibitory input following EC stimulation may have allowed excitation to last longer. However, values for EC-evoked inhibitory duration appeared to fall into three distinct groupings with the respective values for CA1-evoked inhibition appearing far more homogeneous (Fig. 4). Subjecting these EC data to a K-means cluster analysis revealed that they could be separated into three groups. Cluster centres for these were at 69.78 ms, 246.40 ms and 472.80 ms (Fig. 4). The number of cases for these clusters were 37, 5 and 5 respectively. These clusters were significantly different (ANOVA, $df=2, 44; F=469.39; P<0.001$). From this it appears that although most cells responded with weak inhibition after EC stimulation, a
Fig. 3. Latency and duration of excitation and inhibition. (A) Excitatory latency is significantly longer to EC than CA1 stimulation. (B) Excitatory duration to EC stimulation is significantly longer than that to CA1 stimulation. (C) EC stimulation produces inhibition that has a longer latency than that to CA1 stimulation. (D) Inhibition following CA1 stimulation is significantly longer than inhibition produced by EC stimulation. Significance levels for all parts of figure: **$P<0.01$, *$P<0.05$. Error bars represent standard error of the mean.

Fig. 4. Distribution of inhibitory duration following EC and CA1 stimulation. (A) Inhibitory duration after EC stimulation falls into three groups. Most durations are less than 150 ms but a small number are of much longer duration. (B) Inhibitory duration following CA1 stimulation shows a single broad distribution.
significant number of cells were subject to strong inhibition. Interestingly, three of the bursting cells that showed excitation to EC stimulation also expressed clear inhibitory responses (the inhibitory duration in the other two driven cells could not be determined) and the inhibitory duration in these cases was 120 ms, 437 ms and 510 ms. This suggests that cells excited by EC stimulation also show strong inhibition. The longer duration seen in excitatory responses to EC stimulation may simply reflect a more variable conduction time in this longer pathway compared to the CA1 input. It seems unlikely that the level of evoked inhibition was lower in the former cases as responsive cells were all bursting cells yet were prevented (presumably inhibited) from showing evoked burst responses. This is similar to the above cases where CA1 stimulation evoked single spike excitation in cells that fired spontaneous spike bursts.

3.5. Morphology of filled cells

Seven subicular neurons were filled with biocytin during recording. These could be classified by shape as either pyramidal-like \( (n=4\); all bursting neurons) or multipolar \( (n=3\); putative inhibitory neurons; 1 bursting cell and 2 non-bursting cells). Fig. 5 shows the responses for two identified neurons from the same rat. The morphological features of these cells are shown in Fig. 6. The pyramidal-like neurons visualized in the present study all had the general form of hippocampal pyramidal neurons with one long apical dendrite that coursed through the stratum pyramidale towards the subicular molecular layer. This morphology is in agreement with previous reports for subicular pyramidal neurons \cite{20,29,31,49}. The morphology of multipolar neurons was less clear. In general, the cell bodies for all three multipolar neurons were clearly located

![Fig. 5](image_url)

Fig. 5. Evoked responses of cells presented in Fig. 6. (A) Response of multipolar neuron to CA1 stimulation (top) and lateral EC (bottom) stimulation. Averaged responses shown as PSTHs. Burst response to single CA1 stimulation pulse shown as inset. Note the decrement in spike height as the burst progresses. Solid triangles indicate stimulus onset. (B) Responses of pyramidal-like neuron to CA1 (top) and lateral EC (bottom) stimulation. Single spike response to single CA1 stimulation pulse shown in inset. (C) Locations of recorded cells within the subiculum (solid circle in middle panel) and CA1 (solid triangle in left-hand panel) and EC (triangle in right-hand panel) stimulating electrodes. Location of cells was B-7.30 (Paxinos and Watson, 1986). Calibration: data sweep 5 mV, 10 ms; PSTHs 100 spikes/s, 100 ms.
in stratum pyramidale of the subiculum, more towards the border with stratum oriens than stratum moleculare. The best example of the three multipolar neurons is presented in Fig. 6; for the other cells although incomplete filling led to only the cell bodies and proximal processes being visualized these cells were clearly different in morphology from pyramidal cells. The multipolar cell in Fig. 6 had numerous radial processes with prominent varicosities.
These processes had no obvious orientation to any plane within stratum pyramidale. This cell most closely resembles the fast spiking class of interneuron reported by Greene and Totterdell [20].

3.6. Response characteristics of filled cells

In general, the response characteristics for visualized cells were comparable to those for the overall subiculum data. The only statistically significant difference between stimulation sites for identified cells was that for inhibitory duration; pyramidal-like neurons show significantly longer inhibition to CA1 stimulation compared to EC ($P<0.05$, Mann–Whitney $U$). There was a similar trend for multipolar neurons.

Examples of the responses of pyramidal-like neurons are presented in Figs. 5 and 7. In general, the response of pyramidal-like bursting neurons to CA1 stimulation was either inhibition or excitation (single spike) followed by inhibition. The pyramidal-like bursting neuron in Fig. 5

![Fig. 7. Responses of pyramidal-like subicular neuron. (A) Response to CA1 stimulation. PSTHs show excitatory response followed by inhibitory phase (top) and detail of excitatory period (bottom; 44 sweeps). (B) Response to angular bundle stimulation. PSTHs show inhibitory response to angular bundle stimulation (top) and detail of post-stimulus cell firing (bottom; 54 sweeps). (C) Raw data. Single spike response to CA1 stimulation (top) and spontaneous burst (bottom). Almost all spontaneous firing occurred in bursts for this cell. (D) Cell morphology. Reconstruction of pyramidal-like cell and its location in dorsal subiculum (B-6.8). Stimulus occurred at second tick mark from left on horizontal axis in A and B. PSTHs composed of 44 sweeps in (A) and 54 sweeps in (B). Calibration: upper PSTHs in (A) and (B) 100 spikes/s, 100 ms; lower PSTHs in (A) and (B) 100 spikes/s, 25 ms; data sweep 10 mV, 10 ms.](image)
showed a single evoked spike response to CA1 stimulation, followed by inhibition. There was an equivocal response to EC stimulation for this neuron with perhaps a short duration inhibitory phase. Fig. 7 shows the responses of a pyramidal-like neuron to CA1 and angular bundle stimulation. This neuron showed a single-spike response to CA1 stimulation followed by inhibition. The response to angular bundle stimulation showed a slight indication of excitation followed by an inhibitory phase that was more marked when compared to the cells in Fig. 5 with EC stimulation. The data sweeps in Fig. 7c show an example of an evoked spike following CA1 stimulation and a spontaneous burst of spikes.

Following EC stimulation the three recovered multipolar cells showed either inhibition \( (n=2) \) or no response \( (n=1) \). In contrast, all three of these multipolar neurons could be driven by CA1 stimulation. One of these neurons fired spontaneous spike bursts and is shown in Fig. 5. This cell also fired an evoked burst of 2–3 spikes in response to CA1 stimulation. These spikes had a 1.6 ms inter-spike interval and showed a decrease in amplitude with time during the burst. This multipolar neuron displayed a pronounced inhibitory phase after the evoked burst to CA1 stimulation. There was also an inhibitory period following EC stimulation in this neuron, although the duration of inhibition in this case was markedly shorter. The second multipolar cell driven by CA1 stimulation showed an evoked burst of constant amplitude spikes but fired tonic spikes between stimuli. The third multipolar neuron fired a single spike to CA1 stimulation and spontaneous high frequency tonic spikes (spontaneous rate for this cell was 62 spikes per second, the highest rate for all three multipolar neurons).

Overall, the results from morphologically characterized neurons demonstrate that CA1 afferents produce excitation–inhibition sequences in pyramidal and multipolar cells. The responses of these cells to EC stimulation were far less impressive, showing only relatively weak inhibition of cell firing. It appears from the present data that different multipolar neurons show different excitatory responses to CA1 stimulation. These results also suggest that multipolar neurons receive inhibitory inputs (mutual inhibition). It is noteworthy that in the present sample none of the multipolar neurons could be driven by EC stimulation.

### 3.7. Convergent responses

The convergence data for 95 neurons tested with both CA1 and EC stimulation neurons are presented in Fig. 8. Neurons that displayed excitation to CA1 stimulation almost always showed either inhibition or no response to EC stimulation (see Figs. 5 and 7).

In three cases (3% of convergent responses) EC stimulation evoked excitation that could also be tested with CA1 stimulation. In the first of these cases there was excitatory convergence onto a single subicular neuron. Responses from this neuron are shown in Fig. 9; note that this neuron showed one of the few excitatory burst responses to CA1 stimulation. Note also that it was necessary to apply a double-pulse stimulus through the EC electrode in order to evoke activity in this subicular neuron. In the second of these three cases it was possible to overcome EC excitation with (presumed) convergent inhibition produced through simultaneous activation of the CA1 electrode (Fig. 10). In the last of the three cases of EC-evoked excitation the neuron displayed no excitation to CA1.

Two other neurons that showed EC-evoked responses were also tested with dentate gyrus stimulation. In the first of these two cases there was long-latency convergent excitation; excitatory latencies were 15.9 ms for dentate stimulation and 16.8 ms for EC stimulation. In the second of these cases there was an unexpected antidromic response to dentate stimulation; antidromic latency to dentate stimulation was 12 ms, excitatory latency to EC stimulation was 15 ms.

### 4. Discussion

The purpose of this study was to examine whether dorsal subicular neurons receive convergent inputs from lateral EC and hippocampal area CA1. Subicular CA1 and EC afferents were activated electrically and the responses of subicular neurons recorded. The spontaneous firing of subicular neurons could be sub-divided into burst firing and non-burst firing. The excitatory synaptic response of both classes was, in general, a single evoked spike to CA1...
stimulation. The relative proportions of subicular bursting and non-bursting neurons are in general agreement with previous studies in vivo [14,40] and in vitro [5,29,44,49]. Antidromic responses were obtained for both bursting and non-bursting cell types after stimulation of the alveus, indicating that both types are projection neurons. This is in agreement with results from Stewart and Wong [44] using the guinea-pig in vitro slice preparation.

4.1. Responses to CA1 and alveus stimulation

Responses of subicular cells to stimulation of alveus and CA1 were in general agreement with previous in vivo studies of the rat subiculum [11,14]. CA1/alveus activation could evoke excitation, inhibition or antidromic activity. Excitation or antidromic activity could also be followed by a period of prolonged inhibition. The finding
that 39% of subicular neurons responded to CA1 stimulation with excitation demonstrates that the CA1 input to subiculum is very strong. The inhibitory duration in subicular neurons is consistent with the activation of postsynaptic GABAa and GABAb receptors (although we cannot rule out disfacilitation). This is in agreement with Taube [49] who showed that IPSPs observed in subicular pyramidal cells in vitro closely resembled the biphasic IPSPs reported in hippocampal CA1 cells [1,35,36]. It remains to be seen whether application of the appropriate neuroactive compounds (e.g., [18]) can further support the GABAergic nature of this subicular IPSP. It is noteworthy here that Stewart and Wong [44] could only detect GABAa-dependent IPSPs in guinea-pig subiculum in vitro, suggesting that perhaps subicular inhibitory mechanisms differ between species. The strong nature of inhibition in
the present anaesthetized in vivo preparation is evidenced by a low proportion of evoked burst responses to CA1 stimulation in bursting cells.

4.2. Responses to EC stimulation

The predominant response of subicular neurons to EC stimulation was that of weak inhibition, regardless of the response to CA1 input in the same cell. A previous report has described inhibitory potentials in subicular cells in vitro following EC stimulation [22] so it is likely that the inhibition produced with EC stimulation has a direct synaptic origin (see below). Compared to that evoked by CA1 stimulation, inhibitory duration following EC activation was significantly shorter. However, a small but significant number of neurons expressed long duration inhibition to EC stimulation. Stimulation of EC produced very few examples of excitation in subicular neurons. Five neurons could, however, be driven by EC stimulation. Of these, four were bursting neurons and the fifth could not be classified. This is a relatively small number of excitatory responses and it is possible that a more optimal placing of recording and stimulating electrodes in further studies may produce a stronger activation of subicular cells. However, as no previous studies have examined the EC-subiculum pathway in vivo, it is impossible to predict the outcome of such alternate strategies. Neurons that showed excitation to EC stimulation also tended to show the longest duration inhibition. Antidromic activation after EC stimulation was only seen in three subicular neurons and these were all bursting cells. This provides physiological evidence in support of the hypothesis that lateral EC and dorsal subiculum have a functional reciprocal connection. Furthermore, these results suggest that this reciprocal connection may only include subicular bursting neurons. Although we cannot prove that these subicular excitatory responses to EC stimulation were monosynaptic, it is highly likely that this was the case for the following reasons. (1) Mean antidromic and orthodromic latencies in the subiculum-EC pathway were very similar. The shortest possible hippocampal polysynaptic route from EC to subiculum is via CA1. Individual excitatory responses in subiculum following EC stimulation were at most no more than 3–4 ms longer than antidromic responses. For this 4 ms difference to be a conduction delay in a polysynaptic route then the candidate extra connection(s) should show latencies similar to this figure. However, the excitatory latency in subiculum after CA1 stimulation (the shortest candidate polysynaptic route) was substantially longer than 4 ms at approximately 7.5 ms on average (see Fig. 3). It appears, therefore, that the latency for the polysynaptic route via CA1 is substantially longer than that actually observed for excitatory responses. Thus, subicular excitatory responses after EC activation were almost certainly produced by monosynaptic connection between EC and subiculum rather than polysynaptic conduction via CA1. (2) The latency data are supported by the reproducible finding from a number of laboratories that EC afferents are incapable of producing action potential discharge in CA1 pyramidal cells in the anaesthetized rat (see below). This strongly supports the suggestion that the polysynaptic loop between EC and subiculum via CA1 would be non-functional in the anaesthetized animal, again supporting the monosynaptic excitation of subicular neurons by EC activation.

The duration of excitatory responses to EC stimulation was significantly longer than those to CA1 stimulation. This is perhaps due in part to the overall weaker inhibition noted earlier in response to EC stimulation. However, cells driven by EC stimulation tend to show the longest duration inhibition. This suggests that the initial phase of inhibition (GABAa-dependent?) produced by EC stimulation in subicular neurons could be weaker than that evoked following CA1 stimulation. The case in Fig. 10 shows a cell that could be driven by EC stimulation and that showed inhibition to CA1. That the latter actually represented active inhibition is supported by the fact that the EC excitation could be overcome by the presumed strong, short latency inhibition produced during simultaneous CA1 stimulation. The weak inhibitory influence from EC in most subicular recordings resembles the responses seen in rat hippocampus after activation of EC afferents [22,24,25,41] (but see Yeckel and Berger [56,57] for CA1 excitation in the rabbit following EC activation). Although the EC input to CA1 appears weak in the rat, it does express long-term potentiation [24,25] and paired-pulse facilitation [24]. However, despite the prior induction of long-term potentiation in this pathway, activation of the perforant path is still incapable of driving CA1 single-unit activity [24]. In this regard, the EC input to subiculum may be ‘stronger’ than that to CA1 as EC afferents can drive subicular neurons.

Levy et al. [25] have shown that when the EC input to CA1 is co-activated with a weak input from the Schaffer collaterals to CA1, robust associative LTP is seen under conditions of reduced inhibition in vitro. Thus, a weak excitatory input from EC to CA1 can facilitate the responses of CA1 neurons to input from CA3. A similar scheme has been suggested for the mossy fibre input to CA3 from dentate gyrus; activation of single mossy fibre inputs may be capable of firing CA3 cells if there is coincident input from EC to the same CA3 neuron [27]. It would be of some interest to see whether the EC input to subiculum is capable of undergoing long-term potentiation and perhaps modulating the CA1 input in a similar associative fashion. Optimal conditions for this might be during phases of reduced inhibition, e.g., during theta rhythm. It would also be interesting to see whether reducing inhibition via application of GABAergic antagonists is effective in revealing excitation in subicular neurons following EC excitation; removal of inhibition would give a much better estimate for the level of
excitation produced in all subicular neurons by EC stimulation.

4.3. Responses of identified cells

Pyramidal-like and multipolar cells were filled with biocytin and visualized histochemically. Pyramidal neurons all fired spontaneous bursts of spikes. The two pyramidal cells that responded to CA1 stimulation displayed single evoked spikes. It is assumed that the strength of inhibition following CA1 stimulation curtailed the full burst response in these pyramidal cells. The inhibitory duration in pyramidal cells to CA1 stimulation was significantly longer than that to EC stimulation, again highlighting the predominantly weak inhibitory input from EC activation in the present study.

The most fully characterized multipolar cell is presented in Fig. 5.B1. This cell resembles most closely the fast spiking type of interneuron described by Greene and Totterdell [20], with its location in the subicular cell body layer and processes containing numerous varicosities. This cell fired a burst of spikes to CA1 stimulation that decreased in amplitude. The other two subicular multipolar neurons described here fired (a) CA1-evoked spike bursts in one case and (b) a single CA1-evoked spike with high frequency spontaneous tonic firing in the other. These results suggest that there may be more than one class of subicular inhibitory neuron, particularly in stratum pyramidale, and that these classes may be distinguished by response characteristic. The CA1-evoked burst of decreasing amplitude spikes seen in the present fast spiking cell resembles very closely the response of four putative inhibitory subicular cells recorded by Finch and Babb [12].

Thus, inhibitory cells with this particular response characteristic may represent a defined subicular inhibitory cell subtype.

4.4. Sources of inhibition in subiculum

The present results demonstrate that multipolar cells are subjected to inhibition, suggesting that inhibitory neurons, in contrast to pyramidal neurons [40,44], may be strongly interconnected (providing mutual inhibition of inhibitory neurons). The duration of inhibition in multipolar cells was similar to pyramidal cells following CA1 and EC stimulation. The length of the inhibitory response to CA1 stimulation suggests that multipolar cells are also subject to inhibitory inputs acting via postsynaptic GABAa and GABAb receptors. The lack of excitation in multipolar cells following EC activation raises the question of where EC-evoked inhibition is produced. On one hand this may reflect a sampling or topographical issue; recordings from multipolar cells in subiculum may have been in an inhibitory surround, outside the strongest target region of entorhinal projections. This is supported by the observed ‘focal’ nature of the excitatory EC input to subicular principal cells. It is important to note, however, that inhibition in subicular cells produced through EC stimulation may not be a product of inhibitory neurons within the subiculum itself. One candidate extra-subicular source of inhibition is the dentate gyrus [6]. Inhibitory neurons are present throughout the entire dentate outer molecular layer. These neurons project across the hippocampal fissure to the subiculum [6]. Stimulation of EC strongly activates the dentate gyrus, so it is likely to activate this particular inhibitory projection neuron which, in turn, could produce feed-forward inhibition of subicular pyramidal neurons (and possibly subicular inhibitory neurons as well). A second possible route for EC-evoked inhibition is a direct inhibitory input from EC to subiculum; although we know of no direct evidence in support of this claim, the EC does contain GABAergic cells that project to the hippocampus [15,16]. Finally, a third possible mechanism for inhibition following EC stimulation is antidromic activation of inhibitory neurons. Subicular inhibitory neurons have been shown to project to the EC [52]. Stimulation within EC may have activated these cells antidromically, thereby activating their presumed recurrent collaterals within the subiculum.

4.5. Possible significance of EC connections to subiculum

The results presented here provide novel information regarding the convergence of EC and CA1 afferents onto single neurons of the dorsal subiculum. CA1 afferents strongly excite pyramidal cells; the influence of EC afferents is far less impressive, resulting in predominantly weak inhibition. Present recordings from identified multipolar neurons show that these presumed inhibitory neurons are excited by CA1 (but not EC) afferents. We suggest that these putative inhibitory cells both provide and receive strong inhibitory connections. The duration of this inhibition is consistent with the postsynaptic activation of GABAa and GABAb receptors. That only few subicular cells showed excitation and/or prolonged inhibition to EC stimulation suggests that the input from lateral EC may be quite focussed. Thus, stimulation within any part of EC may produce strong excitation and inhibition in only a restricted portion of dorsal subiculum. The relatively small number of antidromic responses seen in subiculum after EC stimulation also supports a similar anatomical restriction in the ‘return’ pathway back to the EC. These neurophysiological findings are consistent with anatomical data showing that the reciprocal EC-subiculum pathway consists of ‘point-to-point’ connections [23,54]. In those cells that did respond to EC stimulation, excitation and antidromic activation was only seen in subicular bursting cells. This neurophysiological evidence supports the hypothesis that lateral EC and dorsal subiculum have a functional reciprocal connection and further suggests that this connection may only involve bursting subicular neu-
rons. It is important to note, however, that this suggested role for bursting cells is based on only a small number of cells that showed excitation to EC stimulation.

In contrast to EC stimulation, activation of CA1 activates a large number of subicular neurons. This is consistent with anatomical data showing that the CA1 output ‘fans out’ over a large dorsoventral extent in the subiculum [3]. Dorsal subicular neurons appear, therefore, to receive a substantial overlapping input from a large region of CA1 but a restricted convergent input from perhaps only a small number of EC neurons. In turn, as described above, the reciprocal output from dorsal subiculum may reach only a limited number of cells in lateral EC. In functional terms, the reciprocal connection between subiculum and EC may represent the neuronal substrate for the hypothesized universal spatial map described by Sharp [39]. In this scheme reciprocal connections between subiculum and EC establish a single spatial map for all environments. The ‘point-to-point’ nature of the EC-subiculum pathway described here is consistent with this mapping hypothesis as this type of connection is thought to preserve similar representation of information between two connected areas (e.g., [10]). The present results further suggest that the production of this putative EC-subiculum universal map involves only bursting cells within dorsal subiculum and that only these bursting cells relay processed information back to lateral EC.

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