Exp Brain Res (2002) 146:155–160
DOI 10.1007/s00221-002-1158-x

RESEARCH ARTICLE

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Physiological evidence for a possible projection from dorsal subiculum to hippocampal area CA1

Received: 14 March 2002 / Accepted: 14 May 2002 / Published online: 23 July 2002
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Abstract The substantial forward projection from hippocampal area CA1 to the subiculum has been comprehensively described, both anatomically and neurophysiologically. There are few data, however, regarding the existence of a backward projection from the subiculum to area CA1. We present here new electrophysiological evidence for the existence of this projection. We demonstrate a positive-going deflection in the evoked synaptic response in area CA1 following stimulation in dorsal subiculum. We also found a small, but significant, paired-pulse facilitatory effect at a 100-ms interstimulus interval. We were unable to induce long-term potentiation following high-frequency stimulation, but were able to induce short-term potentiation.

Keywords Subiculum · CA1 · Paired-pulse facilitation · Long-term potentiation · Short-term potentiation

Introduction

The projection from area CA1 to the subiculum has been described in great detail (Tamamaki and Nojyo 1990; Amaral et al. 1991; Amaral and Witter 1995). This projection is an important link in the cortical-hippocampal-cortical circuit, where the subiculum may serve to further modulate information received from the hippocampus before passing it to cortical and subcortical targets (Canteras and Swanson 1992). The physiological and functional properties of the subiculum have also been described in both in vitro and in vivo studies (see Mason 1993; Taube 1993; Sharpe and Green 1994; O'Mara et al. 2000). The CA1-subiculum projection also sustains various forms of short- and long-term plasticity. Long-term potentiation (LTP) has been described in both in vivo and in vitro preparations using a variety of stimulation protocols (Boeijinga and Boddeke 1996; Commins et al. 1998a, 1999; Dolen and Kauer 1998). Paired-pulse facilitation (PPF), a short-term plastic phenomenon (Zucker 1989), has also been shown in this pathway, in both in vivo and in vitro preparations (Commins et al. 1998b; Dolen and Kauer 1998). Demonstrating that the CA1-subiculum projection is capable of undergoing changes in synaptic strength provides a basis from which theories of hippocampal-cortical interaction can be formulated (O'Mara et al. 2000, 2001).

Within the hippocampal formation a number of reciprocal connections have been described, and these include those between the subiculum and entorhinal cortex (Witter et al. 1989, 1990), the CA1 field and entorhinal cortex (Steward 1976; Lopes da Silva et al. 1990), and the subiculum and perirhinal cortex (Witter et al. 1989, 1990; Naber et al. 1999, 2001). There is, however, a scarcity of reciprocal projections within the hippocampus itself (with the exception of the recurrent collaterals of area CA3 (Ishizuka et al. 1990; Amaral and Witter 1995). Information flow has therefore been long considered to be essentially unidirectional within the hippocampus (Amaral and Witter 1989). One study does, however, point to the existence of a "reentrant bundle" of CA1 fibres (Amaral et al. 1991). An anterograde injection in the distal region of CA1 (close to subiculum) resulted in the labelling of fibres in the proximal subiculum, and from this terminal field a bundle of fibres travelled back into the CA1 field and labelled cells in the area bordering stratum radiatum and stratum lacunosum-moleculare (Amaral et al. 1991). The terminal field of these back projections appeared to be quite extensive involving perhaps up to half the distance of area CA1. Recently Harris and Stewart (2001) reported that subicular pyramidal cells have extensive axon collaterals and that some ascend into the apical dendritic region of CA1. These

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results suggest a significant direct projection from subiculum to CA1. Furthermore, injections of an anterograde tracer in the proximal part of the subiculum result in labelling of fibres in area CA1 (M.P. Witter, personal communication). These fibres leave the subiculum going into the apical direction of the neurons bending backward into CA1, travelling exactly at the border between radiatum and lacunosum-moleculare layers (M.P. Witter, personal communication).

This study examines whether there is physiological evidence to support the limited anatomical data that suggest the existence of a back projection from the dorsal subiculum to area CA1. Furthermore, if such a projection exists, what are the basic characteristics of the evoked response in vivo? Is this projection capable of undergoing changes in synaptic strength? Insight into the nature of this projection may contribute to our understanding of how the interaction between area CA1 and subiculum affects hippocampal processing.

Materials and methods

Adult male Wistar rats (Bio Resources Unit, University of Dublin; weight: 200–300 g) were used. Rats were housed in pairs in a temperature-controlled laminar airflow cupboard, and maintained on a 12:12 light-dark cycle (08:00–20:00). Rats were initially anaesthetised with Sagatal (sodium pentobarbitone; 60 mg/kg, i.p.) and were mounted in a stereotactic holder. Further injections of urethane (ethyl carbamate; 1.5 g/kg, i.p.) were given to sustain anaesthesia throughout the experiments. A local anaesthetic/adrenaline combination was injected under the scalp and an incision was made to visualise the skull. Stimulating electrodes were aimed at

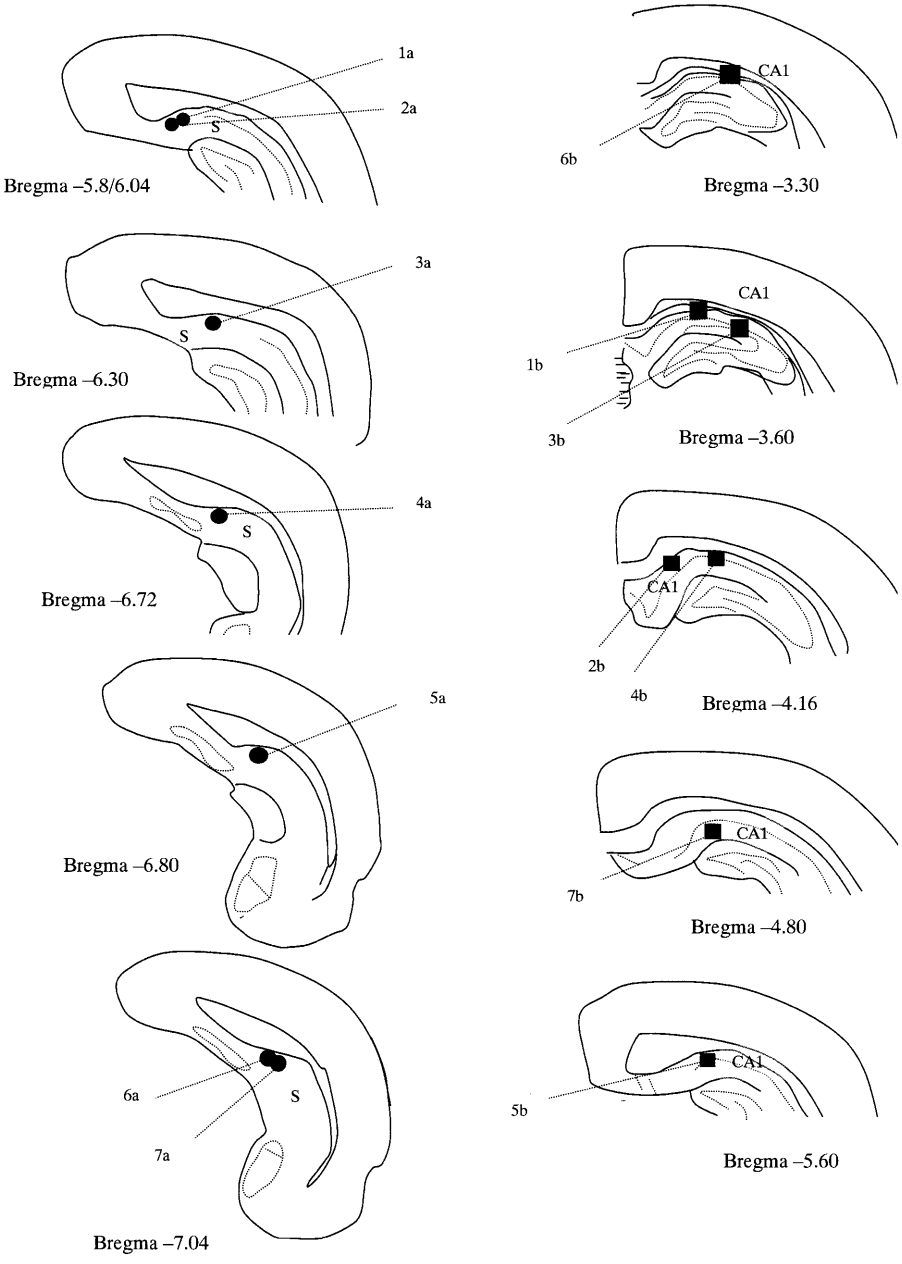

Fig. 1 Examples of stimulating (*closed circles*) and recording (*closed squares*) sites in the dorsal subiculum (*S*) and area CA1 (*CA1*), respectively. *1a* and *1b* indicate the first electrode pairing, *2a* and *2b* the second electrode pairing, etc

dorsal subiculum (6.8 mm posterior to bregma and 4.0 mm lateral to the midline) and recording electrodes at area CA1 (4.5 mm posterior and 2.5 mm lateral to the midline). Electrode implantation sites were identified using stereotaxic coordinates (Paxinos and Watson 1986). Stimulating and recording electrodes consisted of 50 μm tungsten wire, insulated to the tips.

Signals were filtered between 0.1 Hz and 1 kHz and then amplified (DAM-50 differential amplifier; World Precision Instruments, Hertfordshire, UK). Recordings were digitised online using a PC connected to a CED-1401 plus interface (CED, Cambridge, UK). Signals were also monitored using an oscilloscope. Electrodes were slowly lowered to each area, and test stimuli were administered during electrode movement at a rate of 0.05 Hz. The final depths were adjusted until maximal field excitatory postsynaptic potentials (fEPSPs) were obtained, and electrodes were allowed to settle for 10 min before baseline recordings were conducted. Baseline measurements were made for 10 min at a rate of 0.05 Hz.

To examine the effect of paired-pulse facilitation/depression pairs of stimuli were delivered every 20 s for 20, 50 and 100 ms interstimulus intervals (ISIs). The first response and second response elicited by the first and second stimulus of the stimulus pair will be referred to as fEPSP1 and fEPSP2, respectively. The PPF value was calculated by taking the average of amplitude values of fEPSP1, for a given ISI, and normalising the average of values for fEPSP2 with respect to this value in percentage terms.

Induction of LTP was attempted using high-frequency stimulation (HFS; this consisted of ten trains of 20 stimuli at 200 Hz, intertrain interval of 2 s). Stimulation was resumed at baseline stimulation and fEPSPs were recorded for 30 min.

After each experiment the rats were overdosed with sodium pentobarbitone and their brains removed and allowed to sink in 4% formaldehyde. All brains were then sectioned, on a vibratome, along the coronal axis to verify the position of the stimulating and recording electrodes. All data presented here are for stimulating and recording sites that were verified as being in dorsal subiculum and CA1, respectively. Figure 1 provides details of stimulating and recording sites in both areas.

All parameters are given as mean ± SEM. We used a one-way ANOVA and *t*-tests wherever appropriate to test statistical significances between groups. Field potentials drawings are smoothed by graphic correction.

Results

General description

In all cases (n=7) a response was evoked in area CA1 following stimulation in the dorsal subiculum. Figure 1 shows the distribution of the final positions of both stimulating (*filled circles*) and recording (*filled squares*) sites. The stimulating sites were positioned along the entire rostrocaudal extent of the dorsal subiculum, located just below the corpus callosum. Evoked responses were evident along the extent of the rostrocaudal axis of area CA1. The final positions of the recording electrodes were observed in the alveus, stratum oriens, stratum pyramidale, stratum radiatum and moleculare of area CA1.

Depth profile

The recording electrode was lowered 2.5 mm below the surface of the brain. After passing the occipital cortex, corpus callosum and the stratum oriens, the electrode was allowed to settle in the stratum pyramidale of area CA1 [see Fig. 2a*(iii) closed square*]. The stimulating electrode was slowly lowered towards the dorsal subiculum [Fig. 2a*(ii)*]. Stimulation (at a rate of 0.05 Hz) of different sites en route evoked responses in CA1. Stimulation of the overlying cortex (occipital cortex) did not produce a hippocampal response [see Fig. 2a*(i) 1*]. The first hippocampal response was produced following stimulation in the corpus callosum; a small positive-going deflection was observed [see Fig. 2a*(i) 2* and *3*]. As the stimulating electrode passes the border of the corpus callosum and dorsal subiculum [Fig. 2a*(i) 4*] and then settled in the dorsal subiculum, a large positive-going potential was evoked in area CA1 [Fig. 2a*(i) 5*].

A single-pulse stimulation in the dorsal subiculum evoked in the majority of cases (five of seven experiments) a positive-going deflection in area CA1. We measured these potentials in terms of their peak amplitude (defined as the most positive voltage within a specified time interval: usually<50 ms), latency (defined by the time interval between the presentation of the stimulus to the peak amplitude of the evoked response) and slope (defined in terms of the ascending component of the evoked response). The mean peak amplitude of evoked responses in area CA1 was 2.06±0.37 mV, the latency was 7.84±0.62 ms and the slope was 0.52±0.124. In two out of seven experiments, a single-pulse stimulation evoked a dual-component response, an early negative-going and a late positive-going deflection. The early negative deflection occurred at 7.07±0.87 ms and had a mean peak amplitude of –0.44±0.008 mV. The positive deflection occurred later at a mean latency value of 12.44±0.43 ms and a mean peak value of 0.26±0.128 mV.

Although the positive-going deflection was observed in the majority of cases (described above), there was variation in the evoked response which depended on the location of the recording electrode in area CA1. This suggests that the response evoked following stimulation in the dorsal subiculum is polysynaptic in nature. Figure 2b displays two evoked potentials following stimulation in the rostral portion of dorsal subiculum. A short latency synaptic response (6.36 ms) was evoked in the alveus/stratum oriens of rostral CA1, while a longer latency potential (9.38 ms) was evoked in the stratum moleculare of caudal CA1. There were insufficient observations to test for significant differences.

Short- and long-term plasticity

Paired-pulse facilitation was measured at three different ISIs (20, 50 and 100 ms) six times in each of five animals, giving 30 measurements for each interval tested. No change was observed at the 20-ms ISI (–3.2±3.46%; see Fig. 3a); the response evoked by the second stimulus was not significantly different from that evoked by the first stimulus (t=0.89, df=56, P>0.05). No change was observed at the 50-ms ISI (4.0±3.48%; t=1.35, df=58, P>0.05). Paired-pulse facilitation was observed at the 100-ms ISI (see Fig. 3a; 5.9±1.47%), and an independent

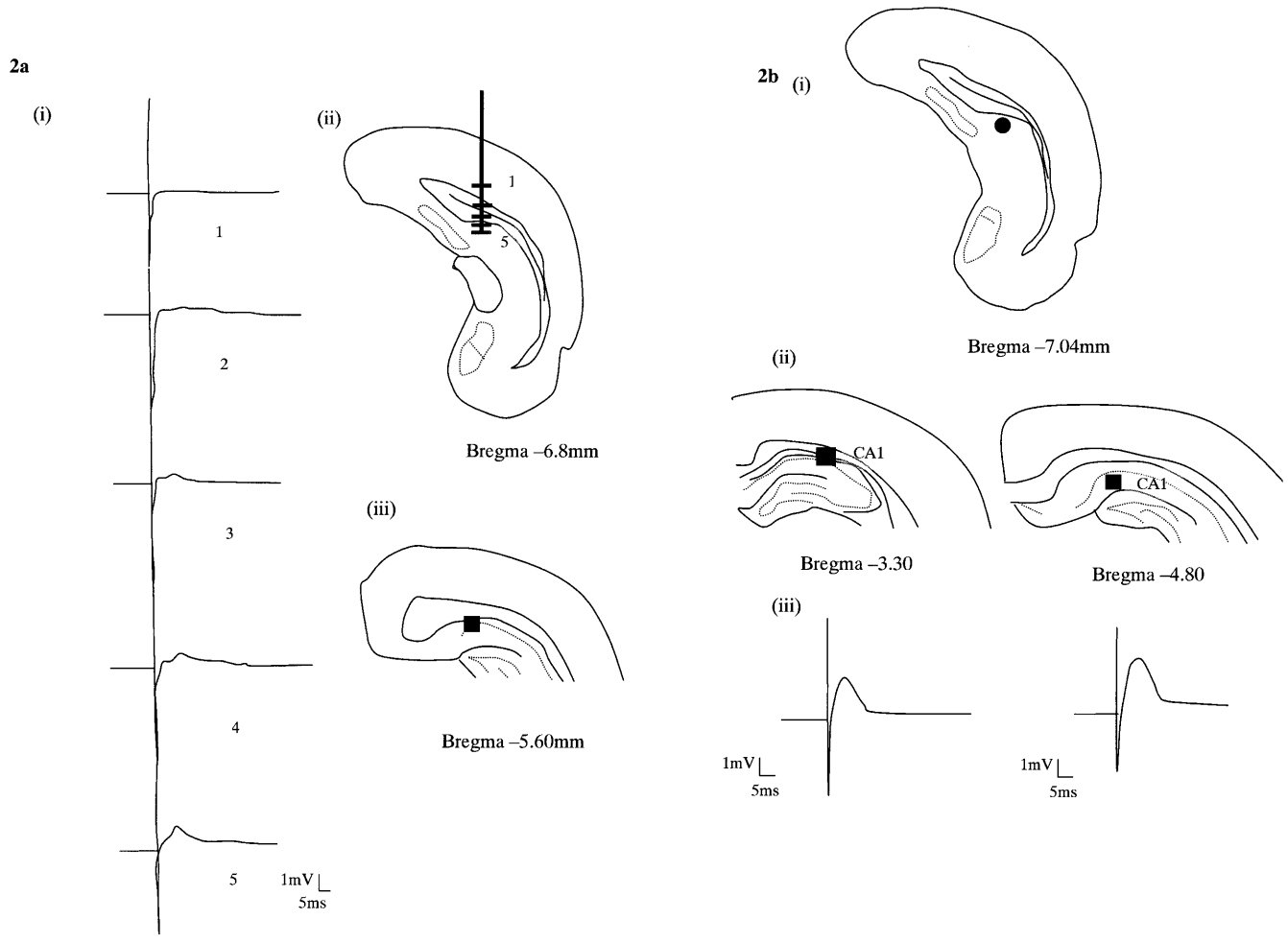

Fig. 2 **a** Depth profile of CA1 field potentials following stimulation in the dorsal subiculum. **a***(i)* A plot of field potentials following stimulation in successive positions from *1* to *5* as the electrode is moved towards dorsal subiculum. *(ii)*, *(iii)* Schematic drawings of the coronal sections indicating the positions of stimulating and recording electrodes located in dorsal subiculum and CA1, respectively. **b** Differences in field potentials recorded in different parts of CA1 along the rostrocaudal axis following stimulation of the same site in dorsal subiculum. *(i)* Schematic drawing of the coronal section indicating the stimulation site in dorsal subiculum. *(ii)* Schematic drawing of the coronal section indicating the recording sites in CA1 at 3.3 and 4.8 mm behind bregma (adapted from Paxinos and Watson 1986). *(iii)* The corresponding field potentials recorded after dorsal subiculum stimulation at the two sites in CA1

t-test confirmed that there was a significant increase in the second synaptic response of the pair, when compared to the first response (t=3.58, df=58, P<0.01). Sample traces at each paired-pulse interval are also given in Fig. 3a.

We examined if the back projection from the dorsal subiculum to area CA1 could sustain LTP (n=5) using HFS. Initially, a baseline was established for 10 min at half-maximal peak amplitude. A short-term potentiation was found immediately following HFS, but the amplitude and slope of the evoked response decreased back to pre-HFS baseline values within 10 min poststimulation. At 5, 15 and 30 min post-HFS amplitude values of the synaptic response stood at 99.04±2.43%, 97.25±4.12% and 96.35±7.24%, respectively, and slope values of the synaptic response at the same time intervals stood at 103.46±6.06%, 99.28±6.5% and 98.01±6.25%, respectively [see Fig. 3b*(i)* and *(ii)*]. A one-way ANOVA was used to compare the 10-min baseline period, 0- to 10-min post-HFS period and 20- to 30-min post-HFS period for synaptic response slope values. An overall significant difference was found between the three time intervals (F=7.22, df=2,87, P<0.01). Subsequent *post hoc* tests (Bonferroni) confirmed that the 0- to 10-min post-HFS values were significantly higher than either the baseline values or the 20- to 30-min post-HFS values (P<0.05). This reflects the short-term potentiation suggested above. Figure 3b also displays representative fEPSP traces taken from baseline, 15 and 30 min post-HFS.

Discussion

The experiments presented here suggest that there is electrophysiological evidence for a back projection or

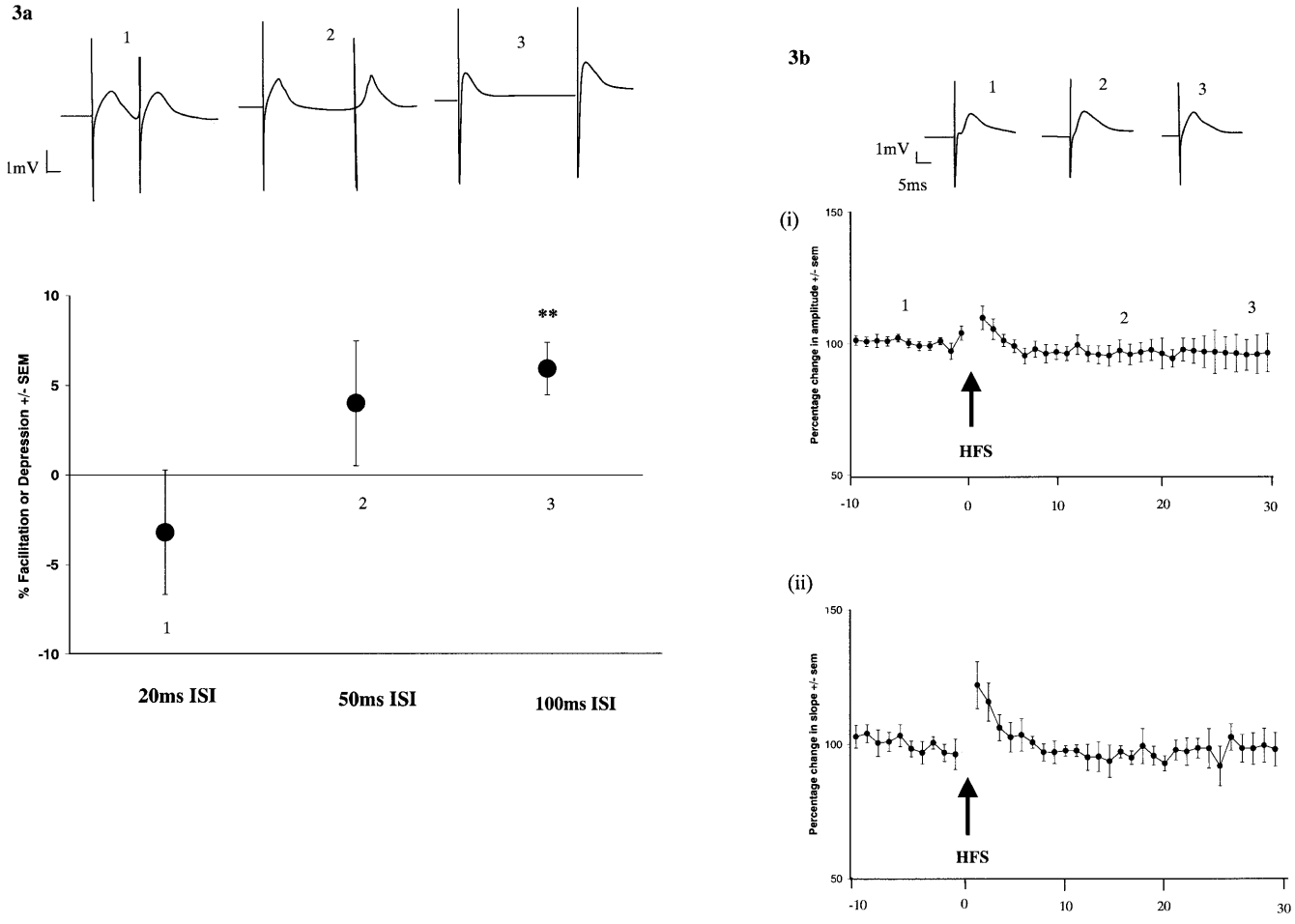

Fig. 3 **a** A plot showing percentage facilitation or depression for 20, 50 and 100 ms interstimulus interval (*ISI*) ± SEM. Sample field potential traces displaying paired-pulse facilitation/depression with *numbers* corresponding to the interval at which the traces were taken. ** $P<0.01$. **b** Effects of high-frequency stimulation (*HFS*) on amplitude *(i)* and slope *(ii)*. The post-HFS values are expressed as a percentage of the prestimulation baseline ± SEM. Responses are averaged over three successive sweeps. Sample field potential traces showing the effects of HFS with *numbers* corresponding to the time where the traces were taken in **b***(i)*

returned projection from the dorsal subiculum to area CA1. Whether this back projection is the "reentrant bundle" of CA1 fibres suggested by Amaral et al. (1991) or is a direct back projection, as suggested by Harris and Stewart (2001), remains controversial. We have been able to describe a physiological response along the extent of the rostrocaudal axis of area CA1. Furthermore, evoked responses were also observed in the alveus, stratum oriens, stratum pyramidale, stratum radiatum and moleculare and not solely in the interface between stratum radiatum and moleculare of area CA1. We would therefore suggest that the responses demonstrated in the above experiments are either oligosynaptic or polysynaptic in nature, as opposed to monosynaptic. The positive-going deflection in the evoked response in the majority of cases and the negative-positive responses also observed may be as a result either of the placement of electrodes with respect to activated synapses or the positive deflections may be a result of activation of local inhibitory circuits. The responses most probably have a mixed mechanism. Although antidromic activity was not observed, the small positive waves may represent a mixture of postsynaptic potentials both by recurrent (antidromically stimulated) and back-projecting (orthodromically stimulated) pathways. Given the presence of a large number of intrinsic bursting neurons in the subiculum (Mason 1993; Taube 1993; O'Mara et al. 2001), this feature of synaptic organisation might prove very useful for setting the gain of hippocampal output. Furthermore, given the short latencies to peak response that we have found, we suggest that this putative projection synapses directly in CA1 and not via an indirect pathway originating in some other area (such as entorhinal cortex).

Although the forward projection from CA1 to the subiculum can sustain both short-term and long-term plastic effects in vivo (Commins et al. 1998a, b, 1999), the back projection does not display such robust plastic effects. We were unable to induce LTP, but did find that there was a short-term potentiation-like effect present. Furthermore, we found that PPF was only evident at the longest ISI tested (100 ms). Both of these results are very much in contrast to the forward projection from area CA1

to subiculum where the PPF effect is evident across a wide range of intervals and LTP can be induced (Commins et al. 1998a, b). Intracellular and extracellular recordings in the subiculum following stimulation in area CA1 have demonstrated that the forward projection is likely to be excitatory (Finch and Babb 1980; Gigg et al. 2000). By contrast, the backward projection may have an inhibitory effect on area CA1, damping the excitatory activity of the forward-projecting CA1-subicular pathway. It is possible that different stimulation protocols such as primed-burst pattern (Rose and Dunwiddie 1986) might induce potentiation. Future experiments should challenge the evoked response by using GABA antagonists such as bicuculline and should also attempt to characterise the neuroanatomy more precisely by using single-unit electrophysiology combined with cell fills.

Acknowledgements This work was supported by the Welcome Trust, the European Commission, the Higher Education Authority/ Enterprise Ireland and the Provost's Fund of Trinity College. We thank John Gigg (Newcastle) and Menno Witter (Amsterdam) for helpful discussions.

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