available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Plastic and metaplastic changes in the CA1 and subicular projections to the entorhinal cortex

*Sarah Craig, Sean Commins**

Department of Psychology, National University of Ireland, Maynooth, Maynooth, Co. Kildare, Ireland

ARTICLE INFO

Article history:
Accepted 2 February 2007
Available online 21 February 2007

Keywords:
Metaplasticity
Functional segregation
Long-term potentiation
Paired-pulse facilitation
Hippocampus

ABSTRACT

The hippocampal formation (HF) is a brain structure critically involved in memory formation. Two major pathways have been identified in the rat; one projection targets the hippocampus via perirhinal cortex and lateral entorhinal cortex (LEC) while another targets the hippocampus via postrhinal cortex and medial entorhinal cortex (MEC). Areas CA1 and subiculum constitute major output structures of HF and target many cortical structures including EC. These return projections are also anatomically segregated with distinct regions of CA1 and subiculum projecting to either the LEC or MEC. We have previously demonstrated that the projections from CA1 and subiculum to the EC are capable of sustaining short- and long-term plastic changes. Here we detail a physiological topography that exists along the hippocampal output projections, equating well with the known anatomy. Specifically, field excitatory postsynaptic potential (fEPSP) responses in LEC are stronger following distal CA1 and proximal subiculum stimulation, compared to either proximal CA1 or distal subiculum stimulation. In addition, fEPSP responses in MEC are stronger following proximal CA1 stimulation compared to distal CA1. We also demonstrate that the distal CA1-LEC, proximal CA1-MEC and proximal subiculum-LEC projections are all capable of frequency-dependent plastic effects that shift the response from LTD to LTP. In addition, responses in distal CA1-LEC projection seem to show metaplastic capabilities. We discuss the possibility of dissociation between LEC and MEC projections, which may suggest two functional circuits from the HF to the cortex and may have implications in information processing, memory research and hippocampal seizure spread to the cortex.

1. Introduction

The hippocampus is a medial temporal lobe structure that is critically involved in the formation of declarative memories (Ogden and Corkin, 1991; Scoville and Milner, 1957). Evidence for this ascertain derives from lesion (Jarrard, 1983, 1993), patient (Cipolotti et al., 2006), imaging (Schacter and Wagner, 1999), and the plastic capabilities of this structure (Martin et al., 2000). Long-term potentiation (LTP), a long-lasting form of synaptic change considered a realistic model of learning and memory was first identified along the perforant pathway (the major input projection to the hippocampus; Bliss and Lomo, 1973; Bliss and Collingridge, 1993). Other forms of activity-dependent changes have been demonstrated throughout the hippocampal circuit, including among others, long-term depression (LTD), paired-pulse facilitation and depression (PPF/D), post-synaptic potentiation (PST) and augmentation (Thomsom, 2000). PPF and PPD are short-term plastic changes

* *Corresponding author*. Fax: +353 1 7084767.
E-mail address: Sean.Commins@nuim.ie (S. Commins).

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.02.016

at the synapse elicited by a brief spike pair. PPF is the phenomenon whereby the field excitatory postsynaptic response (fEPSP) to a second stimulus is enhanced relative to the first, if the second stimulus is delivered relatively quickly after the first (Katz and Miledi, 1970; Zucker, 1989).

It has been increasingly clear that it is not just simply a matter of whether plastic changes can be induced at a particular synapse; rather synapses should be viewed as being dynamic. The dynamic nature of synapses should be seen in terms of the duration of change (short-term changes in the range of milliseconds, in the case of PPF/D to more longer-term changes hours to days, in the case of LTP/D), degree of change and direction of change. Some synapses for example, may demonstrate an increase in efficacy (facilitation and potentiation), while others may decrease (depression). This dynamism is important in the developing cortex (Bienenstock et al., 1982) and is now clear that memory formation and storage may also depend on such changes (Bear et al., 1987).

Traditionally LTP and LTD have been treated as independent entities, evidence, however, now suggests that these processes are bi-directional modifications of the same synaptic mechanism (Castellani et al., 2001; Dudek and Bear, 1993; Heynen et al., 2000). Experimental data obtained from the developing visual cortex have led to a biphasic synaptic modification rule known as the Bienenstock-Cooper-Munro rule (BCM; Bienenstock et al., 1982), with the crossover point from LTD to LTP known as the modification threshold (θm). This threshold is not fixed but varies according to prior postsynaptic activity (Dudek and Bear, 1993). This activity-dependent modulation, termed metaplasticity (Abraham and Bear, 1996) can result from a number of different factors including changes in receptor function, prior synaptic activity and stress (Dudek and Bear, 1992; Garcia, 2001; Gisabella et al., 2003; Van Dam et al., 2004; Wu et al., 2004).

Neuroanatomical research over the last number of years (Witter et al., 2000) has suggested the existence of two parallel pathways through the hippocampal formation that may be involved in separately processing functionally different types of information. The first pathway arises in the perirhinal cortex, projects through the lateral entorhinal cortex (LEC) and terminates in different layers of the dentate gyrus, CA3 and the distal CA1 and proximal subiculum. The second pathway arises in the postrhinal cortex and targets the proximal CA1 and distal subiculum as well as different layers of dentate gyrus and CA3 via the medial entorhinal cortex (MEC). More recent research (Kloosterman et al., 2003) has indicated that the segregation of information is maintained on the return projections from the hippocampus to the cortex. Tracing studies, for example, have shown that the proximal CA1 and distal subiculum target mainly the MEC whereas the distal CA1 and proximal subiculum target the LEC (Kloosterman et al., 2003; Tamamaki and Nojyo, 1995). The importance of identifying hippocampal–cortical projections that are physiologically as well as anatomically connected lies in the suggestion that one or all of these projections may serve as functional routes along which memories may be retained. Indeed, many current theories of memory formation highlight the importance of hippocampal–cortical interactions for the consolidation of declarative memories (Nadel and Moscovitch, 1997; Rolls, 1996; Squire, 1992). Furthermore, some theories (Rolls, 1996) specify that the backprojections from the hippocampus to the neocortex must undergo activity-dependent changes in order for memories to be retained in the long-term.

Recently we have demonstrated that the projection from CA1-EC and subiculum-EC can undergo activity-dependent changes in the form of PPF and LTP (Craig and Commins, 2005, 2006); however, as suggested above it is becoming increasingly recognised that it is not simply a question of whether synapses can become potentiated or not, but rather, it is important to understand the full range of dynamic plastic capabilities of a particular synapse to fully appreciate the role of plasticity in memory formation.

In a first set of experiments we aim to detail electrophysiologically the topographical nature of the CA1 and subicular projections to EC, from this, we wish to examine the plastic and metaplastic capabilities of these projections. Specifically, we aim to elucidate whether the CA1 and/or subicular-EC projections are capable of frequency-dependent plasticity, that is, are the projections capable of shifting from LTD to LTP simply by varying the frequency applied to the particular synapse. In other words, does each projection fit the BCM model of synaptic plasticity and if so what is the modification threshold of each projection? Furthermore once a change has occurred in the responsiveness of a projection, is that projection capable of further change by applying a second stimulation at any give frequency?

2. Results

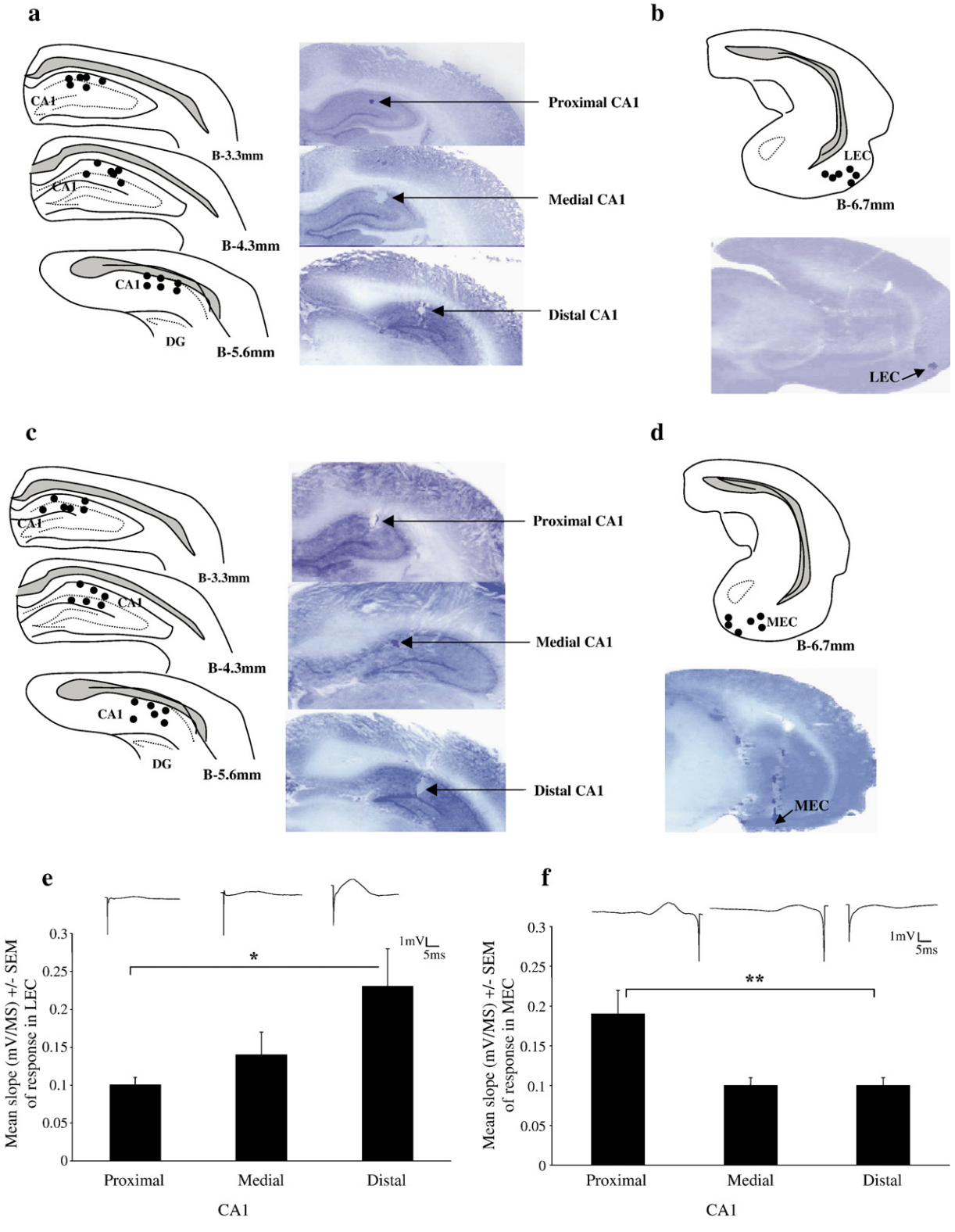
2.1. *General description of electrode placement sites*

2.1.1. *Lateral EC responses following CA1 stimulation*

In all cases ($n=6$) a response was evoked in the LEC following stimulation in area CA1. Figs. 1a (first panel) and b (upper panel) shows the distribution of the approximate final positions of all stimulating and recording sites. Fig. 1a (second panel) shows 3 representative Nissl-stained coronal slices with proximal, medial and distal CA1 electrode tracks, while Fig. 1b (lower panel) shows an electrode track in LEC. The final stimulating sites were positioned along the entire proximo-distal extent of CA1. The positions of the stimulating electrodes were all located between 3.1 mm and 5.8 mm posterior to Bregma. In addition the final positions of the recording electrodes in LEC were all located between 6.7 mm and 7.2 mm posterior to Bregma.

The recording electrode was first lowered to 6 mm below the surface of the brain and allowed to settle in the LEC. Then, the stimulating electrode was slowly lowered towards the proximal CA1 with stimulation conducted at a rate of 0.05 Hz. When the maximal fEPSP response was achieved in the LEC, the stimulating electrode was allowed to settle for 10 min and various features of the fEPSP were noted, including amplitude, slope and latency of response. The response occurred at a mean latency value of 14.67±0.34 ms and had a mean peak amplitude value of 0.34±0.08 mV and a slope of 0.1±0.02 mV/ms. While recording electrode remained in place in LEC the stimulating electrode was then removed

slowly from the proximal CA1 and then lowered towards medial CA1 until a maximal response was again achieved. A single-pulse stimulation in medial CA1 evoked a larger positive-going deflection in the lateral EC. The mean amplitude of entorhinal response for medial CA1 stimulation was 0.48±0.10 mV with a mean latency of 15.12±0.31 ms. The mean slope was 0.14±0.03 mV/ms. Finally, the stimulating electrode was again slowly removed and then lowered to distal CA1 until the maximum response was observed in LEC. The mean amplitude was 1.29±0.15 mV and the latency was 11.15±0.5 ms. The mean slope was 0.21±0.06 mV/ms. A one-way ANOVA was conducted to compare the mean of three fEPSP responses in LEC following stimulation in either the proximal, medial or distal CA1. An overall significant



difference was found in the mean slope of the responses ($F=3.787$, $df=2,17$, $p<0.05$), with subsequent *post hoc* analysis (Tukey, $p<0.05$) demonstrating that the evoked response in LEC was significantly larger following stimulation in distal CA1 compared to the proximal region (Fig. 1e).

2.1.2. *Medial EC responses following CA1 stimulation*

In all cases ($n=6$) a response was evoked in the MEC following stimulation in area CA1. Fig. 1c (first panel) and 1d (lower panel) show the distribution of the final positions of all stimulating and recording sites. Fig. 1c (second panel) shows 3 representative Nissl-stained coronal sections with proximal, medial and distal CA1 electrode tracks, while Fig. 1d (lower panel) shows an electrode track in MEC. The final stimulating sites were positioned along the entire proximo-distal extent of CA1. The positions of the stimulating electrodes were all located between 3.1 mm and 5.8 mm posterior to Bregma. In addition the final positions of the recording electrodes in MEC were again approximately located between 6.7 mm and 7.2 mm posterior to Bregma.

The recording electrode was first lowered to the MEC and allowed to settle, then, the stimulating electrode was slowly lowered towards the proximal CA1 with stimulation conducted at a rate of 0.05 Hz. When the maximal fEPSP response was achieved in the MEC, the stimulating electrode was allowed to settle for 10 min and the fEPSP slope amplitude and latency of response were again noted. As the stimulating electrode settled in the proximal CA1, the response in MEC was characterised by a positive-going deflection that occurred at a mean latency value of 16.82±1.38 ms and had a mean peak value of 1.58±0.24 mV and a slope of 0.19±0.03 mV/ms. The stimulating electrode was removed from the proximal CA1 and slowly lowered towards the medial CA1 while recording continued to take place in MEC. As stimulating electrode settle in the medial CA1 a small positive-going response occurred in MEC. The mean amplitude for the response was 0.52±0.07 mV and the mean latency was 12.83±0.79 ms. The mean slope was 0.10±0.01 mV/ms. Finally the stimulating electrode was placed in the distal CA1; this produced a small response characterised by a mean fEPSP response in the MEC of amplitude of 0.41±0.11 mV and latency of 11.43±0.45 ms. The mean slope was 0.1±0.01 mV/ms. A one-way ANOVA was again conducted to compare the mean of three fEPSP responses in LEC following stimulation in either the proximal, medial or distal CA1. An overall significant difference was found in the mean slope of the responses ($F=7.102$, $df=2,17$, $p<0.01$), with subsequent *post hoc* analysis (Tukey, $p<0.05$) demonstrating that the evoked response in LEC was significantly larger following stimulation in proximal CA1 compared to the distal ($p<0.01$) or medial ($p<0.05$) regions (Fig. 1f).

2.1.3. *Lateral EC responses following subicular stimulation*

In all cases ($n=12$) a response was evoked in the LEC following stimulation in the subiculum. Fig. 2a (first and third panel) shows the distribution of the approximate final positions of all stimulating and recording sites. Fig. 2a (second and fourth panel) shows representative Nissl-stained coronal sections, with stimulating and recording electrode tracks. The stimulating sites were positioned along the entire proximo-distal extent of the subiculum. Proximal electrodes are indicted by a circle, medial electrodes by a triangle and distal electrodes by a square. The positions of the stimulating electrodes were all located between 6.1 mm and 6.4 mm posterior to Bregma. In addition the final positions of the recording electrodes in LEC were all located between 6.7 mm and 7.2 mm posterior to Bregma. A similar procedure to CA1-EC recordings (see above) was adopted here. The recording electrode was allowed to settle in the LEC while the stimulating electrode was slowly lowered towards the proximal subiculum. When the maximal fEPSP response was achieved in the LEC, the stimulating electrode was allowed to settle for 10 min and the fEPSP was characterised. A single-pulse stimulation in the proximal subiculum evoked a positive-going deflection in the LEC with a mean latency value of 13.98±1.36 ms and a mean peak value of 2.10±0.28 mV and a slope of 0.54±0.09 mV/ms. Following this, only the stimulating electrode was removed and aimed towards the medial subiculum. Stimulation in the medial subiculum was again characterised by a positive-going deflection. The mean amplitude was 1.15±0.25 mV, the mean latency of 12.67±2.00 ms and the mean slope of 0.22± 0.05 mV/ms. A smaller response was observed as the electrodes were lowered towards the distal subiculum. The mean amplitude of this response was 0.77±0.30 mV with a latency of 18.93±1.35 ms and a mean slope of 0.22±0.04 mV/ms. A one-way ANOVA was conducted to compare the mean of three fEPSP responses in LEC following stimulation in either the proximal, medial or distal subiculum. An overall significant difference was found in the mean slope of the responses ($F=7.736$, $df=2,35$, $p<0.01$), with subsequent *post hoc* analysis (Tukey, $p<0.05$) demonstrating that the evoked response in LEC was significantly larger following stimulation in proximal subiculum compared to either the distal ($p<0.01$) or medial ($p<0.01$) regions (Fig. 2c).

Fig. 1 – (a, first panel) Distribution of the approximate final positions of all stimulating sites ($n=6$) in proximal, medial and distal CA1. (a, second panel) Three representative Nissl-stained coronal slices highlighting the stimulating electrode track targeting proximal, medial and distal CA1. (b, upper panel) Distribution of the approximate final positions of all recording sites in LEC. (b, lower panel) Shows a representative Nissl-stained coronal slice of an electrode track targeting LEC. (c, first panel) Distribution of the approximate final positions of all stimulating sites in proximal, medial and distal CA1 ($n=6$). (c, second panel) Three representative Nissl-stained coronal slices highlighting the stimulating electrode track targeting proximal, medial and distal CA1. (d) Distribution of the approximate final positions of all recording sites in MEC (upper panel) with a representative Nissl-stained coronal slice of an electrode track targeting MEC (lower panel). (e) Bar chart demonstrating the mean slope of the evoked fEPSP response in the LEC following stimulation in proximal, medial and distal CA1 with representative traces. (f) Bar chart demonstrating the mean slope of the evoked fEPSP response in the MEC following stimulation in proximal, medial and distal CA1 with representative traces.

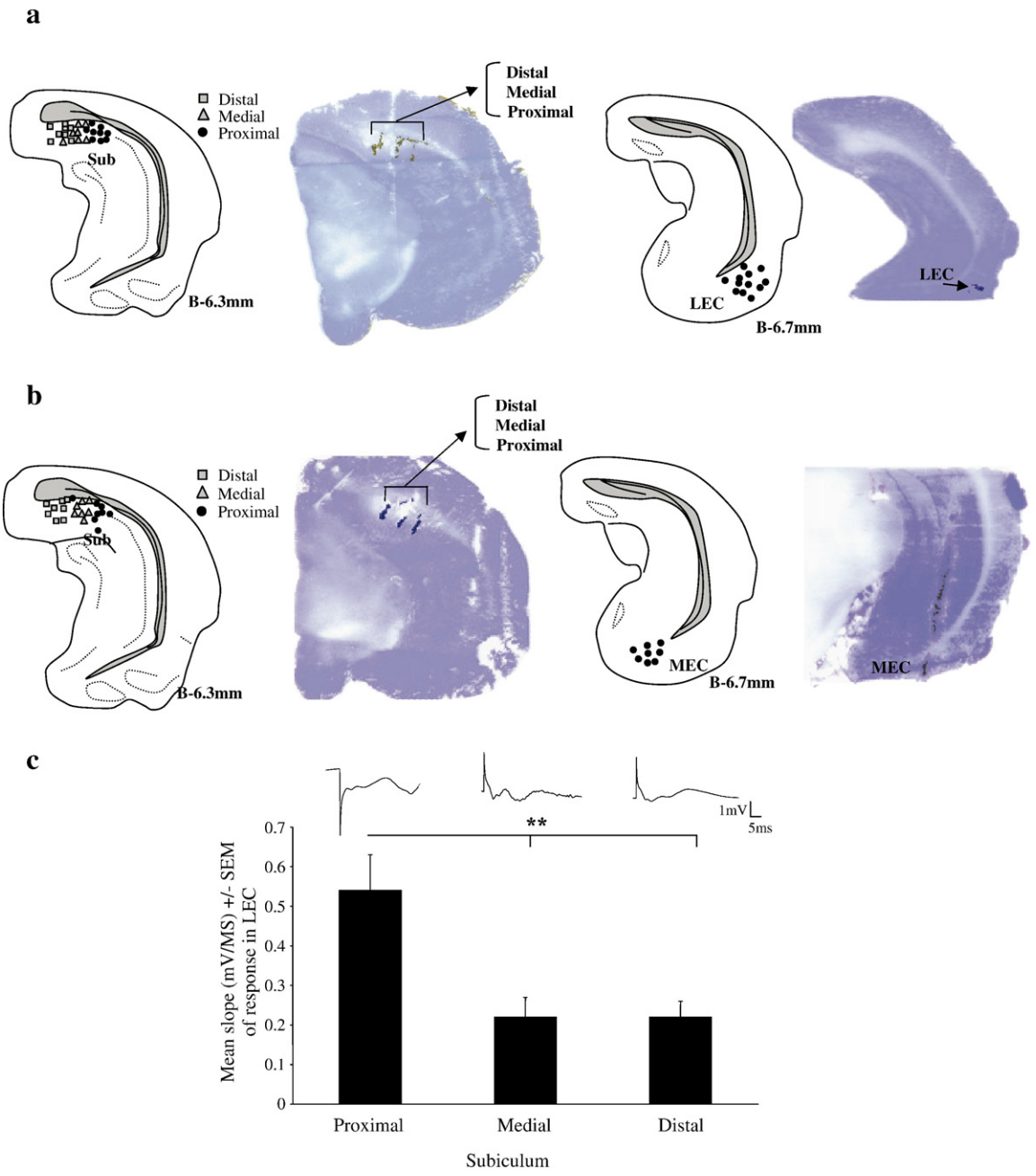


Fig. 2 – (a, first panel) Distribution of the approximate final positions of stimulating sites (n=12) in proximal (filled circle), medial (grey triangle) and distal subiculum (grey square). (a, second panel) A representative Nissl-stained coronal slices highlighting the stimulating electrode track targeting proximal, medial and distal subiculum. (a, third panel) Distribution of the approximate final positions of all recording sites in LEC. (a, fourth panel) Representative Nissl-stained coronal slice of an electrode track targeting LEC. (b, first panel) Distribution of the approximate final positions of stimulating sites (n=8) in proximal (filled circle), medial (grey triangle) and distal subiculum (grey square). (b, second panel) A representative Nissl-stained coronal slices highlighting the stimulating electrode track targeting proximal, medial and distal subiculum. (b, third panel) Distribution of the approximate final positions of all recording sites in MEC. (b, fourth panel) Representative Nissl-stained coronal slice of an electrode track targeting MEC. (c) Bar chart demonstrating the mean slope of the evoked fEPSP response in the LEC following stimulation in proximal, medial and distal subiculum with representative traces.

2.1.4. *Medial EC responses following subicular stimulation*

In all cases (n=8) no response was evoked in the MEC following stimulation in the subiculum. Fig. 2b (first and third panels) shows the approximate distribution of the final positions of all stimulating and recording sites. Fig. 2b (second panel) shows a representative Nissl-stained coronal section with proximal, medial and distal subiculum electrode tracks, while Fig. 2b (fourth panel) shows an electrode track in MEC. The stimulating sites were positioned along the entire proximo-distal extent of the subiculum, located

just below the corpus callosum. The positions of the stimulating electrodes were all located between 6.1 mm and 6.4 mm posterior to Bregma. In addition, the final positions of the recording electrodes in MEC were all located between 6.7 mm and 7.2 mm posterior to Bregma. Unfortunately, in our hands, a single-pulse stimulation in the

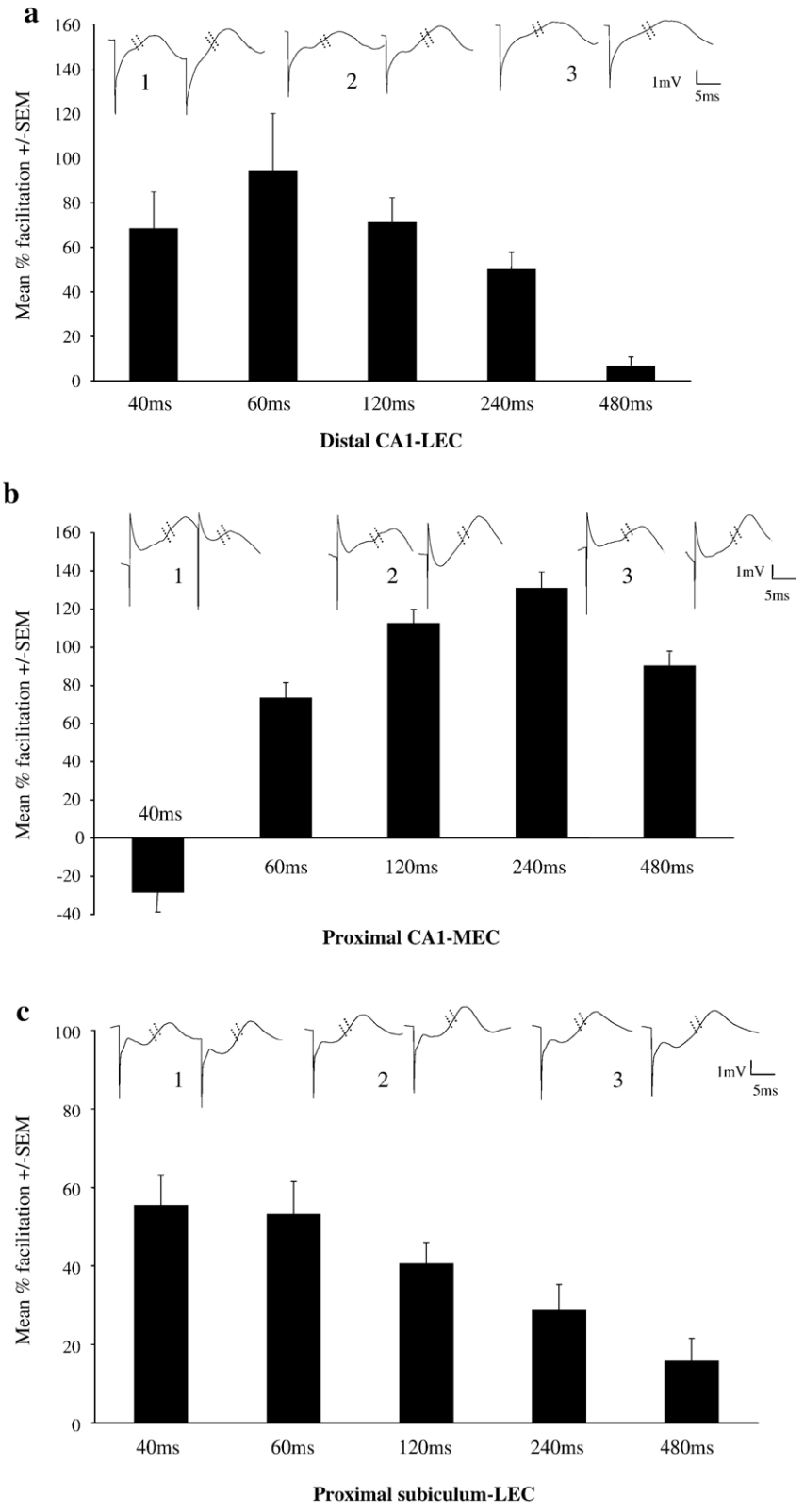


Fig. 3 – Bar chart demonstrating mean percentage facilitation in fEPSP2 normalised to fEPSP1 (100%) with 40, 60, 120, 240 and 480 ms IPIs in the (a) distal CA1-LEC, (b) proximal CA1-MEC and (c) proximal subiculum-LEC projections. Representative traces of fEPSP1 and fEPSP2 for the 20, 60 and 480 ms intervals are illustrated above the graph (1, 2 and 3 respectively).

proximal, medial or distal subiculum failed to evoke a measurable response in the MEC.

2.2. Short-term plastic effects in the hippocampal outputs to EC

In a second set of experiments paired-pulse facilitation or depression was examined in the projections that produced maximal responses, that is, distal CA1-LEC, proximal CA1-MEC and proximal subiculum-LEC.

Using five inter-pulse-intervals (IPIs; 40 ms, 60 ms, 120 ms, 240 ms and 480 ms), PPF was measured at the different IPIs six times in at least 25 animals in the distal CA1-LEC projection. The mean percentage facilitation for 40 ms, 60 ms, 120 ms, 240 ms and 480 ms intervals is 168±16%, 194±25%, 171±11%, 150±7% and 106±4%, respectively (normalised to the first fEPSP of each pair (100%)). A strong PPF effect is evident across all intervals tested (except 480 ms) in this projection ($p<0.01$) (Fig. 3a). A one-way ANOVA was then used to compare the mean percentage facilitation across the different IPIs. An overall significant main effect was found ($F=4.775$, $df=4,20$, $p<0.01$) between the IPIs tested with subsequent *post hoc* tests ($p<0.05$) revealing that the mean percentage facilitation at the 480 ms IPI was significantly lower compared to all other intervals.

Baseline PPF/D was also examined in the proximal CA1-MEC projection using the same five IPIs. The mean percentage facilitation for 40 ms, 60 ms, 120 ms, 240 ms and 480 ms intervals is 72±24%, 173±18%, 212±20%, 230±26% and 190±14% respectively. A strong PPF effect is evident across most intervals tested, including the 480 ms IPI ($p<0.01$), while PPD is observed following the 40 ms IPI ($p<0.05$) (Fig. 3b). Again a one-way ANOVA was used to compare the mean percentage facilitation across the different IPIs. An overall significant main effect was again found ($F=7.404$, $df=4,20$, $p<0.01$) between the IPIs tested with subsequent *post hoc* tests ($p<0.05$) demonstrating that the mean percentage facilitation at the 40 ms IPI was significantly lower than all other intervals as would be predicted from Fig. 3b.

Finally, baseline PPF/D was also examined in the proximal subiculum-LEC projection using the same five IPIs as before. The mean percentage facilitation for 40 ms, 60 ms, 120 ms, 240 ms and 480 ms intervals is 155±7%, 153±8%, 140±5%, 128±6% and 115±5%, respectively (see Fig. 3c). A strong PPF effect is evident across most intervals tested except for 480 ms IPI ($p<0.01$). A one-way ANOVA was conducted to compare the mean facilitation at the different IPIs. An overall significant main effect was found ($F=11.701$, $df=4,20$, $p<0.01$) between the IPIs tested with subsequent *post hoc* tests ($p<0.05$) revealing that the mean percentage facilitation at the 40 ms and 60 ms IPI was significantly higher than the facilitation at 240 and 480 ms, while the mean percentage facilitation the 120 ms IPI was significantly higher than that at the 480 ms IPI.

2.3. Long-term frequency-dependent plasticity in the hippocampal outputs to EC

2.3.1. Distal CA1-LEC

Initially a 3×5 mixed factorial ANOVA was conducted to examine the effects of frequency stimulation and time of application of stimulation on the plastic capabilities in the distal CA1-LEC projection. TIME was used as the within-subjects factor (conducted at three levels; 10 min baseline period, 50–60 min post-first stimulation and 50–60 post-second stimulation). GROUP was used as the between-subject factor (conducted at five levels; 1 Hz, 5 Hz, 10 Hz, 50 Hz and 100 Hz). We found an overall significant main effect for TIME ($F=14.518$, $df=2,46$, $p<0.01$), Group ($F=9.149$, $df=4,23$, $p<0.01$) and also a significant TIME×GROUP interaction effect ($F=4.646$, $df=8,46$, $p<0.01$). Investigation of within-group effects of TIME at each frequency of stimulation revealed the following effects.

The mean slope of the fEPSP response in LEC at 30 and 60 min post-1 Hz stimulation in distal CA1 ($n=6$) stood at 91.17±3.23 and 96.66±4.8%, respectively of pre-stimulation baseline responses. A second stimulation at 1 Hz produced a small increase in fEPSP responses immediately (113.7±7.6%) post-stimulation and remained increased for the next 60 min. Responses at 30 and 60 min post-second stimulation, for example, stood at 124.26±4.5 and 121.6±2.4%, respectively (Fig. 4a). A repeated-measures ANOVA comparing the final 10-min period post-second stimulation to the final 10 min period post-first stimulation and the 10 min baseline period found an overall significant main effect for the three time periods ($F=6.898$, $df=2,10$, $p<0.05$). Bonferroni *post hoc* tests revealed a significant increases in mean responses for the final 10 min post-second stimulation compared to the final 10 min post-first stimulation ($t=-2.942$, $df=5$, $p<0.05$). There was, however, no significant change in mean slope of fEPSPs for the final 10 min of recording post-first ($t=0.209$, $df=5$, $p>0.05$) or second stimulation ($t=-2.372$, $df=5$, $p=0.07$) compared to 10-min baseline period (see Fig. 4a).

Following 5 Hz stimulation ($n=6$), the fEPSP response initially decreased at 15 min (75.79±7.12%) and remained depressed for at least 30 min (80.91±9.2%). This response then gradually increased back to near baseline levels at 45 and 60 min post-stimulation (90.27±7.56 and 90.29±5.18%, respectively). A second 5 Hz stimulation also did not produce significant changes in fEPSP responses. Responses stood at 94.8±6.7 and 89.7±8.5% at 30 and 60 min post-second stimulation respectively (Fig. 4b). Comparisons of the three 10 min periods (final 10-min period post-second stimulation, final 10 min period post-first stimulation and 10 min baseline period) revealed no significant changes in mean fEPSP responses ($F=0.901$, $df=2,10$, $p>0.05$). Bonferroni *post hoc* tests also revealed no significant differences between any of the time periods tested.

Following 10 Hz stimulation ($n=6$), fEPSP responses at 30 and 60 min stood at 66.47±3.89 and 83.83±4.9%, respectively of pre-stimulation response. A second 10 Hz stimulation did not cause further depression but responses gradually returned to baseline levels. Responses for example stood at 89.9±2.55 and 97.2±2.33% at 30 and 60 min post-second 10 Hz stimulation, respectively (see Fig. 4c). A repeated-measures ANOVA comparing the final 10-min period post-second stimulation to the final 10 min period post-first stimulation and the 10 min baseline period did not find an overall significant main effect for the three time periods ($F=2.457$, $df=2,10$, $p>0.05$). *Post hoc* comparisons, however,

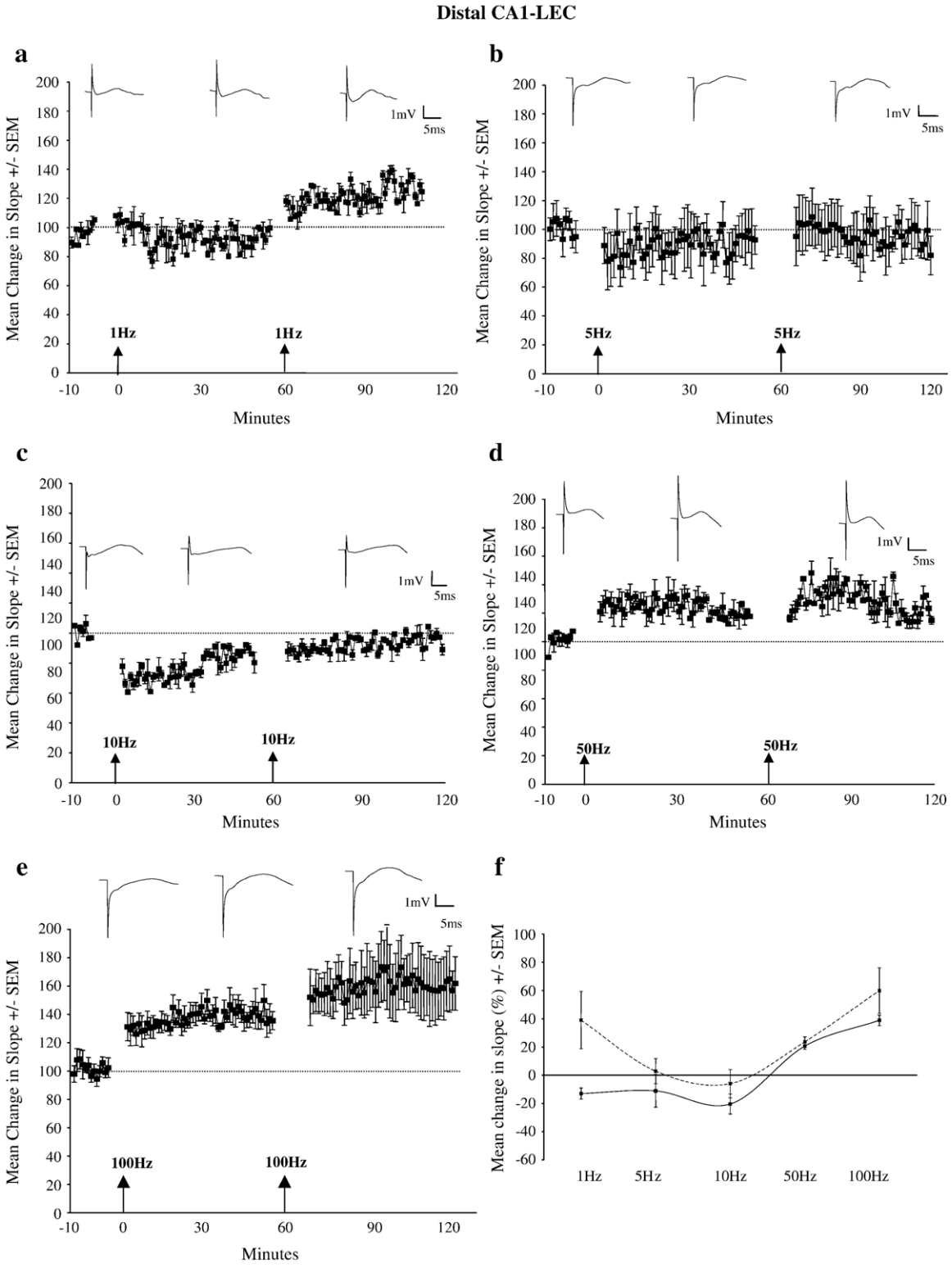


Fig. 4 – Effect of (a) 1 Hz, (b) 5 Hz, (c) 10 Hz, (d) 50 Hz and (e) 100 Hz stimulation on the slope of fEPSPs in the distal CA1-LEC projection (n=6 for each frequency tested). Stimulations are given twice, the first after a 10-min baseline period and the second 60 min post-first stimulation. The post-stimulation values are expressed as percentage of the pre-stimulation baseline ± SEM. Representative traces for baseline, post-first stimulation and post-second stimulation are located above each figure. (f) A line chart plotting the average mean change in fEPSP slope at each frequency for the final 10 min of recording post-first stimulation (black line) and post-second stimulation (dashed line).

revealed that the mean responses of the final 10-min period post-first stimulation were significantly smaller than the mean baseline responses ($t=2.692$, $df=5$, $p<0.05$). No other differences were noted.

Following 50 Hz stimulation ($n=6$) fEPSP responses at 30 and 60 min stood at 116.37±3.28 and 110.51±4.3% respectively that of pre-stimulation responses. While a second 50 Hz stimulation did not produce significant changes in fEPSP responses. Responses remained potentiated at 129± 5.2 and 119±4.9% at 30 and 60 min post-second stimulation respectively (see Fig. 4d). A repeated-measures ANOVA found an overall significant main effect for the three time periods tested ($F=18.178$, $df=2,10$, $p<0.01$). Bonferroni *post hoc* tests revealed significant increases in mean responses for the final 10 min post-first and post-second stimulation compared to baseline period ($t=-5.111$, $df=5$, $p<0.01$ and $t=-4.172$, $df=5$, $p<0.01$, respectively). There was, however, no significant change in mean slope of fEPSPs for the final 10 min of recording post-first compared to final 10 min of recording post-second stimulation ($t=-1.4$, $df=5$, $p>0.05$).

Finally following 100 Hz stimulation ($n=5$) fEPSP responses at 30 and 60 min stood at 143.27±6.4 and 140.22±1.55%, respectively that of pre-stimulation responses. A second stimulation at 100 Hz seemed to enhance this potentiation. Responses stood at 165±16 and 156±13% at 30 and 60 min post-second 100 Hz stimulation, respectively (Fig. 4e). A repeated-measures ANOVA again found an overall significant main effect for the three time periods tested ($F=8.935$, $df=2,8$, $p<0.01$). Bonferroni *post hoc* tests again revealed a significant increase in mean responses for the final 10 min post-first and post-second stimulation compared to baseline period ($t=-8.394$, $df=4$, $p<0.01$ and $t=-3.158$, $df=4$, $p<0.05$, respectively). There was, however, no significant change in mean slope of fEPSPs for the final 10 min of recording post-first compared to final 10 min of recording post-second stimulation ($t=-1.387$, $df=4$, $p>0.05$).

Fig. 4f summaries the results described above by plotting the average mean change in fEPSP slope at each frequency for the final 10 min of recording post-first stimulation (black line) and post-second stimulation (dashed line). The modification threshold occurs at approximately 25 Hz (axis cross-over of black line). As BCM model predicts that synaptic function can vary depending on the previous history of that synapse (Bienenstock et al., 1982), in order to directly test whether this projection was capable of a metaplastic effects, the mean change in response in the final 10 min following the second and first frequency stimulations only was compared. This analysis was carried out using a 2×5 repeated-measures ANOVA. TIME (first stimulation Vs second stimulation) was used for the within-subjects measures and GROUP (1, 5, 10, 50 and 100) was used as the between-subjects measure. An overall significant main effect for TIME ($F=16.13$, $df=1,23$, $p<0.01$) was found suggesting that this projection is capable of metaplastic effects. There was also a main effect for GROUP ($F=8.354$, $df=4,23$, $p<0.001$). Subsequent *post hoc* tests demonstrated that the responses following 100 Hz were significantly higher than those following 5 or 10 Hz. In addition, we found a significant TIME×GROUP interaction effect ($F=3.073$, $df=4,23$, $p<0.05$).

2.3.2. *Proximal CA1-MEC*

Again a 3×5 mixed factorial ANOVA was conducted to examine the effects of frequency stimulation and time of application of stimulation on the plastic capabilities this time in the proximal CA1-MEC projection. TIME was used as the within-subjects factor (again conducted at three levels; 10 min baseline period, 50–60 min post-first stimulation and 50–60 post-second stimulation). GROUP was used as the between-subject factor (conducted at five levels; 1 Hz, 5 Hz, 10 Hz, 50 Hz and 100 Hz). We found no overall significant main effect for TIME ($F=1.201$, $df=2,42$, $p>0.05$), we did, however, find overall significant main effect for GROUP ($F=4.759$, $df=4,21$, $p<0.01$) and also a significant TIME×GROUP interaction effect ($F=3.378$, $df=8, 42$, $p<0.01$). This then allowed us to investigate within-group effects at each frequency of stimulation.

The mean slope of the fEPSP response in MEC following 1 Hz stimulation in the proximal CA1 ($n=5$) decreased initially at 15 min (76.88±1.14%) and then started to increase at 30, 45 and 60 min (88.03±1.2, 91.84±1.83, 92.66±0.74%, respectively). A second stimulation at 1 Hz in this projection leads to an initial depression in response before gradually increasing to just below baseline levels. Responses stood at 72±7% and 77.9±2.8% at 30 and 60 min post-second stimulation, respectively (see Fig. 5a). A repeated-measures ANOVA comparing the final 10- min period post-second stimulation to the final 10 min period post-first stimulation and the 10 min baseline period found no overall significant main effect for the three time periods ($F=1.814$, $df=2,8$, $p>0.05$). Bonferroni *post hoc* tests, however, revealed a significant decrease in mean responses for the final 10 min post-second stimulation compared to the final 10 min post-first stimulation ($t=2.886$, $df=4$, $p<0.05$). No other differences were noted.

Following 5 Hz stimulation ($n=5$), the fEPSP response at 30 and 60 min stood at 69.92±1.3 and 70.30±2.04%, respectively, of pre-stimulation response. A second stimulation did not change this depression. Responses at 30 and 60 min post-second stimulation stood at 71±4% and 72±3.5%, respectively (see Fig. 5b). A repeated-measures ANOVA comparing the three time periods found an overall significant main effect ($F=5.866$, $df=2,8$, $p<0.05$). Bonferroni *post hoc* tests revealed no significant change in mean responses for the final 10 min post-second stimulation compared to the final 10 min post-first stimulation ($t=-0.371$, $df=4$, $p>0.05$). We did find, however, a significant decrease in mean response for both the final 10 min post-first stimulation ($t=5.559$, $df=4$, $p<0.01$) and post-second stimulation compared to baseline responses ($t=2.3$, $df=4$, $p=0.05$).

Following 10 Hz stimulation, fEPSP responses at 30 and 60 min stood at 67.3±1.7 and 72.04±1.42%, respectively of pre-stimulation response ($n=5$). A second stimulation at 10 Hz did not cause any further depression rather responses remained unchanged for a further 60 min. Responses, for example, at 30 and 60 min post-second stimulation remained at 64±4% and 75±2%, respectively (see Fig. 5c). A repeated-measures ANOVA was again conducted to examine the baseline period with the final 10 min of recording post-first stimulation and the mean response of the final 10 min post-second stimulation. An overall significant main effect was found ($F=18.28$, $df=2,8$, $p<0.01$), with Bonferroni *post hoc* tests revealing a significant decrease in the mean fEPSP response for both the final 10 min

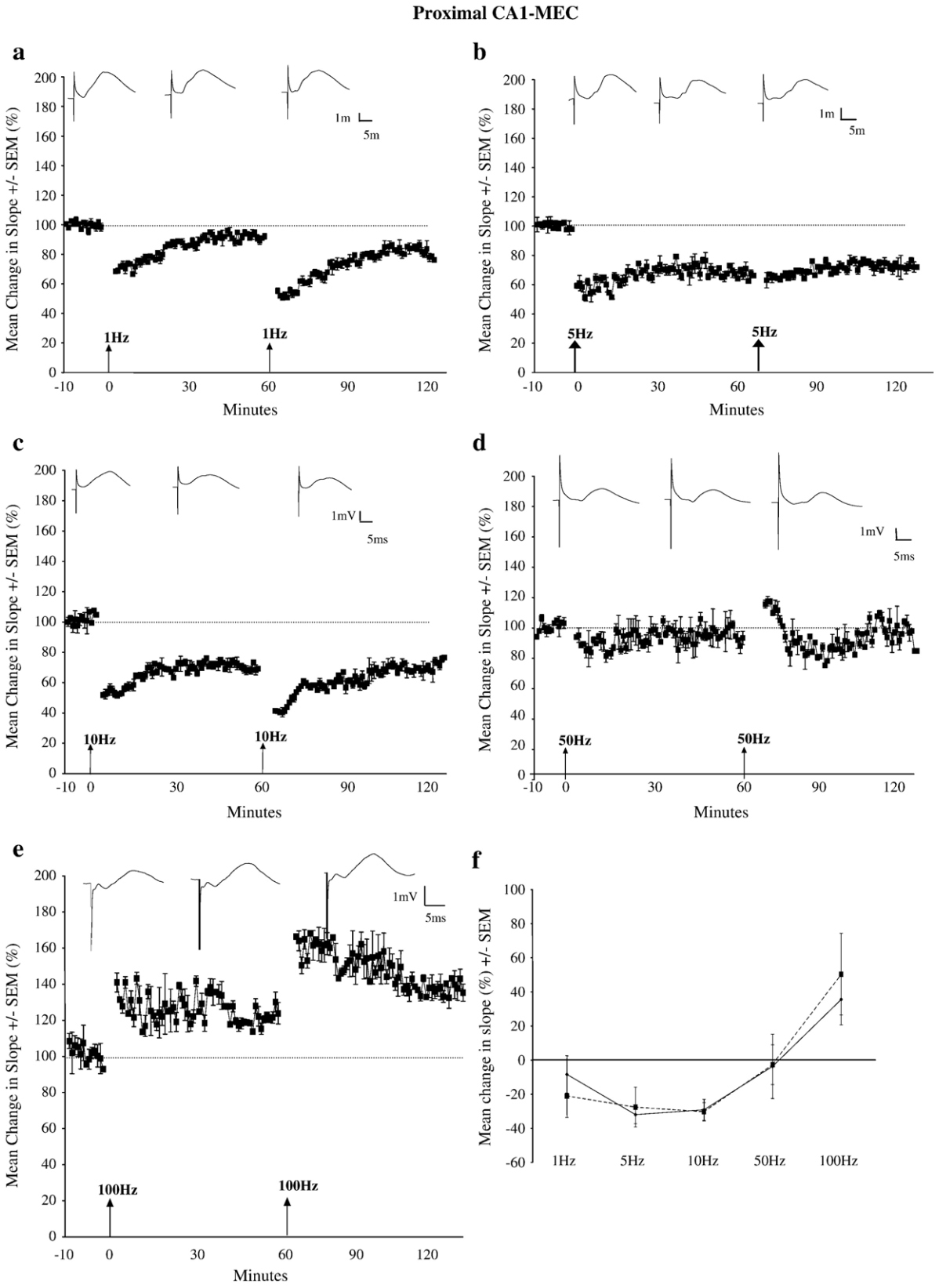


Fig. 5 – Effect of (a) 1 Hz, (b) 5 Hz, (c) 10 Hz, (d) 50 Hz and (e) 100 Hz stimulation on the slope of fEPSPs in the proximal CA1-MEC projection ($n=6$ for each frequency tested). Stimulations are given twice, the first after a 10-min baseline period and the second 60 min post-first stimulation. The post-stimulation values are expressed as percentage of the pre-stimulation baseline ± SEM. Representative traces for baseline, post-first stimulation and post-second stimulation are located above each figure. (f) A line chart plotting the average mean change in fEPSP slope at each frequency for the final 10 min of recording post-first stimulation (black line) and post-second stimulation (dashed line).

recording period post-first stimulation ($t=4.449$, $df=4$, $p<0.01$) and post-second stimulation compared to baseline responses ($t=7.079$, $df=4$, $p=0.05$). No significant differences were noted for the two post stimulation periods (i.e. 50–60 min post-first stimulation and 50–60 min post-second stimulation, $t=0.170$, $df=4$, $p>0.05$).

Following 50 Hz stimulation fEPSP responses at 30 and 60 min post-stimulation stood at 92.68±3.4 and 93.74±2.79%, respectively of pre-stimulation responses ($n=6$). A second stimulation at 50 Hz did not change these responses. Responses at 30 and 60 min post-second stimulation stood at 96±5% and 98±1%, respectively (see Fig. 5d). A repeated-measures ANOVA confirmed these findings with no significant differences noted between the three time periods tested ($F=0.063$, $df=2,10$, $p>0.05$).

Finally, following 100 Hz stimulation, long-term potentiation was induced. Field EPSP responses at 30 and 60 min stood 124.01±3.35 and 124.7±3.02% respectively that of pre-stimulation responses ($n=5$). A second stimulation at 100 Hz seemed to enhance this potentiation further. Responses stood at 149±8% and 135±4% at 30 and 60 min post-second stimulation, respectively (Fig. 5e). A repeated-measures ANOVA found no significant differences between the three time periods tested ($F=3.090$, $df=2,8$, $p>0.05$). However, Bonferroni *post hoc* tests revealed a significant increase in the mean fEPSP response for the final 10 min recording period post-first stimulation compared to 10 min baseline recordings ($t=-2.717$, $df=4$, $p=0.05$). No other differences were noted, due to the variability in responses.

Fig. 5f summaries the results described above by plotting the average mean change in fEPSP slope at each frequency for the final 10 min of recording post-first stimulation (black line) and post-second stimulation (dashed line). The modification threshold for this projection seems to occur at around 50 Hz (black line crossing *x*-axis). Again, to directly examine any metaplastic effects on the proximal CA1-MEC projection we compared the mean change in response following the second and first frequency stimulations at each frequency tested. Using a repeated ANOVA with TIME as the within-subjects and GROUP as the between-subjects measure, we found no significant main effect for TIME ($F=0.82$, $df=1,21$, $p>0.05$) suggesting that metaplastic effects were difficult to achieve on this projection. We did, however, find an overall significant main effect for GROUP ($F=4.672$, $df=4,21$, $p<0.01$). Subsequent *post hoc* analysis revealed that response changes following 100 Hz stimulation was significantly higher than those following 5 and 10 Hz. No interaction effect was revealed ($F=0.905$, $df=4,21$, $p>0.05$).

2.3.3. *Proximal subiculum-LEC*

A 3×5 mixed factorial ANOVA was conducted to examine the effects of frequency stimulation and time of application of stimulation on the plastic capabilities in the proximal subiculum-LEC projection. TIME was again used as the within-subjects factor. GROUP was used as the between-subject factor. We found no overall significant main effect for TIME ($F=1.563$, $df=2,40$, $p>0.05$). We also did not find an overall significant GROUP main effect ($F=0.938$, $df=4,20$, $p>0.05$) or a significant TIME×GROUP interaction effect ($F=1.058$, $df=8,40$, $p>0.05$). However, we decided to investigate each individual group separately to see if any differences would emerge at this level of analysis.

The mean slope of the fEPSP response in LEC following 1 Hz stimulation in proximal subiculum ($n=5$) at 30 and 60 min was 86.26±1.79 and 83.36±3.19% respectively of pre-stimulation baseline responses. A second stimulation at 1 Hz did not change these responses (see Fig. 6a). Responses at 30 and 60 min post-second stimulation stood at 80±7.6% and 87±3%, respectively (see Fig. 6a). Repeated-measures ANOVA found no significant differences between the final 10 min of recording post-second, the final 10 min of recording post-first stimulation and or baseline periods ($F=1.104$, $df=2,10$, $p>0.05$).

Following stimulation in the proximal subiculum at 5 Hz ($n=6$) responses in the LEC initially decreased before increasing back to baseline levels at 60 min post-stimulation. A second stimulation at 5 Hz decreased responses further but these gradually increased again back to baseline levels. Responses at 30 and 60 min post-second 5 Hz stimulation stood at 105±2% and 112±3%, respectively (Fig. 6b). A repeated-measures ANOVA found no significant differences between the three time periods tested ($F=1.271$, $df=2,10$, $p>0.05$).

Following stimulation at 10 Hz ($n=5$) fEPSP responses in the LEC initially decreased before increasing back to baseline levels at 60 min post-stimulation. Responses at 30 and 60 min stood at 82±3% and 104±4%, respectively. A second stimulation at 10 Hz did not produce any changes in fEPSP response (Fig. 6c). A repeated-measures ANOVA again confirmed that there were no significant differences between the three time periods tested ($F=0.123$, $df=2,8$, $p>0.05$).

Stimulation at 50 Hz in the proximal subiculum ($n=6$) produced a small potentiation in fEPSP response in the LEC. Responses increased to 114±2% and 113±3% of pre-stimulation values at 30 and 60 min post-stimulation, respectively. A second stimulation at 50 Hz initially produced an increase in response but these decreased to levels comparable to those post-first stimulations (Fig. 6d). Responses at 30 and 60 min post-second stimulation stood at 124±2% and 118±2%, respectively. Multiple comparisons revealed that the final 10 min of recordings post-first 50 Hz stimulation was significantly higher than baseline recordings ($t=-2.583$, $df=5$, $p<0.05$).

Finally, a 100 Hz stimulation produced an increase in fEPSP response ($n=4$) and this response remained potentiated for at least 60 min ($t=-2.724$, $df=3$, $p<0.05$). Responses at 30 and 60 min post-stimulation stood at 146±7% and 125±5%, respectively. A second 100 Hz stimulation did not cause any change in this response ($t=0.126$ $df=3$, $p>0.05$; see Fig. 6e).

Fig. 6f summarises the results described above by plotting the average mean change in fEPSP slope at each frequency for the final 10 min of recording post-first stimulation (black line) and post-second stimulation (dashed line). The modification for this threshold was hard to determine (see black line). Again, to examine whether the proximal subicular-LEC projection was capable of metaplastic changes, we used a repeated-measures ANOVA with TIME as the between-subjects and GROUP as the between-subjects measure; we found no significant effect for TIME ($F=0.902$, $df=1,20$, $p>0.05$). No overall significant effect was found for GROUP ($F=1.065$, $df=4,20$, $p<0.05$) and no interaction effect was revealed ($F=0.654$, $df=4,20$, $p>0.05$). This finding would

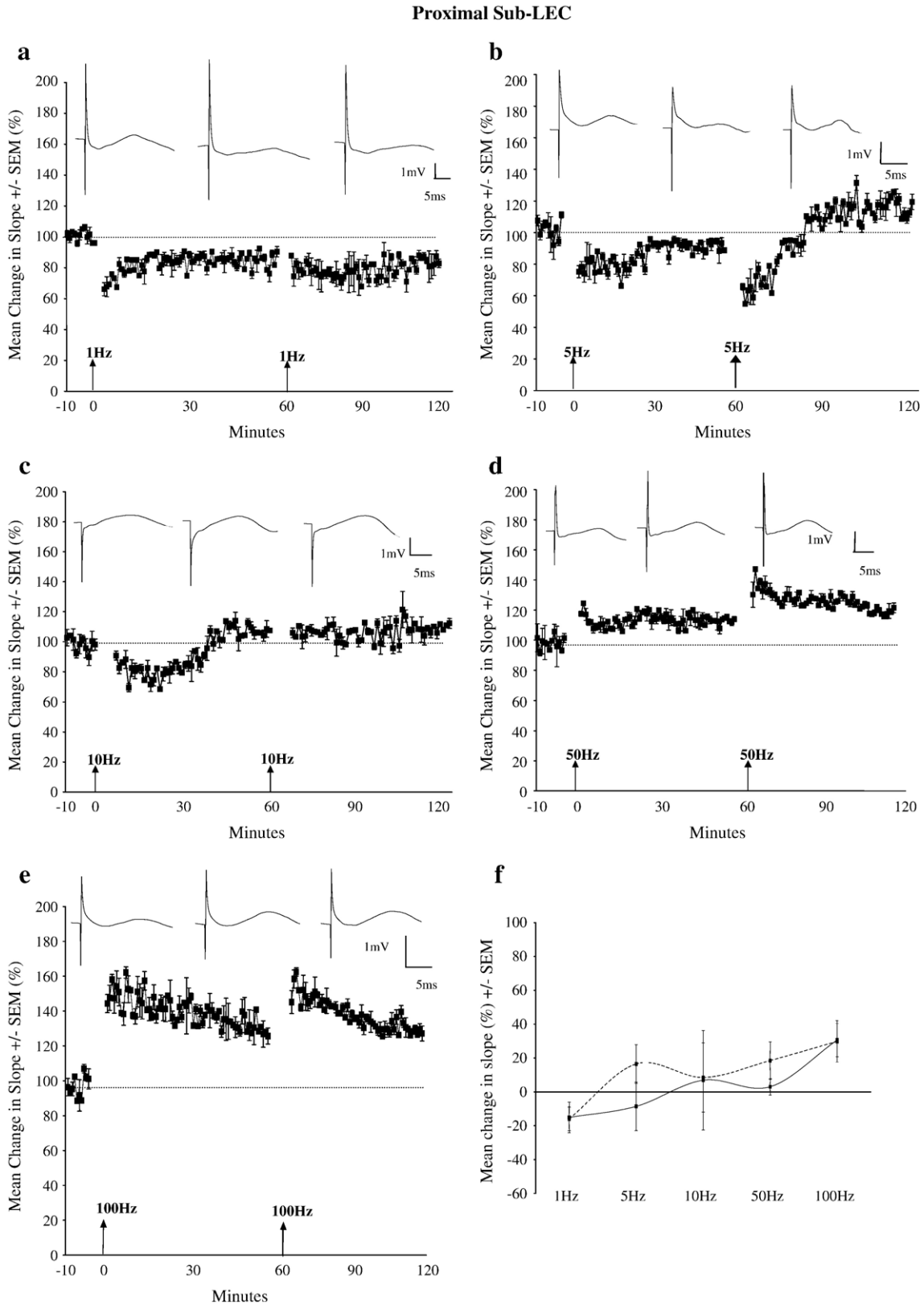


Fig. 6 – Effect of (a) 1 Hz, (b) 5 Hz, (c) 10 Hz, (d) 50 Hz and (e) 100 Hz stimulation on the slope of fEPSPs in the proximal subiculum-LEC projection ($n=6$ for each frequency tested). Stimulations are given twice, the first after a 10-min baseline period and the second 60 min post-first stimulation. The post-stimulation values are expressed as percentage of the pre-stimulation baseline ± SEM. Representative traces for baseline, post-first stimulation and post-second stimulation are located above each figure. (f) A line chart plotting the average mean change in fEPSP slope at each frequency for the final 10 min of recording post-first stimulation (black line) and post-second stimulation (dashed line).

suggest that this projection is not capable of sustaining metaplastic effects using this experimental protocol.

3. Discussion

The results of the experiments reported here reveal important plastic properties along the major output projections of the hippocampal formation. Previous pilot data reported by us, suggested a number of functional projections from the hippocampal areas CA1 and the subiculum to the entorhinal cortex (Craig and Commins, 2003, 2004), here we demonstrate and detail at least three strongly evoked projections, distal CA1-LEC, the proximal CA1-MEC and proximal subiculum-LEC projections, that are organised along topographical lines. These projections equate well with what is known about the anatomy of the region. It is known, for example, that projections from the cortex to the hippocampal formation are anatomically segregated, with the MEC receiving input from the postrhinal cortex and forming the medial perforant path into the hippocampus (Naber et al., 1997). In contrast, the LEC receives information predominately from the perirhinal cortex and in turn forms the lateral perforant path (Burwell et al., 1995; Burwell and Amaral, 1998; McNaughton, 1980, 1982; Witter et al., 2000). Work by Benini and Avoli (2005) and more recently by Kloosterman et al. (2003) would suggest that this segregation is also maintained for the back-projections from the hippocampal formation to the entorhinal cortex. Our findings would also seem to confirm physiologically these results and also suggest two functionally separate pathways from the hippocampus to the EC that would include both CA1 and subiculum to LEC in one circuit and CA1-MEC in the second.

We have recently demonstrated that the CA1-EC projection is capable of sustaining both PPF and LTP (Craig and Commins, 2005). In the series of experiments presented here, we extend these findings by examining the plastic effects for both the distal CA1-LEC and proximal CA1-MEC projections. We were able to demonstrate that both projections are capable of readily shifting from LTD to LTP by simply varying the frequency applied to that synapse. These results fit the BCM model very well and are also similar to results from previous experiments investigating the effect of varying frequency to the Schaffer collaterals *in vitro* (Dudek and Bear, 1992) and in slice cultures of rat hippocampus (Mellentin et al., 2005). We reveal that both CA1 projections seem to show different plastic capabilities, for example, stimulation at both 50 Hz and 100 Hz showed strong potentiation in the CA1-LEC projection, whereas 100 Hz stimulation alone produced potentiation in the CA1-MEC projection. Whereas, stimulation at 5 Hz and 10 Hz in the CA1-MEC projection produced a strong depression of synaptic strength. In addition, dissociable short-term plastic effects were also seen in the two CA1 projections. Facilitation, for example, in the CA1-LEC projection was immediately apparent at the 40 ms IPI, maximising between 60 and 120 ms IPI before the facilitatory effect being lost at the 480 ms IPI. In contrast to this, in the CA1-MEC projection, paired-pulse depression was observed at 40 ms IPI and as the interval lengthened strong facilitation was revealed and this facilitation remained strong even at 480 ms IPI where normally short-term plastic changes would not be observed (see Zucker, 1989). Further differences between the two projections were revealed by their metaplastic capabilities. We observed metaplastic effects in the CA1-LEC projection, whereby a second stimulation at the same frequency produced a general upward shift in synaptic responses (or by a leftward shift in the modification threshold) thereby allowing the synapses to be potentiated more easily. The CA1-MEC projection, in contrast, did not demonstrate any such metaplastic capabilities. We have recently also demonstrated (Craig and Commins, 2006) that the subiculum-EC projection is capable of PPF, LTP and LTD. These effects were observed from recordings made in more lateral areas of the entorhinal cortex, while stimulating more proximal areas of the subiculum. We also reported that we were unable to evoke responses from more medial regions of the entorhinal. In the experiments presented here, we confirm these observations by systematically examining evoked responses in both the LEC and MEC by stimulating proximal, medial and distal regions of the subiculum. We show clearly that the response evoked in the LEC is significantly larger when stimulating proximal subiculum compared to distal subicular stimulations. We were also unable, in these experiments, to evoke a measurable response in the MEC. There are a number of possible reasons for this. It is possible that there are much fewer neurons projecting to MEC from the subiculum, than are projecting to LEC. The anatomical studies that suggest a subiculum-MEC projection, for example, did observe much fewer labelled cells in MEC than in LEC after subiculum stimulation (Kloosterman et al., 2003; Tamamaki and Nojyo, 1995). Thus, it is possible that our electrodes were in the wrong area and we missed the few projecting neurons. Also, it is possible that there is no major projection from the subiculum to MEC and that the few labelled cells observed in MEC in previous studies may have been fibres *en passage* between distal subiculum and postrhinal cortex. We also extend our previous findings by demonstrating that this projection is capable of frequency-dependent plastic changes, shifting readily from LTD to LTP. Finally we demonstrate that this projection at least in our hands was not capable of metaplastic changes. Although the proximal subiculum projection to LEC does not seem to be a dynamic as the CA1 projection, as evidenced by smaller magnitudes in depression and potentiation in general and that there were no significant differences in the long-term responses between any of the frequencies (see Fig. 6f). The plastic properties of the subicular projection do share characteristics more in common with the distal CA1-LEC projection rather than the MEC projection. Stimulations in both projections produce LTP at both 50 Hz and 100 Hz while, facilitation was observed in both projections at 40 ms IPI, maximising at 60 ms IPI and with little or no effect at 480 ms IPI.

Interpretation of our results, however, should proceed with caution. We are unsure as to the exact nature of the evoked response that we have observed in the entorhinal cortex. The positive-going deflection observed in the majority of cases in reported experiments (and indeed, seen in our previous findings; Craig and Commins, 2005, 2006) may be as a result of the placement of electrodes with respect to activated synapses, alternatively, the positive deflection may be a result of activation of local inhibitory circuits. The EC is a complex multilayered structure with intrinsic connectivity (Burwell

and Amaral, 1998) and with hippocampal projections inputting both different layers and subdivisions of the EC, as such, the responses that we have observed most probably have a mixed mechanism. Although the majority of recordings were aimed at the deeper layers of both LEC and MEC, field potential recordings do not allow for precise identification of electrode placement sites.

Recognising the need for more detailed experiments particularly characterising the neuroanatomy more precisely by using single-unit electrophysiology combined with cell fills, our results would putatively suggest two important circuits from the hippocampal region to the entorhinal cortex, one through the LEC and the other through MEC. These circuits may convey dissociable types of information. This is observed along the input projections where Hargreaves et al. (2005), for example, have recently demonstrated that the spatial signal carried by the medial projection is much stronger compared to that carried by the lateral projection. In addition, our results would give credence to the various memory theories that predict that the backprojections to the cortex should be modifiable (see Rolls, 1996). However, our results would suggest that as the CA1-LEC projection and to a lesser extent the subiculum-LEC projection are more capable of potentiation (requiring a lower frequency stimulation) and demonstrate metaplastic effects (in the case of CA1-LEC), that these projections may be more efficient in retaining information compared to the CA1-MEC projection.

In addition to the information processing functions attributed to the permanent plastic changes seen in many hippocampal synapses, permanent excitability synaptic changes may also lead to the promotion of epileptogenesis. Furthermore, it has been recently suggested that synaptic strengthening during epileptiform activity could reinforce the excitatory network and shift the balance of excitation and inhibition towards excitation (Abegg et al., 2004). This shift of balance of excitation and inhibition towards excitation has been used to explain how seizure foci can spread from one brain region to another during secondary epileptogenesis (Abegg et al., 2004). Seizure foci have been shown to spread from the hippocampus to cortical regions (Benini and Avoli, 2005). As both CA1 and subiculum are strongly implicated in temporal lobe epilepsy (Wozny et al., 2005), their cortical projections, may in addition to their role in information processing, also provide potential routes along which seizure-like activity may propagate to parahippocampal structures and further downstream to the neocortex. Our findings would suggest that the proximal CA1-MEC projection might act as an inhibitory mechanism to prevent the spread of the seizure-like activity. While the distal CA1 and to a lesser extent the subiculum-LEC projections may be more efficient in the processing of information, epileptiform activity may also be more capable of being spread easier via these pathways.

In summary, we found clear dissociable effects in the plastic capabilities of the CA1-LEC and CA1-MEC projections, with the CA1-MEC projection demonstrating more depression and a resistance to metaplastic changes. Although the subiculum-LEC projection is not as dynamic as the CA1 projections it does display some plastic properties that seem to be more similar to the CA1-LEC projection as opposed to the CA1-MEC projection. This might suggest a functional circuit through the LEC that is distinct from the projections that pass through the MEC and may have important implications in both information processing and memory research and the spread of seizure-like activity through the medial temporal lobes.

4. Experimental procedures

4.1. *Surgery*

Adult male Wistar rats (Biomedical Facility, University College Dublin; weight; 250–350 g) were anaesthetised with urethane (ethyl carbamate; 1.5 g/kg i.p.) and mounted on a stereotaxic frame. A local anaesthetic/adrenaline combination (xylocaine) was injected under the scalp and an incision was made to visualise the skull. Burr holes were made for electrode access using coordinates according to Paxinos and Watson (1998). Stainless bipolar stimulating electrodes (50 μm in diameter) insulated except at the tip were aimed at the CA1 and subiculum. Stimulating electrodes were aimed at either the proximal, medial or distal CA1 (B −3.3 mm, 2.2 mm L; B −4.3, 2.2 mm L and B −5.6 mm, 4.4 mm L) or proximal, medial or distal subiculum (B −6.3 mm, 4.4 mm L, B −6.3 mm, 3.8 mm L and B −6.3 mm, 3.2 mm L, respectively; Paxinos and Watson, 1998). (The areas distal and proximal CA1 refer to those areas bordering the subiculum and area CA3, respectively. The subiculum is likewise defined with distal and proximal subiculum referring to the regions closest to the presubiculum and area CA1, respectively; see Witter et al., 2000 for further anatomical detail.) Stainless wire recording electrodes (diameter 50 μm) were also used. The recording electrodes were aimed at the following coordinates: B −6.7 mm, 5.8 mm L, and 6.0 mm below the surface for LEC, and B −6.7 mm, 4.2 mm L and 7.2 mm below the surface for MEC (Paxinos and Watson, 1998).

4.2. *Stimulation and data acquisition*

Signals were filtered between 0.1 Hz and 1 kHz and then amplified (DAM-50 differential amplifier, WPI, Hertfordshire, UK). Recordings were digitised online using a PC connected to a CED-1401 plus interface (CED, Cambridge, UK).

In the first set of experiments the recording electrode was slowly lowered towards either LEC ($n=6$) or MEC ($n=6$) and allowed to settle for 10 min. In some cases the recording electrode was lowered at an angle of 20° to ensure no differences were observed due to the oblique nature of the cortical layers in LEC (data not shown). The stimulating electrode was slowly lowered in 100 μm steps until the appropriate area in the proximal CA1 was reached. Field excitatory postsynaptic potentials (fEPSPs) were evoked by stimulating with a square-wave (constant current pulse of 0.1 ms duration at a frequency of 0.05 Hz). The intensity of stimulus was set at a level that evoked a fEPSP slope of 55–65% of the maximum (using individually determined input-output curves). Following characterisation of the fEPSP in this region the stimulating electrode only was slowly removed and then aimed for the medial CA1 and subsequently the distal CA1. A similar procedure was adopted for recordings between the proximal medial and distal subiculum and LEC ($n=12$) and

proximal medial and distal subiculum and MEC ($n=7$). For the purpose of data analysis the field excitatory postsynaptic potential (fEPSP) slope was measured, calculated from the middle one-thirds of the upward-going deflection of the response.

Following identification of the location of maximum responses along the CA1-EC and subiculum-EC projections, three projections emerged: distal CA1-LEC, proximal CA1-MEC and proximal subiculum-LEC. In a second set of experiments, short- and long-term plastic effects were then examined on these maximal responsive projections. Stimulating and recording electrodes were first allowed to settle for 10 min in the appropriate areas in CA1, subiculum and EC (see above). Then PPF was attempted on each of the three projections at inter-stimulus intervals (ISIs) of 40, 60, 120, 240 and 480 ms. The PPF value was calculated by taking the average of six slope values of fEPSP1, for a given ISI, and normalising the average of six values for fEPSP2 with respect to this value in percentage terms (see Commins et al., 1998 for further details). Following this, baseline measurements were induced at a rate of 0.05 Hz for another 10 min. Stimulus intensity during PPF and the different frequency stimulations (see below) was set at baseline intensity. Changes in excitatory postsynaptic potential (EPSP) slope were assessed by measuring the slope of the response compared to baseline response. Induction of longer-term plastic changes in each of the three pathways (distal CA1-LEC, proximal CA1-MEC and proximal subiculum-LEC) was attempted by varying the frequency but keeping the number of stimuli constant (900 pulses) ($n=5/6$ for each frequency for each pathway). The frequencies used were 1, 5, 10, 50 and 100 Hz. Following this, low-frequency stimulation was then resumed at a rate of 0.05 Hz for a further 60 min. A second frequency stimulation (second FS) was then attempted and after which baseline recordings were again resumed for a further 60 min.

4.3. *Histological processing*

After all experiments the rats were overdosed with sodium pentobarbitone and their brains subsequently removed. In order to check the positions of the electrodes the brains were frozen in Tissue-Tek O.C.T. compound (R.A. Lamb, East Sussex, UK). 20 μm coronal slices were cut using a cryostat. Sections with electrode lesions were mounted on slides in order to confirm electrode positioning. The sections were counterstained with cresyl violet (Nissl) to define cytoarchitectonic borders and subsequently coverslipped. All electrode sites and electrode tracks were reconstructed using Paxinos and Watson atlas (Paxinos and Watson, 1998).

4.4. *Ethics*

All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and with Irish Department of Health and Children regulations.

4.5. *Statistics*

The data were analysed using a series of one-way ANOVAs with appropriate *post hoc* tests (Tukey, $p<0.05$). Wherever applicable dependent *t*-tests or repeated-measures ANOVAs using *post hoc* tests with Bonferroni correction were conducted. A star-based system was used: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Acknowledgments

The authors gratefully acknowledge the support of the Health Research Board of Ireland. We would also like to thank Professors Michael Rowan, Shane O'Mara (TCD) and Dr. Richard Roche (NUIM) for their helpful comments and discussions.

REFERENCES

Abegg, M.H., Savic, N., Ehrengruber, M.U., McKinney, R.A., Gahwiler, B.H., 2004. Epileptiform activity in rat hippocampus strengthens excitatory synapses. J. Physiol. 554 (2), 439–448.

Abraham, W.C., Bear, M.F., 1996. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci. 19, 126–130.

Bear, M.F., Cooper, L.N., Ebner, F.F., 1987. A physiological basis for a theory of synapse modification. Science 237, 42–48.

Benini, R., Avoli, M., 2005. Rat subicular networks gate hippocampal output activity in an in vitro model of limbic seizures. J. Physiol. 566 (Pt. 3), 885–900.

Bienenstock, E.L., Cooper, L.N., Munro, P.W., 1982. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J. Neurosci. 2, 32–48.

Bliss, T.V.P., Collingridge, G.L., 1993. A synaptic model for memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Bliss, T.V.P., Lomo, T., 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. J. Physiol. 232, 331–356.

Burwell, R.D., Amaral, D.G., 1998. Cortical afferents of the perirhinal, postrhinal and entorhinal cortices of the rat. J. Comp. Neurol. 398, 179–205.

Burwell, R.D., Witter, M.P., Amaral, D.G., 1995. Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. Hippocampus 5, 390–408.

Castellani, G.C., Quinlan, E.M., Cooper, L.N., Shouval, H.Z., 2001. A biophysical model of bi-directional synaptic plasticity: dependence on AMPA and NMDA receptors. Proc. Nat. Acad. Sci. 98, 12772–12777.

Cipolotti, L., Bird, C., Good, T., Macmanus, D., Rudge, P., Shallice, T., 2006. Recollection and familiarity in dense hippocampal amnesia: a case study. Neuropsychologia 44, 489–506.

Commins, S., Gigg, J., Anderson, M., O'Mara, S.M., 1998. Interaction between paired-pulse facilitation and long-term potentiation in the projection from hippocampal area CA1 to the subiculum. NeuroReport 9, 4109–4113.

Craig, S., Commins, S., 2003. Physiological evidence for a topographical projection from the subiculum to lateral entorhinal cortex. Acta Neurobiol. Exp. 63, 87.

Craig, S., Commins, S., 2004. Physiological evidence for the topographical nature of the output from the hippocampus to the entorhinal cortex. FENS Abstr. 2, A120.9.

Craig, S., Commins, S., 2005. Interaction between paired-pulse facilitation and long-term potentiation in the projection from

hippocampal area CA1 to the entorhinal cortex. Neurosci. Res. 53 (2), 140–146.
Craig, S., Commins, S., 2006. Short- and long-term facilitation of synaptic transmission in the entorhinal cortex following stimulation in the subiculum. Behav. Brain Res. 174 (2), 281–288.
Dudek, S., Bear, M., 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc. Nat. Acad. Sci. 89, 4363–4367.
Dudek, S., Bear, M., 1993. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J. Neurosci. 13, 2910–2918.
Garcia, R., 2001. Stress, hippocampal plasticity and spatial learning. Synapse 40, 180–183.
Gisabella, B., Rowan, M.J., Anwyl, R., 2003. Mechanisms underlying the inhibition of long-term potentiation by preconditioning stimulation in the hippocampus in vitro. Neuroscience 121, 297–305.
Hargreaves, E.L., Rao, G., Ler, I., Knierim, J.J., 2005. Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. Science 308, 1792–1794.
Heynen, A.J., Quinlan, E.M., Bae, D.S., Bear, M.F., 2000. Bidirectional, activity-dependent regulation of glutamate receptors in the adult hippocampus in vivo. Neuron 28, 527–536.
Jarrard, L.E., 1983. Selective hippocampal lesions and behaviour: effects of kainic acid lesions on performance of place and cue tasks. Behav. Neurosci. 97, 873–889.
Jarrard, L.E., 1993. On the role of the hippocampus in learning and memory in the rat. Behav. Neural Biol. 60, 9–26.
Katz, B., Miledi, R., 1970. Further study of the role of calcium in synaptic transmission. J. Physiol. 207, 789–801.
Kloosterman, F., Witter, M.P., van Haeften, T., 2003. Topographical and laminar organization of subicular projections to the parahippocampal region of the rat. J. Comp. Neurol. 455, 156–171.
Martin, S.J., Grimwood, P.D., Morris, R.G.M., 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. Annu. Rev. Neurosci. 23, 649–711.
Mellentin, C., Moller, M., Jahnsen, H., 2005. Properties of long-term synaptic plasticity and metaplasticity in organotypic slice cultures of rat hippocampus. Exp. Brain Res. 1–10.
McNaughton, B.L., 1980. Evidence for two physiologically distinct perforant pathways to the fascia dentate. Brain Res. 199, 1–19.
McNaughton, B.L., 1982. Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. J. Physiol. (London) 324, 249–262.
Nadel, L., Moscovitch, M., 1997. Memory consolidation, retrograde amnesia and the hippocampal complex. Curr. Opin. Neurobiol. 7, 217–227.
Naber, P.A., Caballero-Bleda, M., Jorritsma-Byham, B., Witter, M.P., 1997. Parallel input to the hippocampal memory system through peri- and postrhinal cortices. NeuroReport 8, 2617–2621.
Ogden, J.A., Corkin, S., 1991. Memories of H.M. In: Abraham, W.C., Corballis, M.C., White, K.G. (Eds.), Memory Mechanisms. Lawrence Erlbaum Associated, Hillsdale, N.J.
Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego.
Rolls, E.T., 1996. A theory of hippocampal function in memory. Hippocampus 6, 601–620.
Schacter, D.L., Wagner, A.D., 1999. Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. Hippocampus 9 (1), 7–24.
Scoville, W.B., Milner, B., 1957. Loss of recent memory after bilateral hippocampal lesions. J. Neurol., Neurosurg. Psychiatry 20, 11–21.
Squire, L.R., 1992. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol. Rev. 99 (2), 195–231.
Tamamaki, N., Nojyo, Y., 1995. Preservation of topography in the connections between the subiculum, field CA1 and the entorhinal cortex in rats. J. Comp. Neurol. 353, 379–390.
Thomsom, A.M., 2000. Facilitation, augmentation and potentiation at central synapses. Trends Neurosci. 23, 305–312.
Van Dam, E.J.M., Kamal, A., Artola, A., de Graan, P.N.E., Hendrik Gispen, W., Ramakers, G.M.J., 2004. Group I metabotropic glutamate receptors regulate the frequency-response function of hippocampal CA1 synapses for the induction of LTP and LTD. Eur. J. Neurosci. 19, 112–118.
Witter, M.P., Naber, P.A., van Haeften, T., Machielsen, W.C.M., Rombouts, S.A.R.B., Barkhof, F., Scheltens, P., Lopes da Silva, F.H., 2000. Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways. Hippocampus 10, 398–410.
Wozny, C., Knopp, A., Lehmann, T.N., Heinemann, U., Behr, J., 2005. The subiculum: a potential site of ictogenesis in human temporal lobe epilepsy. Epilepsia 46 (5), 17–21.
Wu, J., Rowan, M., Anwyl, R., 2004. Synaptically stimulated induction of group I metabatropic glutamate receptor-dependent long-term depression and depotentiation is inhibited by prior activation of metabotropic glutamate receptors and protein kinase C. Neuroscience 123, 507–514.
Zucker, R.S., 1989. Short-term synaptic plasticity. Annu. Rev. Neurosci. 12, 13–31.