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The effects of single and multiple episodes of theta patterned or high frequency stimulation on synaptic transmission from hippocampal area CA1 to the subiculum in rats

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Abstract

Long-term potentiation (LTP) is a popular model for the synaptic changes that may occur during learning and memory; it involves a strengthening of synaptic response and is readily induced in the hippocampus, an area of the brain implicated in learning and memory. Previous research on LTP has focused on 'early' components of the hippocampal circuitry, that is, the dentate gyrus and areas CA1 and CA3. This paper examines the plasticity of the CA1-subiculum pathway; we extend our previous work in this area demonstrating that the projection from area CA1 to subiculum sustains theta-patterned stimulus-induced LTP in vivo. We show that this pathway remains potentiated over a long period (3 h). Furthermore, once this projection is potentiated, it seems resistant to further episodes of high-frequency stimulation. We discuss the implications of these findings for theories of hippocampal-cortical interaction during the biological consolidation of memory.

Keywords: Synaptic plasticity; Subiculum; Long-term potentiation; High-frequency stimulation; Hippocampal formation; Learning and memory

Long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy resulting from stimulation of afferent fibres. LTP is rapidly induced and once stabilized, it persists for hours to days, depending on the preparation [2]. These properties and others are consistent with the idea that an LTP-like mechanism is involved in learning and memory [13]. Most previous research has concentrated on the analysis of LTP in the 'early' components of the hippocampal circuit, for example, dentate gyrus and area CA1 [3,12], whereas the 'later' components of the hippocampal formation, such as the subicular complex, have been relatively neglected. The subiculum is the primary output structure of the hippocampal formation; it receives a massive unidirectional projection from hippocampal area CA1 [1,9]. The subiculum plays a pivotal role in pathophysiological diseases such as epilepsy [7] and Alzheimer's disease [4] and also plays a major role in processing spatial information and spatial memory, as indicated by lesion studies [18] and neuronal recordings of the subiculum [19]. There has been one study examining the plasticity of the projection from area CA1 to the subiculum in vivo [5]. The authors found that HFS produced a clear potentiation that lasted 30 min. Here we examine if LTP can be induced using a more 'natural' stimulation protocol, that is, theta-patterned stimulation. It is well known that LTP can be induced by afferent stimulation that is patterned at theta frequency (4–12 Hz), a frequency band dominating hippocampal EEG during locomotor behaviour such as exploration in rats. Thus, one strategy to attempt to induce LTP in a more physiologically plausible way is to give short bursts of stimuli with an inter-burst interval of approximately 200 ms, so-called theta-patterned stimulation [10,20]. We also examine whether the changes in synaptic efficacy are long-lasting, that is, if the induced potentiation lasts at least 3 h using HFS. Finally, we examine the effects of multiple episodes of stimulation, also using HFS, in an attempt to determine whether this pathway, once potentiated, can undergo further changes in synaptic strength.

Adult male Wistar rats (weight: 200–300 g) were anaesthetized with urethane (ethyl carbamate: 1.5 g/kg, i.p.) and mounted in a stereotactic holder. A local anaesthetic/adre-

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naline combination was injected under the scalp and an incision was to visualize the skull. Stimulating electrodes were aimed at area CA1 and the recording electrodes at the dorsal subiculum. Electrode implantation sites were identified using stereotaxic coordinates relative to bregma [15]; recording and stimulating electrodes were 6.8 mm posterior and 4.0 mm lateral to the midline and 4.5 mm posterior and 2.5 mm lateral to the midline respectively. Bipolar stimulating and monopolar recording electrodes consisted of two pieces of twisted 50 μm Tungsten wire, insulated to the tips. Signals were filtered between 0.1 Hz and 1 kHz and then amplified. All the recordings were digitized 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 a depth of 2.5 mm; test pulses were administered during electrode movement at a rate of 0.1 Hz. The final depths were adjusted until maximal extra-cellular field excitatory post-synaptic potentials (fEPSPs) were obtained. Initially, input-output (I/O) analyses were conducted to determine the maximal response to constant increments in stimulus intensity. Baseline measurements were conducted at half-maximal intensity for a minimum of 10 min. Induction of LTP was then attempted using a number of protocols. Theta-patterned stimulation consisted of five trains of five stimuli, intertrain interval of 200 ms (5 Hz), and stimulus intensity during LTP induction set at baseline levels. Baseline stimulation was then resumed at a rate of 0.1 Hz for 30 min. Experiments were also conducted using multiple trains of HFS. HFS consisted of 10 trains of 20 stimuli at 200 Hz, intertrain interval of 2 s; this protocol identical to that used by [5]. Four separate trains of HFS were delivered at 15-min intervals to see if potentiation could be enhanced further. Our final experiment examined if potentiation could last over an extended period (that is, more than half an hour). After induction of LTP, using a single HFS train, baseline stimulation was resumed at a rate of 0.1 Hz for 3 h. Unless stated otherwise, all data are expressed as mean ± SEM percentage baseline fEPSP peak amplitude. We recorded fEPSP amplitude rather than fEPSP slope because we have previously found that in the CA1-subiculum pathway [5] fEPSP amplitude measures are a more conservative and less variable measure than slope in this projection. I/O curves were reconstructed across the range of stimulus intensities used to derive the original I/O curves. After each experiment the rats were overdosed with sodium pentobarbitol. Their brains were removed and allowed to sink in 4% formaldehyde; these were examined to verify the positions of the stimulating and recording electrodes. All data presented here are for stimulating and recording sites that were verified as being in CA1 and subiculum, respectively.

The first experiment examined LTP using theta-patterned stimulation. Initially, a baseline was established for 15 min at half-maximal peak amplitude. A rapidly-stabilizing potentiation was induced using theta-patterned stimulation ($n = 6$). The level of potentiation measured by fEPSP amplitude, stood at 118.39 ± 4.5% (relative to baseline), 5 min post-theta patterned stimulation. Potentiation remained stable over a 30-min period as demonstrated by the fEPSP amplitude at both 15 and 30 min post-stimulation, which were 116.25 ± 1.9 and 116.88 ± 4.2% respectively (Fig. 1a). A representative example of an fEPSP pre- and post-stimulation is presented in Fig. 1b. A representative I/O curve pre- and post-LTP from a single experiment is presented in Fig. 1c. The I/O curve has shifted to the left ($t = 4.16$; d.f. = 1,8; $P < 0.01$) across all stimulus intensities, suggesting that LTP was successfully induced. A one-way ANOVA was conducted to compare the last 5-min period of the baseline, with; (i) the first 5 min post-HFS; (ii) 10–15 min post-HFS, and (iii) the final 5 min of the 30-min period. The overall analysis for the peak amplitude was found to be significant ($F = 97.86$; d.f. = 3,119; $P < 0.0001$). Post-hoc (Newman–Keuls) analyses were then conducted to determine the significance between these groups. The 5 min pre-HFS baseline period was found to be significantly lower than the other time periods ($P < 0.05$) indicating that LTP was successfully induced.

The second experiment examined LTP over a 3-h period. A baseline was again established for 15 min at half-maximal peak amplitude. LTP was induced ($n = 5$), using the HFS protocol and fEPSP levels were monitored over a 3-h period. Potentiation was again rapidly induced and reached a peak within the first half-hour; average fEPSP amplitude stood at 140.9 ± 13.3% after 30 min. The response then decreased in magnitude, as seen by the peak amplitude measurement after 1 h, which stood at 122.2 ± 16.5%. The response then stabilized; average fEPSP amplitude at

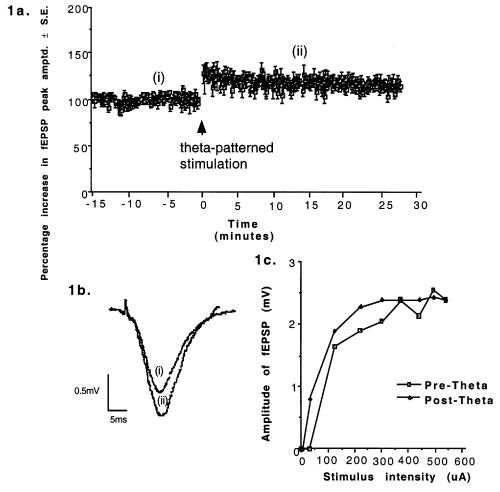

Fig. 1. Effects of theta-patterned stimulation on the amplitude of fEPSs. The post-stimulation values are expressed as percentage of the pre-stimulation baseline ± SEM. Trace (i) is an example of a representative fEPS prior to LTP induction; trace (ii) is a representative fEPS post-stimulation (taken from point (i) and (ii) on (a), respectively. Example of I/O curve (stimulus intensity vs. amplitude of fEPSP) before and after LTP induced by theta-patterned stimulation.

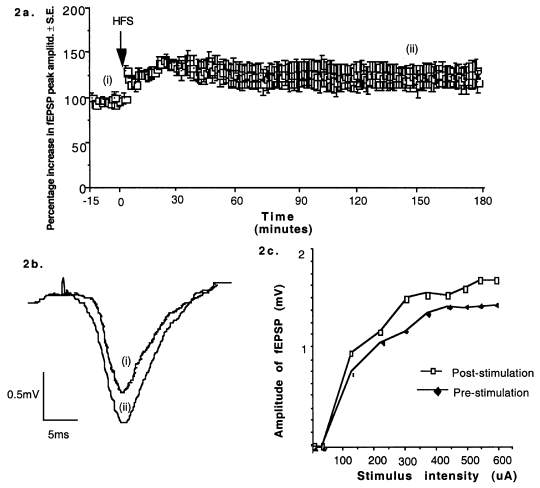

Fig. 2. Effects of HFS on the amplitude of fEPSPs, recorded over a 3-h period (recorded very minute). The post-stimulation values are expressed as a percentage of the pre-stimulation baseline ± SEM. Trace (i) is an example of a representative fEPSP pre-LTP induction: trace (ii) is a representative fEPSP post-LTP induction (taken from point (i) and (ii) on (a), respectively). Example of an I/O curve (stimulus intensity vs. amplitude of fEPSP) before and after the 3-h post-stimulation period.

two hours post-HFS and three hours post-HFS was 125.9 ± 16.5 and 120.1 ± 2.6%, respectively (Fig. 2a–c). The I/O curve shifted upward and leftward ($t = 4.47$; d.f. = 1,8; $P < 0.01$) across all stimulus intensities, suggesting that LTP was successfully induced. A one-way ANOVA was conducted to compare the last 5 min of the baseline period, with the 25–30 min period post-HFS, and the last 5 min of the first, second and third hours. The overall analysis, for peak amplitude of the groups, was found to be significant ($F = 93.9$; d.f. = 4,149; $P < 0.0001$). Post-hoc analyses (Newman–Keuls) revealed that the baseline period was significantly lower than the other time periods ($P < 0.05$). No significant difference was found between the first 30 min post-HFS and the 150–180 min period post-HFS ($F = 2.8135$; d.f. = 1,59; $P > 0.05$). This indicates that the level of potentiation was relatively stable over the 3-h post-HFS period.

The third experiment examined the effect of multiple HFS trains. A baseline was initially established for 15 min with stimulation at half-maximal peak amplitude. LTP was induced ($n = 6$) using the HFS protocol. Potentiation was allowed to stabilize for 15 min before the second stimulation was attempted. Initial potentiation was rapidly induced demonstrated by the peak amplitude response which stood at 133.21 ± 9.8% of baseline at 10 min post-first HFS. Further episodes of HFS did not lead to further increases in potentiation. The peak-amplitude response 10 min after the second HFS stood at 129.76 ± 7.47%. At 10 min after the third and final HFS, potentiation stood at 131.88 ± 6.0 and 133.58 ± 11.2%, respectively (Fig. 3a,b). A one-way ANOVA was conducted to compare the different time periods, i.e. the 15 min pre-HFS, 0–15, 15–30, 30–45 and 45–60 min post-HFS (periods immediately after the first, second, third and fourth HFS, respectively). The overall analysis for peak amplitude was found to be significant ($F = 154.77$; d.f. = 4,217; $P < 0.0001$). Post-hoc analyses (Newman–Keuls) showed that the baseline period was significantly different from the other periods ($P < 0.05$). There were no significant differences between the other potentiated responses, suggesting that the synaptic response was saturated following the initial episode of high-frequency stimulation.

These results extend our earlier in vivo work in this area [5] and are comparable to results reported elsewhere [6]. Long-term potentiation in the CA1-subiculum pathway can be obtained by using a more natural stimulation protocol, namely, theta-patterned stimulation. This is an encouraging result, even though the level of potentiation achieved was modest. The results found on this pathway are somewhat different to those obtained by other researchers. Staubli and Lynch [20] for example, found a 34.7 ± 4.8% increase in CA1, which lasted several days, after applying a theta-patterned stimulation protocol. Theta rhythm occurs naturally during motor activity or novelty perception and is believed to be important for memory formation because blocking theta rhythm impairs the ability of rats to learn spatial tasks [21]. Furthermore, theta-patterned stimulation resembles natural firing patterns such as complex spike activity, which occurs naturally in theta-frequency EEG rhythms. Complex spike activity may play a role in the induction of LTP-like changes of synaptic strength in vivo. Further experiments are needed to examine the CA1-subiculum pathway could be potentiated over a long

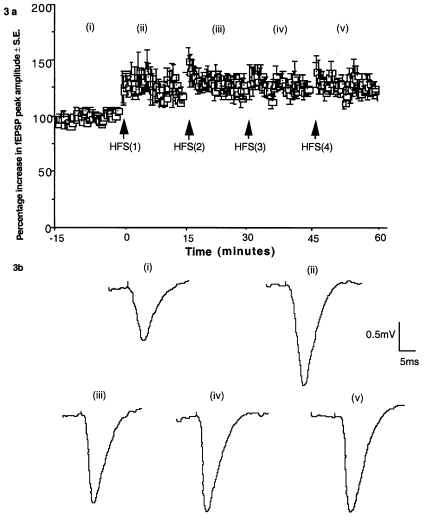

Fig. 3. Effects of multiple episodes of HFS. The post-HFS responses are expressed as a percentage of the pre-stimulation baseline ± SEM. Trace (i) is a representative fEPSP from the baseline period; trace (ii) is a representative fEPSP after the first HFS; trace (iii) is a representative fEPSP after the second HFS and traces (iv) and (v) are representative fEPSPs after the third and fourth HFS, respectively. (These are taken form the points (i–v) on the figure).

period (hours to days), using this more natural stimulation protocol. We have shown that potentiation persists in a stable state for at least 3 h. This is comparable with other hippocampal areas. LTP can persist from hours to several weeks depending on the induction parameters and stimulated pathways; Staubli and Lynch [20], for example, report that in CA1, LTP can last for at least 7 days. A systematic examination of the effect of multiple periods of stimulation is important for understanding the nature of the plasticity of a particular set of synapses. Disruption of the ability of synapses to alter their weights, by forcing synaptic weights to their maximum value, may interfere with the types of learning for which the hippocampal formation is necessary [11]. Our present data show that the CA1-subiculum projection attains maximum synaptic strength after a single period of HFS perhaps implying that the subiculum does not manipulate the information it receives from CA1 but passes it directly to the neocortex for further processing. It is widely believed that long-term memories are laid down in the neocortex and that the hippocampal formation plays an important role in the biological consolidation of long-term memory [14,17]. There are differing views on the nature of the processes of consolidation by the hippocampus. Nadel and Moscovitch [14] regard it as an instantaneous process, whereas Rolls and O'Mara [17] suggest that it may take a much longer period of time. Furthermore, Rolls [16] predicts that the CA1-subiculum and subiculum-entorhinal projections should exhibit LTP thereby suggesting that the subiculum plays an important role as the primary interface between the hippocampus and cortex. We have confirmed the first of these predictions. Further experiments are required to test the second prediction and to examine the plasticity of other loci en route to the cortex [8].

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