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The effects of low frequency and two-pulse stimulation protocols on synaptic transmission in the CA1-subiculum pathway in the anaesthetized rat

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Abstract

The downregulation of synaptic efficacy is referred to as long-term depression (LTD). Recent work has shown that a two-pulse stimulation (TPS) protocol is successful at inducing LTD in vivo in area CA1 of the hippocampus. Here, we examine the ability of two TPS protocols and two low-frequency stimulation (LFS) protocols to induce LTD in the projection from hippocampal area CA1 to the subiculum in the anaesthetized rat. We find no evidence of LTD induction with TPS or LFS protocols. Instead, with three of the protocols (both TPS protocols and 1 Hz LFS), a late-developing potentiation is observed.

Keywords: Synaptic plasticity; Hippocampus; Long-term depression; Learning and memory; Low-frequency stimulation; Two-pulse stimulation

Long-term depression (LTD) of synaptic transmission is the persistent decrease in the amplitude and slope of evoked field excitatory postsynaptic potentials (fEPSPs) below the level observed in the naive pathway, as a result of repeated low-frequency stimulation (LFS) of the pathway. Like long-term potentiation (LTP), LTD is regarded as a biologically plausible model of information storage in the mammalian central nervous system [14].

LTD has been most investigated in the hippocampal slice with long trains of single pulse LFS typically delivered at 1 or 10 Hz, and it is more reliably induced in slices prepared from immature rats [10]. The inability to induce LTD in vivo using protocols that have been successful in vitro has brought the biological significance of LTD into question [11]. Recently, however, different protocols have been successful in inducing LTD in vivo. Low frequency trains of paired stimuli successfully induced LTD in area CA1 of the anaesthetized adult rat (150–200 paired pulses at 0.5 Hz, interpulse interval (IPI) of 25 ms [19]), and in the awake adult rat (200 paired pulses at 0.5 Hz, IPI of 25 ms [7]).

The subiculum may play an important part in the consolidation of long-term memory, possibly via hippocampal-cortical interactions. It is surprising, therefore, that to date there have been relatively few studies of the physiology or functions of the subiculum. The subicular complex, (comprising the subiculum proper, the presubiculum and the parasubiculum), is the primary output structure of the hippocampal formation, and projects to a variety of cortical and sub-cortical areas [1]. We have previously demonstrated in vivo that the projection from hippocampal area CA1 to the subiculum expresses both LTP and paired-pulse facilitation [3–5]. This is a monosynaptic projection, as confirmed by our own dual stimulation experiments which have examined both single-unit and fEPSP responses [13] and by other data [12]. We examine here, for the first time, if LTD can be induced in this projection using two standard LFS protocols (900 stimuli delivered at 1 and 10 Hz) and two two-pulse stimulation (TPS) protocols (450 pairs of stimuli delivered at 1 Hz with IPIs of 5 and 40 ms).

Adult 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/adrenaline combination was injected under the scalp and the skull was visualized. Stimulating electrodes were aimed at CA1 (4.5 mm posterior to bregma and 2.5 mm lateral), recording electrodes at dorsal subiculum (6.8 mm posterior and 4.0 mm lateral) [16]. Bipolar stimulating and monopolar recording electrodes consisted of two pieces of twisted 50 μm tungsten wire, insulated to the tips. Signals were filtered

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(0.1–1 kHz) and amplified. All recordings were digitized on-line using a PC connected to a CED-1401+ interface (CED, Cambridge, UK). Signals were monitored using an oscilloscope. The recording electrode was lowered through the cortex to 2.5 mm. The stimulating electrode was lowered to 2.5 mm while test pulses were administered at 0.1 Hz. The final depths were adjusted until maximal fEPSPs were obtained. Field EPSPs stabilized over 10 min before baseline recordings were conducted. Baseline measurements were conducted for 10 min at an intensity to produce half-maximal fEPSPs. The results are averaged over 1 min of data and expressed as a percentage of the baseline amplitude ± standard error of the mean. A total of 24 animals was used in this study: no animals were discarded from the results. Group 1 ($N = 6$) received LFS (900 pulses at 1 Hz), while group 2 ($N = 6$) received LFS (900 pulses at 10 Hz). Group 3 ($N = 6$) received TPS (450 pairs of pulses at 1 Hz with an IPI of 5 ms), while group 4 ($N = 6$) received TPS (450 pairs of pulses at 1 Hz with an IPI of 40 ms). All protocols were delivered at baseline intensity. Recording was resumed for 30 min at baseline frequency. After each experiment the rats were overdosed with sodium pentobarbitol and their brains were removed and allowed to sink in 4% formaldehyde. Electrode positions were verified histologically.

A one-way ANOVA was conducted for all groups to compare the last 5 min of the baseline period with (i) the first 5 min post-treatment, (ii) 15–20 min post-treatment, and (iii) 25–30 min post-treatment. Important comparisons were then made using post-hoc tests (Newman–Keuls).

At 5, 15 and 25 min post-LFS (1 Hz), the amplitude of fEPSPs stood at 103.3 ± 3.2, 111.2 ± 2.1 and 115.8 ± 4.7%, respectively. An increase in fEPSP amplitude post-LFS is evident (see Fig. 1a). The ANOVA confirmed these findings ($F = 68.99$; d.f. = 3,36; $P < 0.0001$). No evidence of LTD was found: the 15 and 25 min post-LFS amplitudes were both significantly larger than the baseline amplitude (both $P < 0.01$). The 25 min post-LFS amplitude was significantly larger than the 15 min post-LFS amplitude ($P < 0.01$). These results indicate a late-developing potentiation of fEPSP amplitude after 1 Hz LFS.

At 5, 15 and 25 min post-LFS (10 Hz), the amplitude of fEPSPs stood at 86.0 ± 4.4, 102.0 ± 4.3 and 101.9 ± 3.3%, respectively. No increase in fEPSP amplitude was evident (see Fig. 1b). The ANOVA was significant ($F = 79.642$; d.f. = 3,36; $P < 0.0001$). No evidence of LTD was found, nor indeed of any potentiation. There was short-term depression confirmed by post-hoc tests; the 5 min post-LFS amplitude was significantly smaller than the baseline amplitude ($P < 0.01$). However, the 15 and 25 min post-LFS amplitudes were not significantly different to the baseline amplitude. Field EPSP amplitude rapidly recovered to baseline levels and stabilized.

At 5, 15 and 25 min post-TPS (5 ms IPI), the amplitude of fEPSPs stood at 76.5 ± 6.1, 103.0 ± 3.1 and 115.0 ± 1.4%, respectively. An increase in fEPSP amplitude post-TPS is evident (see Fig. 2a). The ANOVA confirmed these findings ($F = 172.29$; d.f. = 3,36; $P < 0.0001$). No evidence of LTD was found. Again, there was short-term depression confirmed by post-hoc tests; the 5 min post-TPS amplitude was significantly smaller than the baseline amplitude ($P < 0.01$). However, fEPSP amplitude recovered and by 25 min post-TPS, fEPSP amplitude was significantly larger than the 5 min pre-TPS amplitude ($P < 0.01$), indicating a significant degree of a late-developing potentiation.

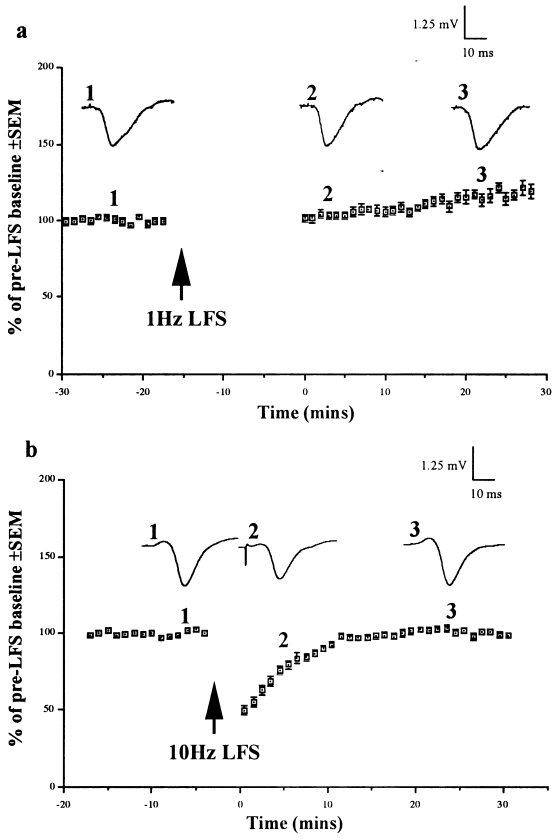

Fig. 1. Effects of low-frequency stimulation (LFS) on the amplitude of fEPSPs. (a) LFS delivered at 1 Hz induces late-developing potentiation, (b) LFS delivered at 10 Hz does not induce LTD or late-developing potentiation. Sample fEPSP traces are displayed above each graph with numbers corresponding to the number above the plot to indicate the time at which the traces were taken. No samples were taken during the LFS delivery period.

At 5, 15 and 25 min post-TPS (40 ms IPI), the amplitude of fEPSPs stood at 89.4 ± 2.5, 112.9 ± 3.5 and 118.9 ± 4.1%, respectively. An increase in fEPSP amplitude post-TPS is evident (see Fig. 2b). The ANOVA confirmed these findings ($F = 125.44$; d.f. = 3,36; $P < 0.0001$). No evidence of LTD was found. Again, there was short-term depression confirmed by post-hoc tests: the 5 min post-TPS amplitude was significantly smaller than the baseline amplitude ($P < 0.01$). However, both the 15 and 25 min post-TPS amplitudes were significantly different to the baseline amplitude (both $P < 0.01$), showing again a significant degree of potentiation.

The major finding of this study is that LFS protocols that

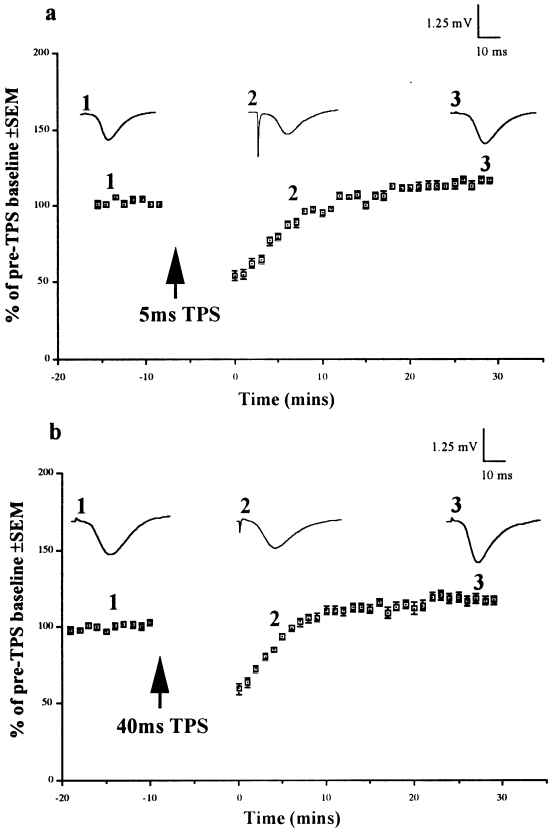

Fig. 2. Effects of two-pulse stimulation (TPS) on the amplitude of fEPSPs. (a) TPS with an IPI of 5 ms induces late-developing potentiation. (b) TPS with an IPI of 40 ms also induces late-developing potentiation. Sample fEPSP traces are displayed above each graph with numbers corresponding to the numbers above the plot to indicate the time at which the traces were taken. No samples were taken during the TPS delivery period.

have been successful in inducing LTD in other hippocampal areas in vitro [9] and in vivo [14], and TPS protocols that have been successful in inducing LTD in vivo [7,19,20] do not induce LTD of the CA1-subiculum projection. Instead, three of the four tested protocols (1 Hz LFS, TPS (5 ms IPI), and TPS (40 ms IPI)) induced a moderate but significant level of potentiation in this pathway. The other protocol, the 10 Hz LFS, induced neither LTD nor late-developing potentiation; we found instead that the amplitude of fEPSPs was reduced for the period directly after the delivery of the LFS, and that baseline levels of synaptic transmission were rapidly re-established. This late-developing potentiation is dependent on protocol delivery, not the duration of the experiment or the time under anaesthesia, since baseline fEPSP amplitudes in this pathway remain very stable over at least one hour (unpublished observations).

That LFS delivered at 1 Hz can induce potentiation rather than depression in the in vivo preparation has been previously observed in the projection from area CA1/subiculum to prefrontal cortex. Burette et al. [2] delivered LFS at 1 Hz to the hippocampal projection to the prefrontal cortex (PFC) and reported a significant increase in fEPSP amplitude within 10 min of LFS delivery which rose to 11.9 ± 3.8% above baseline in the 30–60 min following the LFS [2]. Other reports indicate that LFS at 1 Hz neither potentiates nor depresses fEPSP amplitude in the awake or anaesthetized adult rat [8,17].

The TPS protocols tested here induced a moderate but significant level of potentiation in the CA1-subicular projection. This is contrary to the findings reported in area CA1 where a TPS of 200 pulses delivered at 0.5 Hz with an IPI of 25 ms consistently produced LTD in the anaesthetized adult rat [19] and in the awake adult rat [7]. However, the same protocol failed to induce LTD in dentate gyrus of both adult and young (10–11 days) rats [7]. In the CA1/subiculum-prefrontal cortex projection, as with LFS, late-developing potentiation is observed following the delivery of either of two TPS protocols (450 pairs of pulses delivered at 0.5 Hz with an IPI of 35 ms; 900 pairs of pulses delivered at 1 Hz with an IPI of 5 ms [2]).

Burette et al. [2] suggest that the late-developing potentiation in the CA1/subiculum-PFC projection could be due to a reduction in GABAergic inhibition during low-frequency stimulation. It is well-documented that repetitive stimulation of hippocampal pyramidal cells in vitro can lead to an activity-dependent disinhibition [6]. It is possible that repetitive stimulation of the CA1-subiculum projection, with LFS or TPS, also leads to a reduction in IPSP amplitude. It would be of interest in further experiments to include an analysis of subicular cell response following LFS and TPS in the presence of a GABAergic agonist. Another possible explanation of the potentiation following LFS and TPS concerns the ratio of bursting to non-bursting cells within the subiculum. In the subiculum bursting cells outnumber non-bursting cells by about two to one [18]. This heterogeneity of cell type within the subiculum is in contrast to the homogeneity of cell type within area CA1 (regular firing) and area CA3 (bursting). Bursting activity in the subiculum following repetitive stimulation in area CA1 (by LFS or TPS) may lead to LTP-like effects within local subicular circuits (where rhythmic bursting activity acts similarly to a tetanus) that cause long-term synaptic enhancement. A single burst of stimulation can produce long-term synaptic modifications in the hippocampus [15]. It is harder to explain why such a mechanism would work with 1 Hz LFS and TPS but not with 10 Hz LFS.

Late-developing potentiation may occur via a modified system to that recruited in the induction of LTP. We have previously shown that, using a standard high-frequency stimulation protocol (10 trains of 20 stimuli at 200 Hz; intertrain interval of 2 s), maximal LTP of the CA1-subiculum projection is induced within 5 min of HFS delivery [3], while here, the potentiation we refer to as 'late-developing' occurs at least 15 min following the 1 Hz LFS, 5 ms TPS, and 40 ms TPS (and fEPSP amplitude continues to increase for the rest of the recording period).

We have shown that it is difficult to obtain homosynaptic LTD in the CA1-subiculum projection in vivo. However,

heterosynaptic LTD may occur. A suitable protocol may require concurrent stimulation of a different projection, such as the entorhinal input to the subiculum. If LTP is to be regarded as a biologically-plausible model of information storage then some mechanism for the downregulation of synaptic efficacy must also exist. This may occur via the depotentiation of previously potentiated pathways. It would be of interest in future experiments to investigate the ability of LFS and TPS protocols to depotentiate a previously potentiated fEPSP.

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