



Induction and duration of long-term potentiation in the hippocampus of the freely moving mouse

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Abstract

We describe a simple method, using readily available minaturised components, for inducing and monitoring long-term potentiation (LTP) at perforant path-granule cell synapses in the dentate gyrus of the freely moving mouse. Tetanic stimulation induced LTP of the field EPSP and the population spike which persisted for more than 24 h but was not present 10 days after the tetanus. The potentiation of the population spike was proportionately greater than the potentiation of the EPSP (E-S potentiation). Induction of LTP was blocked by intraperitoneal injections of the *N*-methyl-D-aspartate (NMDA) receptor antagonist, 3-((RS)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP). © 1997 Elsevier Science B.V.

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1. Introduction

Mice with targeted gene mutations are being increasingly used to study the molecular basis of hippocampal long-term potentiation (LTP) and its relation to spatial memory. Electrophysiological analysis of LTP in transgenic or knockout mice is normally performed in vitro, using the hippocampal slice preparation. However, certain problems associated with the slice preparation, such as the severing of neuronal connections (Matthews et al., 1976) and deterioration of the slice over time, have been documented (Teyler, 1980). LTP has also been documented in the anaesthetised mouse (Payne et al., 1982; Bliss and Errington, 1984; Namgung et al., 1995) and in a more recent study, significant differences were found between LTP in the dentate gyrus in vitro, and LTP in the same pathway of the anaesthetised animal (Nosten-Bertrand et al., 1996). Anaesthetics, however, also have profound and complex effects on cellular physiology and in particular on inhibitory

transmission (Zimmerman et al., 1994). In terms of the relationship between LTP and behaviour that have been studied in genetically modified mice, the important question is whether or not LTP is blocked or impaired in the freely-moving mouse. Although Jaffard and his colleagues have studied learning-related changes in evoked responses in the hippocampus (Jaffard and Jeantet, 1981) and lateral septum (Garcia et al., 1993) of freely moving mice, hippocampal LTP has not so far been studied in this preparation. We describe here a method using readily available miniaturised components which has allowed us to monitor LTP for several days in the dentate gyrus of the freely moving mouse.

2. Methods

To test the feasibility of making long-term electrophysiological recordings from perforant path evoked response in the dentate gyrus of the freely moving mouse, five male adult hybrid mice (F1: C57B1/6 × DBA) weighing between 20 and 30 g were used.

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2.1. Electrodes

Recording electrodes were made from a single nichrome wire (62 μm in diameter) cut flush at the end and placed inside a stainless steel microtube (200 μm external diameter). The tube was insulated except for its base which was used as a reference. The wire extended past the base of the tube by 1.2 mm. Concentric bipolar stimulating electrodes consisted of a stainless steel wire (150 μm) placed inside a 300 μm stainless steel tube. The wire was bevelled at the end and the bevelled tip protruded from the tube. Both the tip of the wire and the base of the tube were stripped of insulation. A small silver sphere placed on the cortical surface served as a ground. All electrodes were mounted on female miniature sockets (Harwin, USA, ref.: M22-6120522). The recording electrode was mounted on a 4×4 mm socket with three active pins: active, reference and ground. The stimulating electrode was mounted on 4×2 mm socket with two active pins (Fig. 1B).

2.2. Chronic recording

The connecting socket on the cable was the same as that used for the electrodes. The pins corresponding to the active and reference electrode pins were connected to a chip containing a dual differential low noise JFET input operational amplifier (Motorola, USA, ref.: TL 072) to which active and reference electrodes were connected (Fig. 1A). Also mounted upon the chip were two miniature resistors (Bournes, USA, CMS technology, ref.: CR 1206-JW, 0.25W 5%, 100 Ω ; dimension: $3.2 \times 1.6 \times 1$ mm) that acted to reduce spontaneous oscillations resulting from the capacitance of the connecting wire (≈ 100 –250 pF/m). The circuitry on the chip also contained two miniature capacitors (AVX, USA, CMS technology, 100 nF, dimension: $3.2 \times 1.6 \times 1$ mm) to decouple the battery power supply (± 6 V) from the FETs (Fig. 1A). The resistors and capacitors were secured to the FET chip with a layer of dental acrylic. All parts were obtained from Radiospare Composants, Beauvais, France. The female sockets on the head of the mouse and the cable were coupled by a double male connecting pin (Fig. 1C). The rationale for using this interface was to help safeguard the circuitry from damage or breakage of the pins whilst connecting the mouse to the cable and during the recording session.

2.3. Stimulating and recording parameters

All low frequency stimulation consisted of single (50 μs) monopolar pulses, that were negative going with respect to the reference electrode and driven from a photically isolated constant current unit, delivered to the perforant path. The evoked response was recorded

through the FET on the mouse's head and the signal was fed through a Grass amplifier (P511) and filtered between 0.1 Hz and 3 kHz. Single test stimuli were delivered at 30 s intervals at an intensity which evoked an EPSP (mean: 220 μA ; range: 130–450 μA), the slope of which was 40% of its maximum. High frequency stimulation to induce LTP consisted of six trains of six pulses (50 μs) at 400 Hz, 100 ms between each train, repeated six times at a 20 s interval. A similar protocol produces reliable LTP in the anaesthetised mouse (Nosten-Bertrand et al., 1996). During the tetanus the stimulation intensity was raised (mean: 265 μA ; range: 180–520 μA) to produce a population spike height that was 80% of its maximum. Input-output curves were generated, starting at a point where no response was observed with increasing increments until

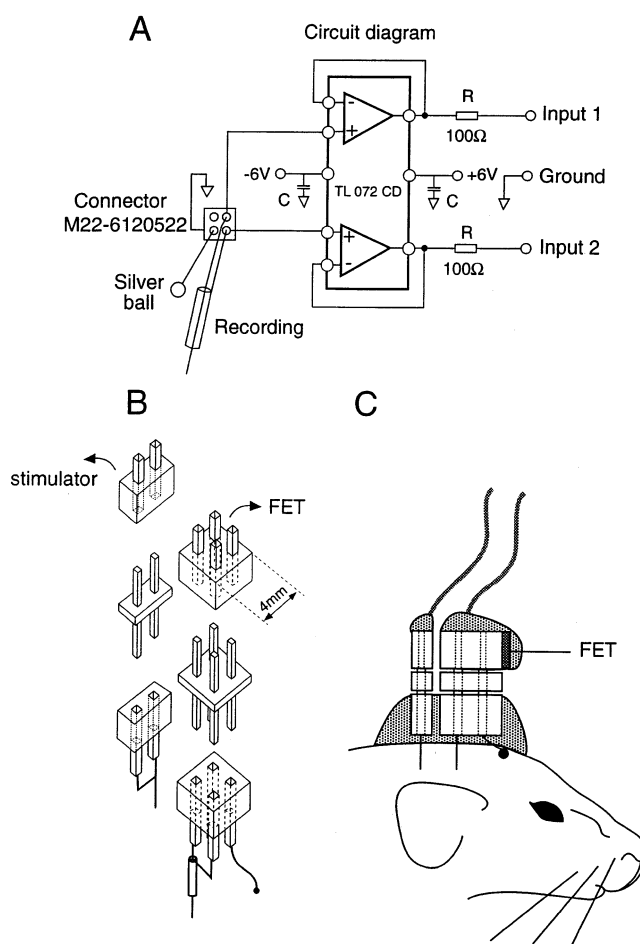


Fig. 1. A, circuit diagram of the headstage based on the Motorola TL072CD dual JFET chip and two miniature resistors, which are mounted on the side of a female connector (not shown to scale on the left). Leads to the preamplifier are attached at Inputs 1 and 2. B, blow-up diagram showing the arrangement for connecting the lower female connectors, to which stimulating and recording electrodes are attached, via double male adapters to corresponding female connectors at the top of the assembly. C, diagram of the electrode and cable assembly in place upon the mouse's head, position (FET) on headstage is also shown.

saturation of the response was reached (Fig. 3 for individual intensity range and increments). Stimulation was delivered every 10 s, and after the response to ascending increments had reached saturation, the intensity was reduced in the same decremental steps. All responses were stored as averages of four responses on computer.

2.4. Analysis

The EPSP slope was measured at a fixed latency early on the rising phase of the response. The spike height was measured from the negative peak with a vertical line drawn between the two positive peaks. Spike onset latency was taken from the initial positive peak of the spike.

2.5. Drugs

3-((RS)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, Tocris Cookson, UK.) was dissolved in saline to yield a dose of 10 mg/kg and injected i.p. two and a half hours before tetanic stimulation.

2.6. Surgical procedures

Mice were anaesthetised with pentobarbital (6 μ g/g; i.p.) and supplemented throughout surgery as required. They were mounted in a stereotaxic frame used for rat surgery, using finer ear bars (0.75 mm tip) and a nose piece for mice. An incision was made along the midline, the skin retracted and the skull scraped free of connective tissue. Two holes were drilled in the skull, the dura removed and a recording electrode lowered into the hilus of the dentate gyrus (2.1–2.3 mm caudal to Bregma; 2.1 mm lateral to the midline; approximate depth from the brain surface: 1.5 mm). The stimulating electrode was lowered into the angular bundle of the perforant path, perpendicular to the brain surface (0 mm to lambda; 3.0 mm lateral to lambda, approximate depth from brain surface: 1.5 mm). A third hole in the frontal plate of the skull allowed a silver sphere to be placed upon the surface of the cortex to form the ground electrode. Electrodes were lowered to the point where a maximal EPSP was evoked by low frequency stimulation.

After placement of electrodes drops of dental adhesive resin cement (Superbond C & B, from Sun Medical, Japan) were placed upon the skull to act as an adhesive interface between the skull surface and the dental cement used to hold the electrodes in place. The use of this specialised glue is an important factor in maintaining the electrode and socket assembly in place over long periods of time, as the mouse skull is too thin and brittle to support the jewellers screws used for this purpose in the rat. It also reduces the total weight.

Dental acrylic (Hesa, Chimie Dentaire, S.A., Switzerland) was used to encase the electrodes and sockets and hold them securely upon the mouse's head (total weight of electrodes and socket in the dental cement was no more than 1.0 g).

2.7. Experimental procedure

Following surgery, mice were allowed to recover for several days, during which time they were habituated for several hours a day to the recording chamber. On commencement of recording, mice were placed in the recording chamber for approximately 40 min prior to connecting and then left for a further 30 min, with cables connected, before recording. On day 1 an input-output curve was generated to ascertain the test and tetanus intensities, and for comparison with post LTP input-output curves. Following the input-output curve, a 20 min baseline period was recorded. Tetanic stimulation was delivered and responses were recorded for a further 60 min period at test intensity. After that, a second input-output curve was generated. On the ensuing days, after habituation to the chamber and the cables, a 30 min period was recorded at test intensity, followed by an input-output curve. In one mouse it was not possible to record a response and was therefore not tested.

3. Results

The mice were healthy after surgery and appeared to suffer no discomfort from carrying the extra weight on their heads. Climbing behaviour was observed both in their home cages and whilst connected to the cables in the recording chamber, suggesting the head caps did not restrict normal exploratory behaviour.

Samples of evoked responses recorded in the hilus and the dendritic layer of the dentate gyrus are shown in Fig. 2A,B. In three mice the recording electrode was placed in the hilus, giving a positive field EPSP and in one mouse a negative response in the dendritic layer was recorded. Typically the mice showed very large spike amplitudes and in some cases at high intensity stimulation or after LTP induction there was the appearance of multiple spikes. The spike also tended to have an early onset, appearing quite low down on the slope of the EPSP (Fig. 2A).

The magnitude of LTP, measured 60 min after the tetanus was approximately 45% for the slope of the EPSP and 400% for the population spike height (Fig. 2C and 2D). The spike onset latency was reduced by approximately 40%. These changes are also reflected in the input-output curves before and after LTP for each of these measures (Fig. 3). LTP persisted at the same

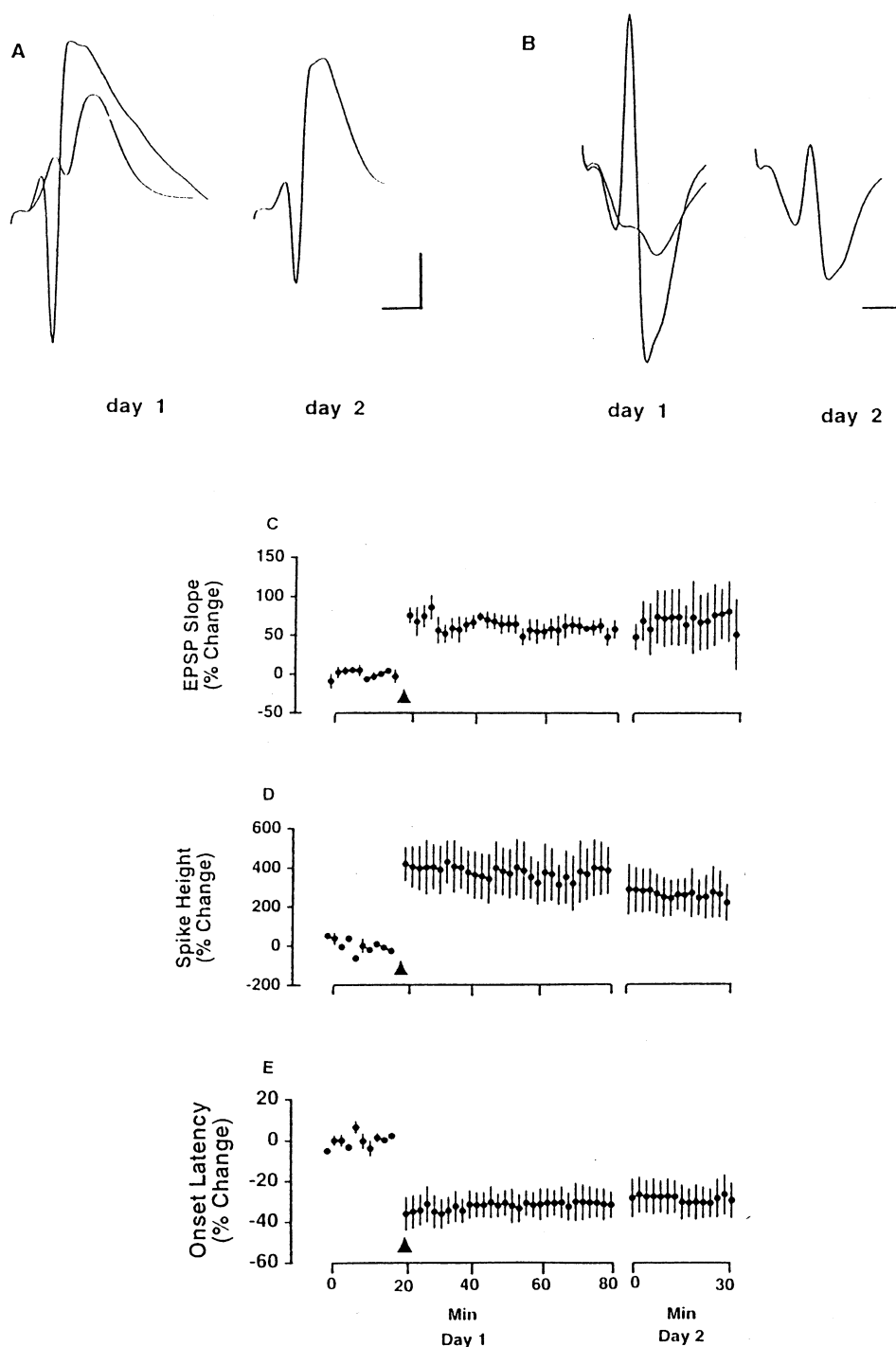


Fig. 2. LTP in awake mice. A, B: Traces from 2 animals in one of which the recording electrode was positioned in the hilus (A) and, in the other, the molecular layer (B). In each case, the superimposed pairs of traces on the left were collected before and 58–60 min after the tetanus, and the single traces on the right approximately 24 h post-tetanus. Each trace is the average of four consecutive responses. Calibration: A, 1.5 mV, 4 m/s; B, 1 mV, 5 ms. C–E: normalised plots (group means \pm S.E.M.) of the EPSP (C), spike height (D) and spike onset latency (E) for the three animals with responses recorded in the hilus. Data from the day of induction and the following day are plotted. For each animal, four consecutive responses were averaged to provide data points for the group average. The tetanus is indicated by an arrow.

level for at least 1 day after induction (Fig. 2). After this time, the response became more variable where the EPSP and the spike in two of the mice had begun to descend towards baseline values, whereas for the other two the response remained at the same potentiated level

(data not represented). In two mice, recordings were made again 10 days after the induction of LTP and the response had returned to baseline values. It is interesting to note that there was no evidence of post-tetanic potentiation.

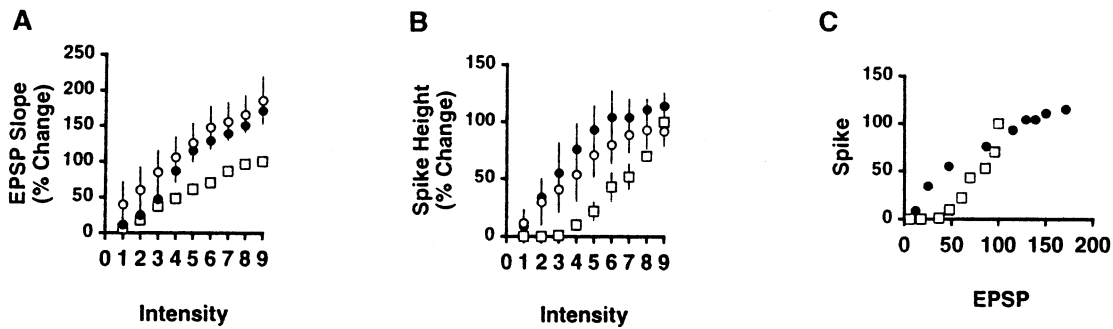


Fig. 3. Input-output curves for EPSP (A), spike height (B), and EPSP-spike (E-S) relationship (C), generated before and after the induction of LTP. All points were normalised to the maximum pre-tetanus value and the abscissa represents the nine increments used. The range of intensities and corresponding increments used in each of the three animals with hilar responses were: 120–200 μ A, 10 μ A increments; 90–170 μ A, 10 μ A increments; 240–560 μ A, 40 μ A increments. Input-output curves (means \pm S.E.M.) are plotted from values obtained before the tetanus (open squares), 60 min after the tetanus (filled circles) and 24 h after the tetanus (open circles). E-S curves are plotted for the group mean data obtained before (open squares) and 1 h after (open circles) the tetanus.

The input-output curves showed a typical increase in the EPSP and the spike amplitude for a given stimulus intensity, 60 min and 24 h after the induction of LTP. There was, on average, an 80% increase in the slope of the EPSP at the highest intensity. With the spike, although it was saturated both before and after induction of LTP, the input-output curve following LTP reached saturation earlier and at all intensities it was greater (Fig. 3A,B). The E-S relationship showed a dramatic shift to the left, implying that after LTP induction spike potentiation is relatively greater than EPSP potentiation (Fig. 3C).

In two of the mice, after the response had returned to baseline levels, a tetanus was delivered in the presence of the NMDA receptor antagonist, CPP, which blocked induction of LTP in both the spike and the EPSP. Two days following the blockade of LTP, a tetanus was once again delivered and LTP was induced, indicating that the compound had been metabolised and normal LTP could again be induced (Fig. 4).

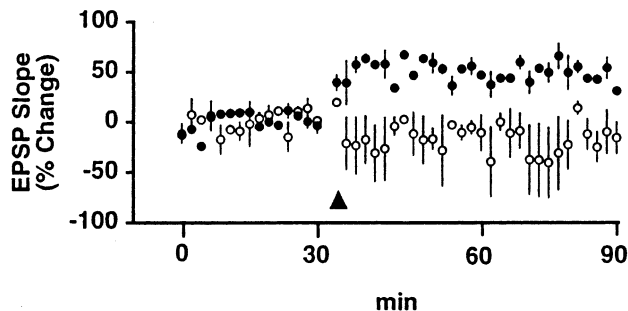


Fig. 4. The NMDA receptor antagonist CPP blocks the induction of LTP in awake mice. The mean slope of the EPSP, normalised to baseline values, is plotted for two animals in which CPP (10 mg/kg, i.p.) was injected 2.5 h before the tetanus, (arrow) blocking the induction of LTP (open circles). Two days later, a tetanus was again delivered, and LTP was now induced (filled circles). For each animal, four consecutive responses were averaged to provide data points for the group average.

4. Discussion

We have shown that it is possible to make chronic implantation of electrodes in mice without causing impairment to their health or behaviour and to record from them in an awake, freely moving state for many days. The dentate gyrus responses evoked by perforant path stimulation are much the same as that seen in the freely moving rat with the exception that the spike appears earlier on the rising phase of the EPSP. LTP, including an E-S component of potentiation, can be induced that lasts for several days and is NMDA receptor-dependent.

There are several advantages of measuring LTP or LTD *in vivo* rather than *in vitro*, of which the most obvious is the ability to follow the full time course of the effect. The possibility that the magnitude of LTP in the artificial environment of the hippocampal slice chamber, may be very different from the same region in the anaesthetised animal has already been documented (Nosten-Bertrand et al., 1996). We describe here a technique for recording the evoked response in the dentate gyrus and monitoring LTP in the freely moving mouse. We foresee little problems in extending this technique to other regions of the hippocampus and to other parts of the brain. This technique has far reaching implications for investigating the role of proteins and the molecular mechanisms of synaptic plasticity and learning in genetically engineered mice.

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