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Review

Comparative plasticity of brain synapses in inbred mouse strains

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Summary

One niche of experimental biology that has experienced considerable progress is the neurobiology of learning and memory. A key contributor to such progress has been the widespread use of transgenic and 'knockout' mice to elucidate the mechanisms of identifiable phenotypes of learning and memory. Inbred mouse strains are needed to generate genetically modified mice. However, genetic variations between inbred strains can confound the interpretation of cellular neurophysiological phenotypes of mutant mice. It is known that altered physiological strength of synaptic transmission ('synaptic plasticity') can modify and regulate learning and memory. Characterization of the synaptic phenotypes of inbred mouse strains is needed to identify the most appropriate strains to use for generating mutant mouse models of memory function. More importantly, comparative electrophysiological analyses of inbred mice *per se* can also shed light on which forms of synaptic plasticity underlie particular types of learning and memory. Many such analyses have focused on synaptic plasticity in the hippocampus because of the critical roles of this brain structure in the formation and consolidation of long-term memories. Comparative electrophysiological data obtained from several inbred mouse strains are reviewed here to highlight the following key notions: (1) synaptic plasticity is influenced by the genetic backgrounds of inbred mice; (2) the plasticity of hippocampal synapses in inbred mice is 'tuned' to particular temporal patterns of activity; (3) long-term potentiation, but not long-term depression, is a cellular correlate of behavioural memory performance in some strains; (4) synaptic phenotyping of inbred mouse strains can identify cellular models of memory impairment that can be used to elucidate mechanisms that may cause specific memory deficits.

Key words: synaptic plasticity, hippocampus, inbred mice, mouse strain, long-term potentiation (LTP), long-term depression (LTD), learning, memory.

Introduction

All of experimental biology is, in one way or another, aimed at identifying and characterizing the mechanisms that produce identifiable phenotypes in cells, tissues and organisms. This is especially true of neuroscience research. At multiple levels of experimental analysis, the diversity of behavioural, neurophysiological and neurochemical phenotypes seen in mammals is, in large part, a result of the complexity of numerous brain functions. These functions include cognitive processes such as learning, memory and perception, and cellular actions such as activity-dependent modifications of synaptic strength ('synaptic plasticity').

Understanding the mechanisms of synaptic plasticity and memory is an important goal of neuroscience. Murine transgenic and gene-targeting techniques are invaluable methods for elucidating the roles of genes and intracellular signalling pathways in synaptic plasticity and memory (Wehner et al., 1996; Picciotto and Wickman, 1998; Micheau and Riedel, 1999; Martin et al., 2000). Within specific brain regions, single genes may be artificially overexpressed (Jaenisch and Mintz, 1974; Constantini and Lacy, 1981; Palmiter et al., 1982; Mayford et al., 1996), or their expression may be reduced or eliminated by targeted mutagenesis (Thomas and Capecchi, 1986; Bradley, 1993). These molecular strategies have been effectively applied to generate genetically modified mice for mechanistic investigations of synaptic plasticity and memory (Picciotto and Wickman, 1998).

Two inbred strains of mice are commonly used to generate genetically modified mice. An inbred strain is one in which matings between siblings have been performed for at least 20 generations, resulting in a population of genetically homogeneous animals (Lyon and Searle, 1989). One inbred strain supplies a viable genetic background for breeding and survival, whereas a second inbred strain provides stem cells for genetic manipulation (Hogan et al., 1994). An important consideration inherent in all of these studies is that disruption

or overexpression of a single gene can lead to compensatory changes in the expression of other genes, the presence or absence of which can vary according to the genetic backgrounds of the mouse strains used to generate a genetically modified line of mice (Crawley et al., 1997). Valid interpretation of the neurophysiological phenotypes that emerge from genetically modified mice therefore requires knowledge of the synaptic properties of relevant neurons in the parent strains used to produce genetically modified lines of mice. Hence, the characterization of synaptic phenotypes of neurons in relevant brain structures of inbred mice is an important step towards defining the genetic and molecular bases of synaptic plasticity. It can also lead to the compilation of physiological databases (mouse 'physiomes') needed to construct mouse models of synaptic and cognitive dysfunction.

Long-term potentiation (LTP) and long-term depression (LTD) constitute activity-dependent enhancement and reduction, respectively, of excitatory synaptic strength (Lomo, 1966; Bliss and Lomo, 1973; Dudek and Bear, 1992). These two types of synaptic plasticity are believed to play important roles in mediating learning and memory (Martin et al., 2000; Lynch, 2004), perception (Klein et al., 2004), and the refinement of synaptic circuitry (Kirkwood et al., 1995). In humans and mice, area CA1 (cornu ammonis-1) of the hippocampus is vital for the formation of long-term memory (Zola-Morgan et al., 1986; Tsien et al., 1996). Genetic modifications of key signalling molecules within area CA1 of the mouse hippocampus can impair long-term memory and LTP (reviewed by Lynch, 2004). Some comparative data showing strain-associated variations of hippocampal memory *and* LTP in area CA1 of *in vitro* slices have been reported (Nguyen et al., 2000a; Nguyen et al., 2000b; Schimanski et al., 2002; Schimanski et al., 2005a; Schimanski et al., 2005b) [for *in vivo* data, see (Bampton et al., 1999; Jones et al., 2001)]. However, the mechanisms underlying strain-dependent variations in LTP are mostly undefined, and conjoint characterization of LTP and memory in inbred mouse strains is still nascent. Also, it should be emphasized that comparative analysis of inbred strains can shed light on which particular types of synaptic plasticity are critical for expression of specific forms of learning and memory. The question, 'Does LTP=memory?' can be effectively addressed by using inbred mice: they provide an experiment of nature to test this hypothesis in a less biased manner than experiments that use reverse genetic approaches.

One of the goals of this article is to provide newcomers to this field with information to 'get started' with phenotyping of hippocampal synapses in mouse strains. A second goal is to provide a succinct, selective overview of comparative *in vitro* synaptology of inbred mice. Some *in vitro* electrophysiological methods for probing hippocampal synaptic plasticity are described, along with comparative data from selected inbred mouse strains. Brief coverage of memory function is provided to underscore the notion that some types of behavioural memory can be correlated with particular forms of synaptic plasticity in inbred strains. Readers seeking more in-depth coverage of memory functions in mice, and quantitative genetics of mouse behaviour, should consult additional sources (Crawley, 2000; Wehner et al., 2001; Wahlsten et al., 2003; Greenspan, 2004; Schimanski and Nguyen, 2004).

Methods for assessing synaptic plasticity in mouse hippocampal slices

C57BL/6J ('B6') is frequently used as a 'background' strain for breeding congenic and transgenic mice, and as a 'control' for inter-strain comparisons of synaptic plasticity, learning and memory. Mice of various strains, aged 8–13 weeks, were used for most of the experiments reviewed here (Nguyen et al., 2000a; Nguyen et al., 2000b; Schimanski and Nguyen, 2005a; Schimanski and Nguyen, 2005b).

Many aspects of the methods described below are used in several laboratories (e.g. Nayak et al., 1998; Bozdagi et al., 2000; Matsushita et al., 2001; Knapp and Klann, 2002; Vanhoose and Winder, 2003; Ferguson et al., 2004; Ho et al., 2004; Sajikumar et al., 2005; Wood et al., 2005; Young and Nguyen, 2005). There are variations in these methods. These usually pertain to the type of slice chamber used (interface *versus* submerged), the temperature at which slices are maintained, and the stimulation protocols used to induce synaptic plasticity. I describe extracellular methods only, because they are relatively simple to learn (and thus, are used extensively) and they can provide stable recordings of synaptic potentials over several hours of experimentation. I also present some whole-cell patch clamp data; methods for single-cell patch clamp recording are described elsewhere (Nguyen et al., 2000b).

Hippocampal slice preparation and interface slice chambers

Mice are sacrificed by rapid cervical dislocation followed by decapitation. The isolated brain (Fig. 1A) is transferred to a beaker of ice-cold artificial cerebrospinal fluid (ACSF; composition in mmol l^{-1}: 124 NaCl, 4.4 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, 10 glucose). It is important that all ACSF used during the dissection is bubbled with a mixture of 95% O_2 and 5% CO_2. This 'carbogen' mixture is needed to maintain proper pH balance of the bicarbonate-buffered ACSF. The hippocampus is isolated from the adjoining brain hemisphere and is placed on an acrylic platform lined with a piece of filter paper. Once oriented with its longitudinal axis perpendicular to a razor blade that is mounted on a manual tissue chopper (Stoelting, Woodale, IL, USA), the hippocampus is cut into 400 μm-thick slices. A drop of ACSF is applied to the isolated whole hippocampus immediately prior to sectioning so that slices can adhere to the blade. A fine paintbrush is used to gently transfer slices from the blade to a small glass Petri dish containing ice-cold oxygenated ACSF.

Transversely sectioned hippocampal slices (Fig. 1B) are then transferred to an interface recording chamber (Fine Science Tools, Vancouver, Canada; Fig. 1C). Slices are continuously perfused with ACSF (flow rate of 1 ml min^{-1})

aerated with carbogen. Suction is provided to continually remove circulated ACSF from the slice recording wells in the chamber. With sufficient practice, this entire procedure, from decapitation to slice transfer, should take no more than 7–10 min. In order to obtain robust and stable synaptic field recordings, 1–2 h after slicing, it is critical to minimize warming of hippocampal tissue and to prevent its contact with blood plasma during dissection.

An interface chamber allows maintenance of partially submerged, physiologically viable brain slices at pre-set temperatures within a humidified environment. Many labs maintain slices at temperatures ranging from room temperature up to 34°C. Generally, with an interface chamber, higher temperatures tend to increase the rate of moisture condensation on the surfaces of glass recording electrodes. This condensation can drop onto slices during an experiment. When this occurs, field EPSP recordings can be obliterated. One solution to this problem is to use a fine thread or strand of facial tissue to gently wipe away small drops of condensation on the electrode shank during experiments. Also, rubbing dental wax onto the shanks of glass microelectrodes can provide an absorbent surface that reduces the amount of free condensation formed on these electrodes.

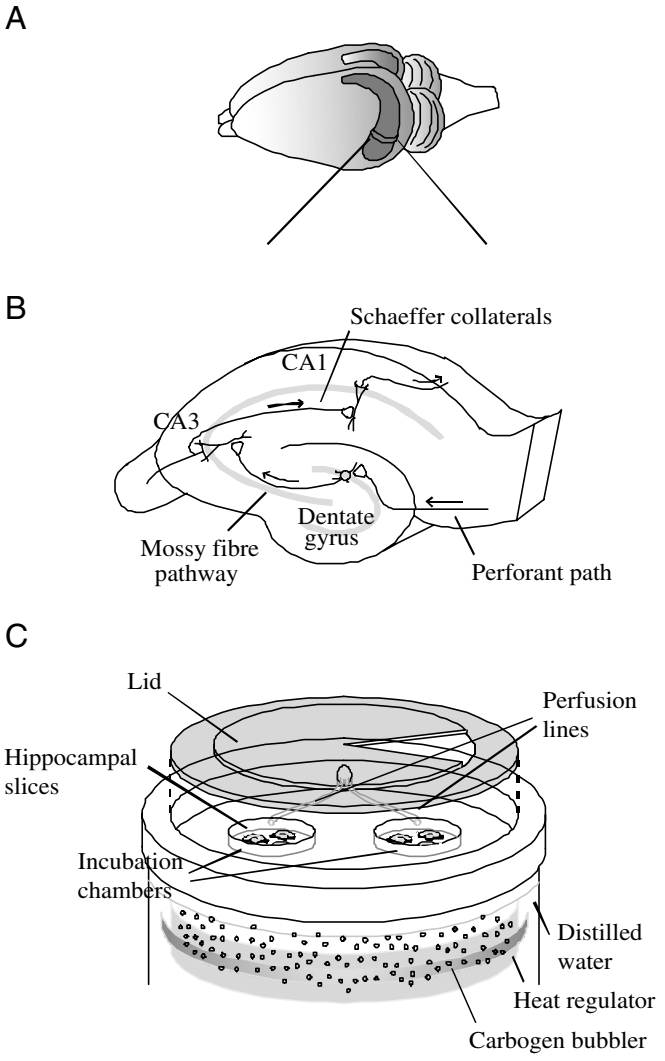

Fig. 1. Hippocampal slices in an interface chamber. (A) Diagram of a mouse brain showing the positions of the hippocampus and a transverse hippocampal slice. (B) Circuitry and anatomical subregions within a hippocampal slice. (C) Schematic diagram of an interface recording chamber. Hippocampal slices are placed on meshed rings positioned within individual wells. This temperature-regulated chamber allows slices to be perfused with artificial cerebrospinal fluid (ACSF) while exposed to a humidified, oxygenated environment. Adapted from (Young, 2005).

After a recovery period of at least 1 h, extracellular field excitatory postsynaptic potentials (fEPSPs) are recorded with an ACSF-filled glass microelectrode (see below) positioned in the stratum radiatum of area CA1. These fEPSPs represent the collective synaptic responses generated by activation of populations of postsynaptic CA1 neurons. Evoked fEPSPs are elicited by stimulation of Schaeffer collateral fibres (Schaeffer, 1892) using an extracellular bipolar nickel–chromium electrode (see below). These fibres provide synapses that excite the apical dendrites of CA1 pyramidal neurons. Substantial information on synaptic transmission has been derived from studies of these synapses; they are prototypical chemical synapses in the mammalian central nervous system.

Electrodes and data analysis

Bipolar stimulating electrodes are created by twisting two fine nickel chromium wires (A-M Systems, Carlsborg, WA, USA) together and sealing them within a glass capillary tube. Because the thin wires are coated with formvar, one end of the wires is briefly flamed to strip away the coating, and then the stripped segment is connected *via* an isolator to a stimulator (e.g. Grass Instruments model S88, West Warwick, RI, USA). The final diameter of the stimulation electrode is approximately 130 μm. After repeated usage over several weeks, a noticeable increase in the threshold for eliciting fEPSPs usually occurs. A razor blade is then used to trim off the distal tip of wires in order to obtain a new electrode surface for tissue stimulation. With daily usage, this process is generally required every 2–4 weeks. At the end of each day's experiments, the distal tip of the electrode that had contacted brain tissue should be cleaned by brief immersion in ethanol followed by distilled water. This procedure appears to decrease the frequency of electrode trimming required to 'refresh' these electrodes.

Glass recording electrodes are produced from a micropipette puller (e.g. Flaming Brown P-87 puller, Sutter Instruments, Novato, CA, USA). The recording electrode can be filled with ACSF and it should have an electrical resistance of 1–3 MΩ.

The stimulation intensity (0.08 ms pulse width) is adjusted to give fEPSP amplitudes that are approximately 40% of maximal fEPSP sizes. Control 'baseline' responses are elicited once per minute at this intensity. Slices that show maximal fEPSP sizes smaller than 3 mV are rejected.

For two-pathway experiments, two stimulating electrodes (S1 and S2) are placed in the stratum radiatum on opposite sides of the recording electrode to stimulate two separate

groups of Schaeffer collateral fibres. The independence of the two pathways is demonstrated by the absence of paired-pulse facilitation of fEPSPs when two successive stimuli are delivered to the two pathways at a 50 ms interpulse interval.

Evoked fEPSPs are low-pass filtered (1–3 kHz) and amplified (e.g. IE-210 amplifier, Warner Instruments, Hamden, CT, USA). These fEPSPs are digitized at a rate of 20 kHz by a Digidata-1200 acquisition system (Molecular Devices, Union City, CA, USA), and they are recorded with pCLAMP software (Molecular Devices). Offline data analysis is performed with Clampex software (Molecular Devices), by measuring the initial slopes of fEPSPs.

Stimulation protocols

LTP is induced by applying one of several different protocols, including a single 1 s train of 100 Hz (henceforth referred to as 'single-train'), four 1 s trains of 100 Hz (henceforth referred to as 'multi-train') spaced at various intervals, or theta-burst stimulation. A theta-burst stimulation protocol can consist of 15 bursts of four pulses each, delivered at a pulse frequency of 100 Hz with a 200 ms interburst interval. These protocols are used because they induce distinct forms of LTP that have been correlated with particular types of hippocampus-dependent memory. In mouse hippocampal slices, a single-train stimulus induces 'early' LTP (E-LTP) that is strongly correlated with hippocampus-dependent short-term memory for contextual fear conditioning (Abel et al., 1997), whereas multi-train stimulation induces a long-lasting, 'late' phase of LTP (L-LTP, Fig. 2A) that is correlated with hippocampal long-term memory (Abel et al., 1997). L-LTP, but not E-LTP, requires translation and transcription (Fig. 2B) (reviewed by Nguyen and Woo, 2003). Theta-burst stimulation mimics hippocampal spike discharge patterns that occur during some types of exploratory behaviour in rodents (Larson et al., 1986; Otto et al., 1991).

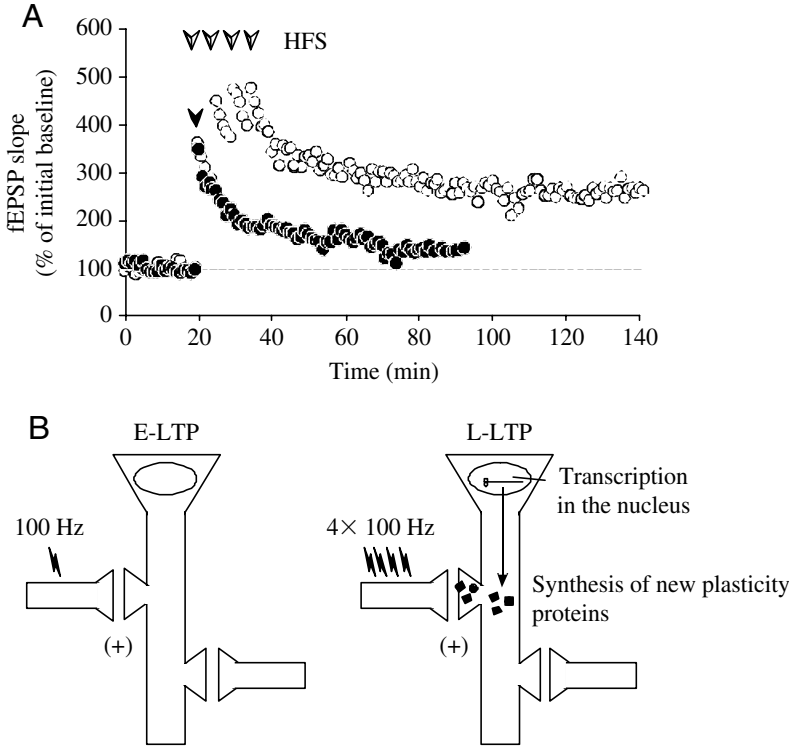

Fig. 2. Different phases of long-term potentiation (LTP). (A) The early phase of LTP (E-LTP; solid circles) is usually induced by one train of high frequency stimulation (HFS; arrowhead) and lasts 1–2 h. A more enduring, late phase of LTP (L-LTP) is induced by three or more trains of HFS (open circles) and is associated with larger amplitudes and steeper initial slopes of fEPSPs for a longer period of time. Note the flat, stable plateau of elevated fEPSP slopes, which is LTP. (B) Different phases of LTP have different mechanisms. E-LTP involves covalent modifications of pre-existing molecules, whereas L-LTP requires transcription and *de novo* protein synthesis. Adapted from (Woo, 2003).

The converse of LTP, long-term depression (LTD), can be induced by giving 1 Hz stimulation for 15 min to slices cut from mice aged 4–5 weeks.

Strain-related variations in hippocampal LTP and LTD: mechanisms and insights

Comparative studies of hippocampal memory in several inbred mouse strains have demonstrated strain-dependent variations of hippocampal memory expression (Paylor et al., 1993; Paylor et al., 1994). These studies prompted conjoint analyses of hippocampal memory and hippocampal synaptic plasticity in area CA1 of these strains (Nguyen et al., 2000a), as well as detailed investigation of the cellular bases for strain-related deficits in CA1 LTP (Nguyen et al., 2000b). These investigations focused on four inbred strains: C57BL/6J ('B6'), CBA/J ('CBA'), DBA/2J ('DBA') and 129/SvEms/J ('129'). These were selected because they have been used to generate mutant mice for neurobiological research, and therefore, the phenotypes of these parental strains should be of broad interest to neuroscientists. The objective of these studies was to identify cellular correlates of the memory deficits seen in some of these strains. Collectively, these studies produced the following observations and conclusions:

(1) There are significant variations in the induction and maintenance of LTP in area CA1 of the four selected strains of inbred mice (Nguyen et al., 2000a; Nguyen et al., 2000b). Hippocampal slices from CBA, 129 and DBA mice showed less robust induction and maintenance of LTP than B6 slices following multi-train (Fig. 3) or theta-burst stimulation. Hence, defective LTP in mutant mice generated from these strains may result from the genetic background of the parent strain rather than from the genetic manipulation *per se*.

(2) The temporal pattern of synaptic stimulation critically modulates hippocampal LTP in a strain-specific manner (Nguyen et al., 2000b). LTP was enhanced in slices from strain 129 following repeated stimulation using 3 s interburst

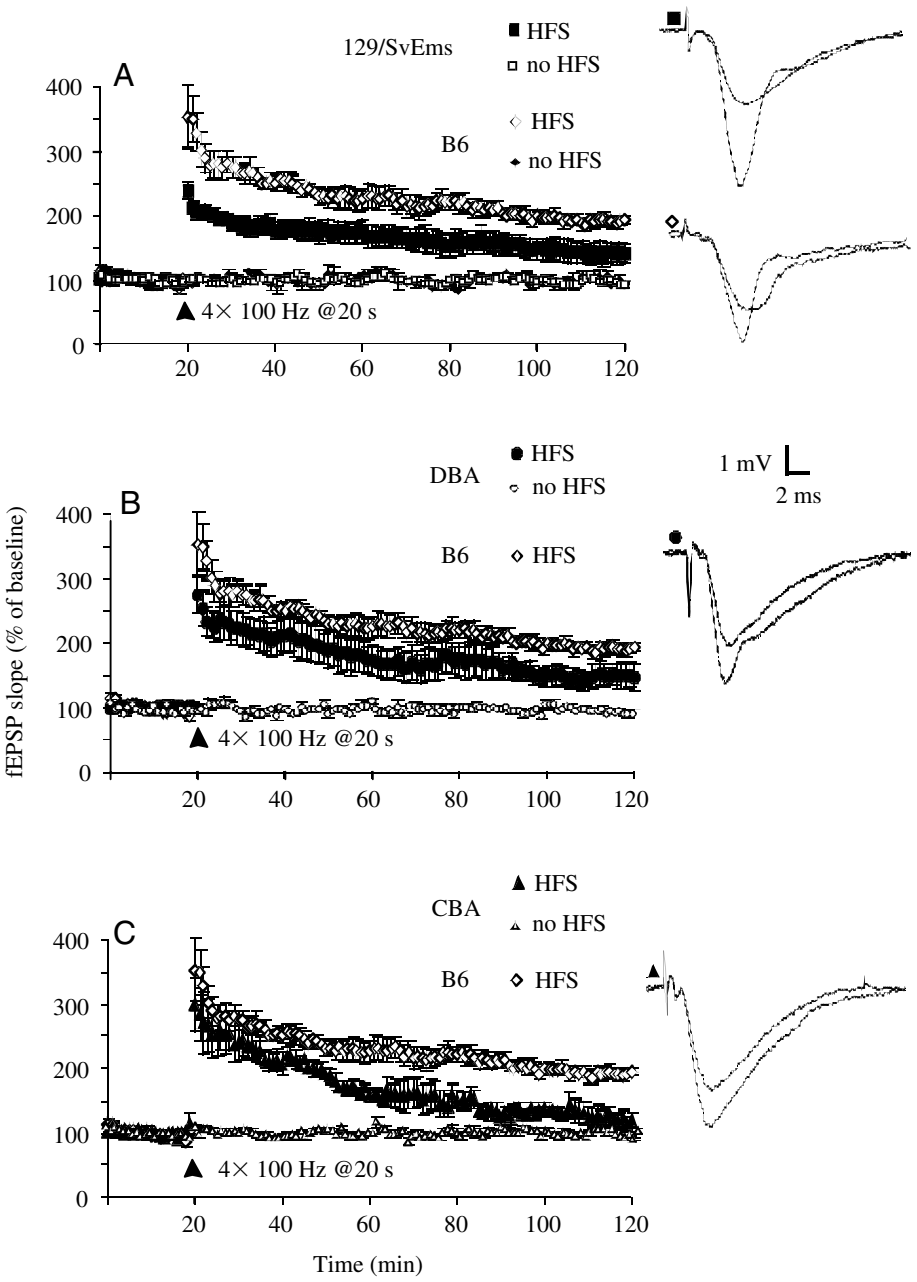

Fig. 3. Long-term potentiation (LTP) evoked by multiple bursts of stimulation is deficient in some strains of mice. LTP elicited by four 1 s bursts of 100 Hz, interburst interval 20 s, (4× 100 Hz@20s) is reduced in 129/SvEms (A, *N*=7 mice, 7 slices), DBA (B, *N*=7 mice, 7 slices) and CBA (C, *N*=10 mice, 12 slices) as compared to B6 (*N*=5 mice, 5 slices) hippocampi. Baseline fEPSPs measured during stimulation of a neighbouring pathway that did not experience high-frequency stimulation (no HFS) were unaffected in each strain tested. The B6 curve in A is repeated in B and C for comparison with other strains. Sample sweeps to the right are synaptic responses measured at 90 min post-induction. Taken from (Nguyen et al., 2000b). (Copyright 2000, The American Physiological Society, used with permission.)

intervals (Fig. 4), whereas the same total amount of imposed activity produced less robust LTP when a 20 s interburst interval was applied (Fig. 3). In slices from CBA, DBA and B6 mice, changing the interburst interval from 20 s to 3 s, while keeping the total imposed activity constant, did not significantly affect LTP (Nguyen et al., 2000b). However, changing interburst intervals had opposite effects on maintenance of LTP in 129 and DBA slices: in 129, decreasing the interburst interval increased LTP (Fig. 3A, Fig. 4A), whereas in DBA, decreasing the interval reduced LTP (Fig. 3B, Fig. 4B). In other words, the hippocampal neurons of some inbred mouse strains are more 'tuned' to particular temporal patterns of synaptic activity because of strain-related variations in genetic background. These findings also emphasize the importance of using various temporal patterns of synaptic stimulation to probe for altered synaptic plasticity.

(3) Membrane biophysical properties and spike frequency accommodation (Fig. 5) of hippocampal CA1 pyramidal neurons did not vary significantly between these four strains (Nguyen et al., 2000b). There were no significant strain-specific differences in membrane input resistance, spike frequency accommodation, and membrane depolarization (during 100 Hz stimulation) in CA1 pyramidal cells from these strains. Thus, genetic variation among these four strains did not significantly alter membrane biophysical properties or spiking efficacy of CA1 neurons.

(4) Glutamatergic receptors, such as *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, are important for

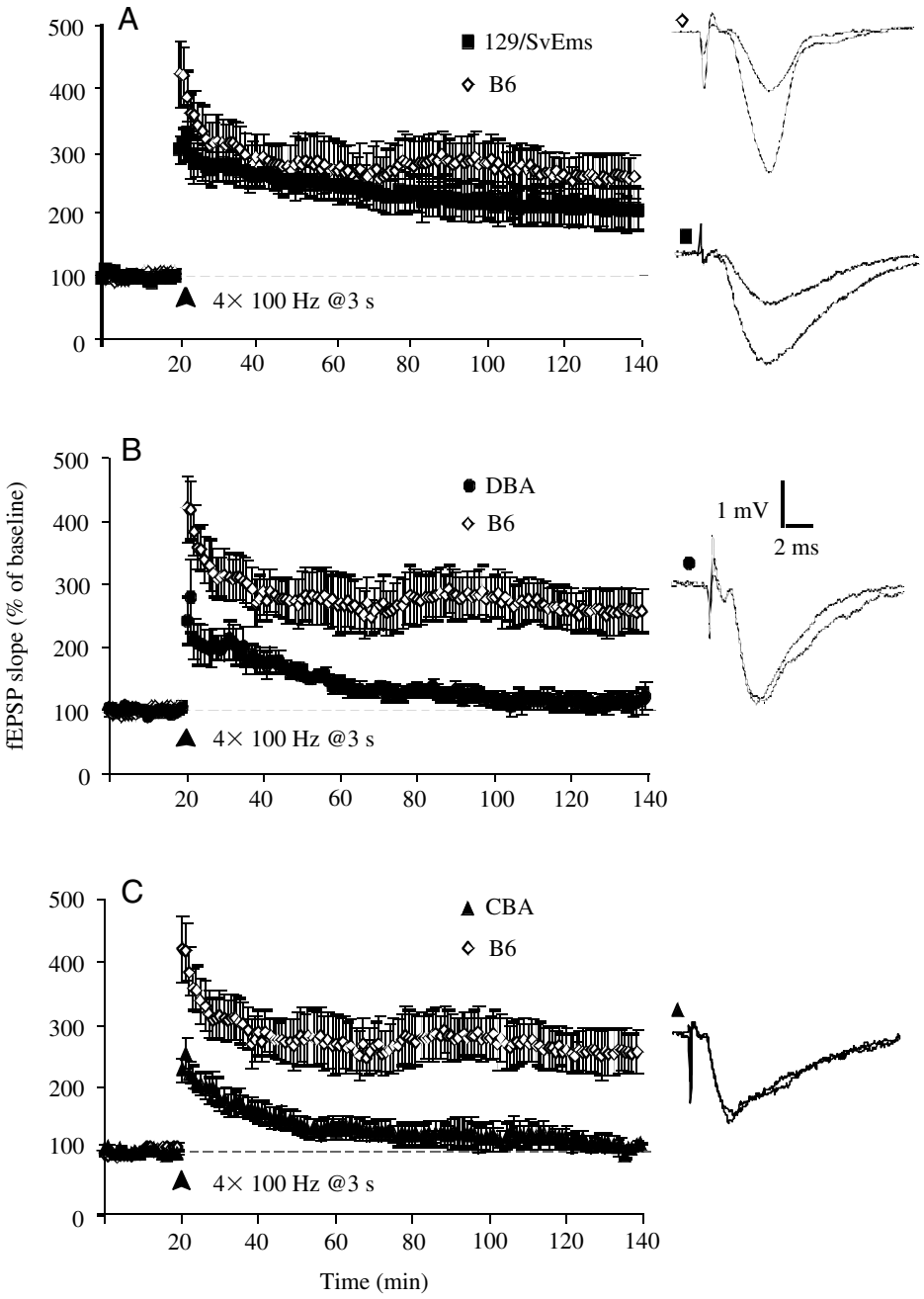

Fig. 4. Strain-selective enhancement of long-term potentiation (LTP) by temporally compressed stimulation. (A) There was no significant difference in LTP between B6 (*N*=7 mice, 7 slices) and 129/SvEms (*N*=5 mice, 5 slices) hippocampi when the interburst interval was reduced to 3 s. (B,C) LTP in DBA (*N*=6 mice, 6 slices) and CBA (*N*=7 mice, 7 slices) strains remained deficient relative to that of the B6 strain. The B6 curve in A is repeated in B and C for comparison with other strains. Sample sweeps were recorded at *t*=130 min (110 min post-induction). Taken from (Nguyen et al., 2000b). (Copyright 2000, The American Physiological Society, used with permission.)

induction of hippocampal LTP, but no significant strain-dependent variation in the sizes of synaptically evoked NMDA and non-NMDA currents was observed (Fig. 5). These data are consistent with those reported elsewhere (Jia et al., 1998), which showed that, for some inbred and hybrid strains (129/Sv, 129/SvXC57BL/6 and 129 SvXCD1), LTP of AMPA- and NMDA-type currents was independent of genetic background. Thus, the genetic backgrounds of many inbred mouse strains do not significantly alter the amplitudes of synaptically evoked glutamatergic currents.

(5) By contrast to the LTP deficits seen in these four strains, there were no significant differences in LTD (Nguyen et al., 2000b). DBA and CBA mice displayed deficits in both hippocampus-dependent spatial memory and fear conditioning (Nguyen et al., 2000a), but they showed intact LTD (Nguyen et al., 2000b). Thus, LTP, but not LTD, may be a cellular mechanism for hippocampal memory in these strains.

Variations in hippocampal LTP are certainly not confined to these four inbred strains. Other inbred mouse strains also display deficits in particular forms of LTP in hippocampal and amygdalar circuits, as assessed using *in vitro* techniques (Schimanski and Nguyen, 2004; Schimanski and Nguyen, 2005a; Schimanski and Nguyen, 2005b). It should be noted that many studies using genetic methods have reached similar conclusions, and that inducible systems, e.g. doxycycline-mediated expression of transgenes (Kistner et al., 1996) are

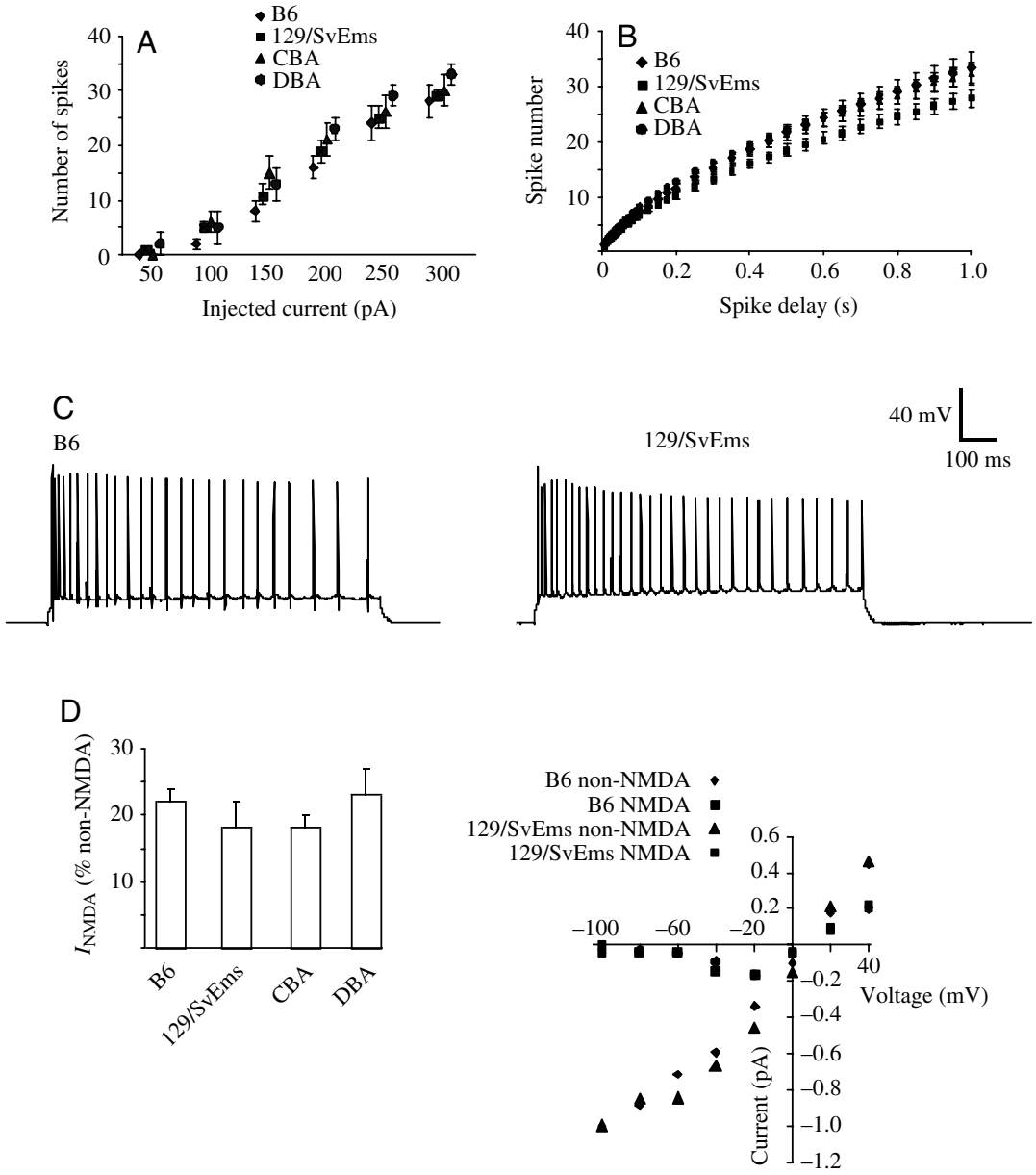

Fig. 5. Spike discharge properties and glutamatergic receptor currents of CA1 pyramidal neurons are not strain dependent. (A) Membrane excitability: plot of the number of action potentials produced during a 1 s current pulse *versus* current amplitude in each of the four mouse strains. (B) Spike frequency accommodation: plot of spike number (order in a train) *versus* delay from onset of current injection. These are averages of fifth-order polynomial curve fits of data obtained from B6 (N=13 cells) 129/SvEms (N=28), DBA (N=14) and CBA (N=21) neurons. (C) Sample traces showing spike frequency accommodation in B6 and 129/SvEms cells. Current injections of 300 pA were used to elicit spiking. (D) Left: ratios of *N*-methyl-D-aspartate (NMDA) to non-NMDA glutamatergic currents were not significantly different between strains (B6, N=14 cells; 129/SvEms, N=12; DBA, N=5; CBA, N=5). Right: a current–voltage plot comparing the voltage dependence of glutamatergic currents measured in B6 and 129/SvEms neurons. Taken from (Nguyen et al., 2000b). (Copyright 2000, The American Physiological Society, used with permission.)

also effective means of addressing the question, 'Does LTP=memory?'.

Hippocampal synaptic plasticity and memory function

Conjoint measurements of synaptic and mnemonic phenotypes have been accomplished for several inbred mouse strains (Table 1). These data underscore the notion that it is difficult to make broadly based, generic correlations between fear conditioning (a key memory task used in mouse behavioural research) (see Crawley, 2000; Schimanski and Nguyen, 2004) and LTP. Some consistent correlations exist (Table 1), but not every strain tested has yielded data that would definitively establish generic correlations between particular

forms of LTP and fear conditioning. For example, some forms of CA1 LTP are excellent cellular correlates of contextual fear memory in many, but not all, strains that have been tested; one exception is strain 129/SvEms/J (Table 1). Also, LTP in the medial perforant pathway (MPP) is not a good cellular correlate of contextual fear memory, because in two strains, DBA/2J and C3H/HeJ, defective memory is present alongside intact MPP-LTP. In order to generalize these conclusions, more thorough examination of other inbred strains is necessary.

Synaptic phenotyping of inbred mice can shed light on the synaptic mechanisms that contribute to information processing by specific subregions of the mammalian hippocampus. Different computational functions, such as pattern association and temporal pattern completion, may be mediated by distinct hippocampal subregions (Kesner et al., 2000). Synaptic plasticity in CA1 and the dentate gyrus may be correlated with these functions, but this idea can only be rigorously tested by combining detailed electrophysiological analysis with well-defined behavioural tests that substantially target selected subregions of the hippocampal formation. Such targeting has been accomplished at the molecular level, using subregion-specific knockouts of key molecules, such as the NMDA receptor in areas CA1 (Tsien et al., 1996) and CA3 (Nakazawa et al., 2002). These studies implicated critical roles in memory processing and memory recall for these subregions of the hippocampus.

Additionally, other forms of activity induced synaptic plasticity might be modified in inbred mice. For example, altered 'metaplasticity', i.e. plasticity of synaptic plasticity (Abraham and Bear, 1996), of synapses might correlate with behavioural performance on some memory tasks, such as extinction. In slices from B6 mice, low-frequency stimulation that does not alter synaptic strength *per se* can still suppress future L-LTP, but only when such stimulation is given within a critical time window before L-LTP induction (Fig. 6) (Woo and Nguyen, 2002). It is unclear whether other inbred strains would display similar anterograde metaplasticity of L-LTP, but deficits in such metaplasticity might contribute to the inability of some strains to form stable long-term memories (cf. Abraham and Robins, 2005). If deficits are found, experimentation with pharmacological activators of key neuromodulatory systems can be done to try to rescue or enhance metaplasticity (e.g. Gelinas and Nguyen, 2005). This would shed light on the mechanisms that regulate metaplasticity and might reveal potentially useful strategies for enhancing types of synaptic plasticity that may control neural information processing.

Potential gains from phenotyping inbred mice

Inbred mouse strains do not offer the empirical precision that is the hallmark of directed mutagenesis (i.e. transgenic and genetic deletion technologies). Reverse genetics is still the

Table 1. *Comparisons of behavioural memory and long-term potentiation in several inbred mouse strains*

	Fear conditioning			Hippocampal LTP		Amygdalar LTP
Strain	Contextual	Cued	Contextual extinction	CA1	MPP	Lateral-basolateral
C57BL/6J	√	√	√	√ All protocols	√	√
CBA/J	↓	↓	n/a	↓ Multi-train ↓ Theta LTP √ One-train	n/a	n/a
DBA/2J	↓	↓	n/a	↓ Theta LTP √ Multi-train	√	↓
129/SvEms/J	√	√	n/a	↓ All protocols	n/a	n/a
129S1/SvImJ	√	√	n/a	√ All protocols	√	√
A/J	√	↓	√	√ All protocols	√	↓
BALB/cByJ	√	↓	↓	√ One-train √ Theta LTP ↓ Multi-train	√	↓
C57BL/10J	√	√	√	√ All protocols	√	√
SM/J	√	↓	↓	√ All protocols	√	√
C3H/HeJ	↓	↓	n/a	√ Theta LTP ↓ One-train ↓ Multi-train	√	↓

LTP, long-term potentiation; CA1; cornu ammonis-1 region; SC, Schaeffer collateral pathway; MPP; medial perforant pathway; n/a, not yet analysed.

Original data were published elsewhere (Nguyen et al., 2000a; Schimanski and Nguyen, 2005a; Schimanski and Nguyen, 2005b).

↓ indicates less robust than B6. √ indicates similar to B6. Amygdalar LTP was assessed in the lateral-basolateral pathway.

paradigm *par excellence* for elucidating the roles of specific molecules in brain physiology and cognition. Nonetheless, studies of the physiology and behaviours of inbred mice can facilitate the *unbiased* discovery of biological and genetic correlations that may help identify the genes and molecular mechanisms that cause specific phenotypes. That inbred strains show diverse behavioural and synaptic phenotypes suggests that natural variation, and variation under laboratory conditions, exist at the genetic level. This genetic variance allows for the elucidation of the genetic bases of these phenotypes (Wehner et al., 2001; Schimanski and Nguyen, 2004). More importantly, it allows for a less biased test of the relationship between LTP and memory.

There are some disadvantages to studying inbred strains. Because genetic differences between strains are not imposed by the experimenter, it can be difficult to discern exactly which genes are different between strains. Also, because many phenotypes are polygenic, it is a challenge to determine which genes are responsible for these phenotypes. These difficulties can be partially resolved by incorporating sophisticated, and sometimes complex, genetic analyses (e.g. quantitative trait loci, or QTL, analysis; microarray techniques) (for reviews, see Wehner et al., 2001; Schimanski and Nguyen, 2004).

Phenotypic analysis of inbred strains should use multidisciplinary approaches. For example, biochemical techniques may be used to quantify the activities of key protein kinases and protein phosphatases in hippocampal tissue derived from inbred strains. These enzymes are important for mediating synaptic plasticity and memory function (Micheau and Riedel, 1999; Nguyen and Woo, 2003). Proteomic approaches that include mass spectrometric methods aimed at identifying phosphorylated proteins should be used to identify the substrates

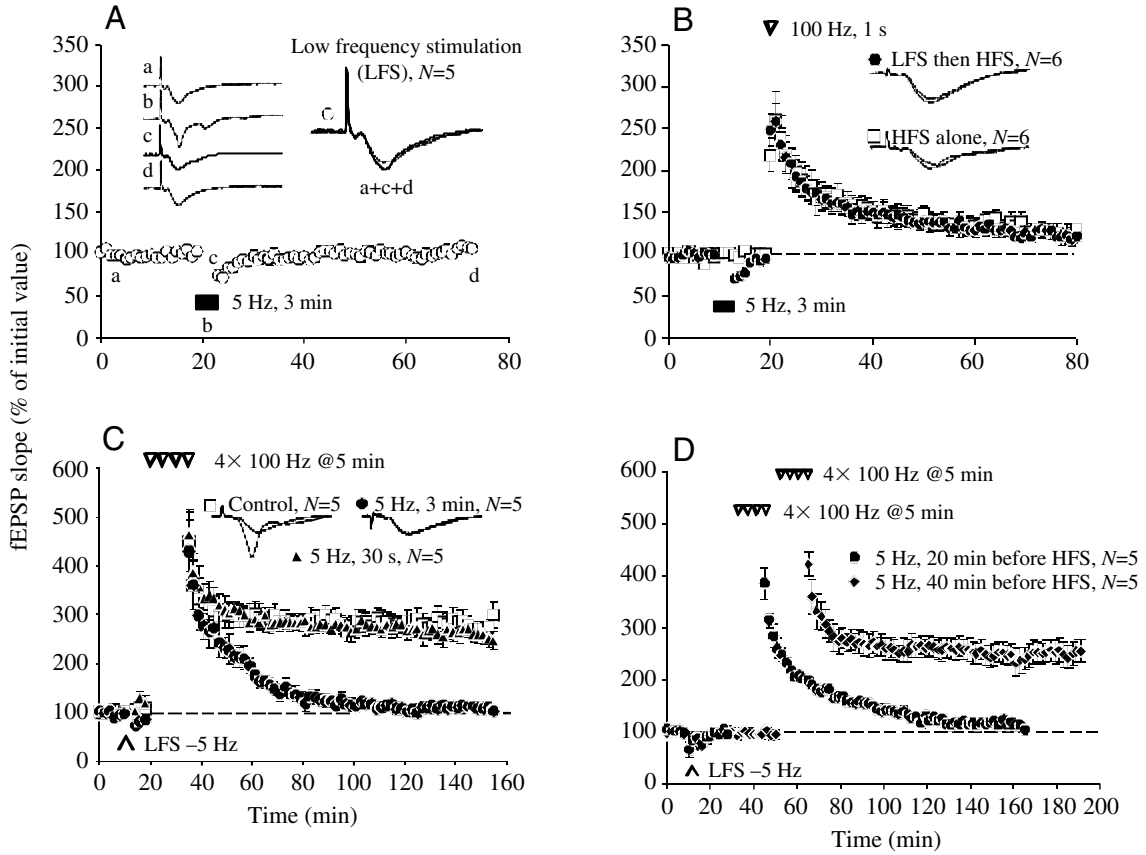

Fig. 6. Metaplastic inhibition of the late phase of long-term potentiation (L-LTP). (A) Transient depression was observed after 5 Hz low-frequency stimulation for 3 min (LFS) was applied to area CA1 of hippocampal slices from B6 mice. However, fEPSP slopes recovered within 7 min after the end of LFS (open circles). Sample fEPSP traces from one experiment are shown; these were recorded 15 min before (a), during (b), immediately after (c), and 20 min after (d) LFS. (B) Prior LFS at 5 Hz does not affect early LTP induced by a single 100 Hz train. Control slices (open squares) generated LTP that was similar in magnitude and time course to LTP induced in slices that received LFS prior to tetanization (solid circles). Inset: sample fEPSP traces from one experiment, measured 10 min before and 60 min after E-LTP induction. (C) Four successive trains of 100 Hz, spaced 5 min apart, induced robust L-LTP in control slices (open squares) and in slices that received a brief prior episode of LFS (5 Hz, 30 s; solid triangles). No L-LTP was seen in slices that received more prolonged LFS (5 Hz, 3 min; solid circles) prior to HFS. Inset: sample traces from an experiment, measured 10 min before (Control) and 2 h after L-LTP induction. (D) Increasing the time interval between LFS and L-LTP induction abolishes anterograde metaplasticity of L-LTP. Defective L-LTP was observed when LFS was applied 20 min before L-LTP induction (solid circles). By contrast, normal L-LTP was seen when the time interval between LFS and HFS was extended to 40 min (solid triangle). Adapted from (Woo and Nguyen, 2002). (Copyright 2002, Cold Spring Harbor Laboratory Press, used with permission.)

of protein kinases that may be altered in memory-impaired inbred strains (Oda et al., 2001; Mann et al., 2002). Gene expression analysis may identify transcripts that are altered in particular inbred strains and that may cause behavioural or synaptic phenotypes. Variations in the structure of specific brain regions need to be compared among strains, as they may have an important influence on behaviour (e.g. Crusio et al., 1987). Functional magnetic resonance imaging (Small et al., 2000) (see also Weissleder et al., 2000) may be employed to identify brain regions with structural or functional modifications that may be associated with altered cognitive performance in some inbred mouse strains. An example of the utility of inbred mice for linking specific brain structures to particular memory functions is the study by Schimanski et al. (Schimanski et al., 2002). Inbred mice lacking intact hippocampal commissures displayed robust long-term memory for contextual fear, but showed impaired extinction of contextual fear memory; both processes rely on hippocampal information processing. Defective memory extinction was correlated with deficits in hippocampal short-term synaptic facilitation. Thus, the use of multidisciplinary approaches can reveal novel links between brain and behaviour.

Identification of mouse models of memory function is needed to establish the causes of impaired memory. Presently, some inbred strains that possess specific memory impairments have been identified (reviewed by Schimanski and Nguyen, 2004) (see also Table 1). These strains should be phenotyped further, by using multiple tests of hippocampal learning and memory (e.g. radial arm maze, object recognition, social transmission of food preference). These tests are needed to confirm that memory deficits are products of hippocampal dysfunction *per se*. Murine 'physiome' databases may be generated, and then used with murine genomic data, to identify the genetic and molecular bases of synaptic alterations and mnemonic deficits. Specific mechanistic hypotheses, based on data obtained from studies performed on mutant mice and on other animal species, can then be formulated to drive and refine experimentation. Thus, phenotyping of inbred mice should add to, and consolidate, the knowledge gained from studies of mutant mice. There is also the promising prospect of testing treatments for memory deficits in many of these inbred strains. This might involve rescuing memory by genetic or pharmacological treatments that target specific proteins that have been identified by phenotypic and proteomic analyses as likely causes of memory dysfunction.

Abbreviations

ACSF	artificial cerebrospinal fluid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate
CA1	cornu ammonis-1
E-LTP	early phase of LTP
fEPSP	field excitatory postsynaptic potential
HFS	high-frequency stimulation
LFS	low-frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
L-LTP	late phase of LTP
MPP	medial perforant pathway
NMDA	*N*-methyl-D-aspartate
QT	quantitative trait loci
SC	Schaeffer collateral

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