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Kainate receptors and the induction of mossy fibre long-term potentiation

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There is intense interest in understanding the molecular mechanisms involved in long-term potentiation (LTP) in the hippocampus. Significant progress in our understanding of LTP has followed from studies of glutamate receptors, of which there are four main subtypes (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), mGlu and kainate). This article summarizes the evidence that the kainate subtype of glutamate receptor is an important trigger for the induction of LTP at mossy fibre synapses in the CA3 region of the hippocampus. The pharmacology of the first selective kainate receptor antagonists, in particular the GLU_{K5} subunit selective antagonist LY382884, is described. LY382884 selectively blocks the induction of mossy fibre LTP, in response to a variety of different high-frequency stimulation protocols. This antagonist also inhibits the pronounced synaptic facilitation of mossy fibre transmission that occurs during high-frequency stimulation. These effects are attributed to the presence of presynaptic GLU_{K5}-subunit-containing kainate receptors at mossy fibre synapses. Differences in kainate receptor-dependent synaptic facilitation of AMPA and NMDA receptor-mediated synaptic transmission are described. These data are discussed in the context of earlier reports that glutamate receptors are not involved in mossy fibre LTP and more recent experiments using kainate receptor knockout mice, that argue for the involvement of GLU_{K6} but not GLU_{K5} kainate receptor subunits. We conclude that activation of presynaptic GLU_{K5}-containing kainate receptors is an important trigger for the induction of mossy fibre LTP in the hippocampus.

Keywords: synaptic plasticity; long-term potentiation; hippocampus; glutamate

1. INTRODUCTION

Since its discovery, first documented in full 30 years ago (Bliss & Lømo 1973; Bliss & Gardner-Medwin 1973), LTP has become the most popular experimental model for understanding the synaptic processes that are involved in learning and memory and many other functions of the nervous system (Bliss & Collingridge 1993). Two distinct forms have since been identified in the mammalian CNS, which are distinguished by their induction mechanisms. The most widely expressed form of LTP is induced by the synaptic activation of NMDA receptors (Collingridge *et al.* 1983). The mechanism of induction of NMDA receptor-dependent LTP is established, and has been described elsewhere (Collingridge 1985; Bliss & Collingridge 1993; see also Collingridge 2003). The other form of LTP is distinguished by its independence from the synaptic activation of NMDA receptors. The best characterized form of NMDA receptor-independent LTP is at mossy fibre synapses in the hippocampus (Harris & Cotman 1986). Recent experiments have begun to shed light on the induction mechanisms of LTP at this synapse, and in particular the role of kainate receptors. We summarize our experiments that led to the discovery of the role of kainate receptors in the induction of LTP, and in the related synaptic facilitation, at mossy fibre synapses, and present previously unpublished information obtained during the course of these experiments.

Early work suggested that mossy fibre LTP was independent of the activation of glutamate receptors since it could be induced during application of glutamate receptor antagonists such as kynurenic acid and CNQX (Ito & Sugiyama 1991; Castillo *et al.* 1994; Weisskopf & Nicoll 1995; Yeckel *et al.* 1999). Indeed, one simple model proposed that mossy fibre LTP was triggered by Ca^{2+} entry into the presynaptic terminal via entry through voltage-gated Ca^{2+} channels. However, we were always intrigued by the observation that the mossy fibre pathway was strikingly different from other hippocampal pathways in that it was associated with a relatively low density of NMDA receptor binding sites and an extremely high density of kainate receptor binding sites (Monaghan & Cotman 1982). We therefore wondered whether kainate receptors might serve an analogous function to NMDA receptors at other hippocampal pathways; namely, act as a trigger for the induction of mossy fibre LTP. However, to explore this possibility required the development of selective kainate receptor antagonists.

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One contribution of 30 to a Theme Issue 'Long-term potentiation: enhancing neuroscience for 30 years'.

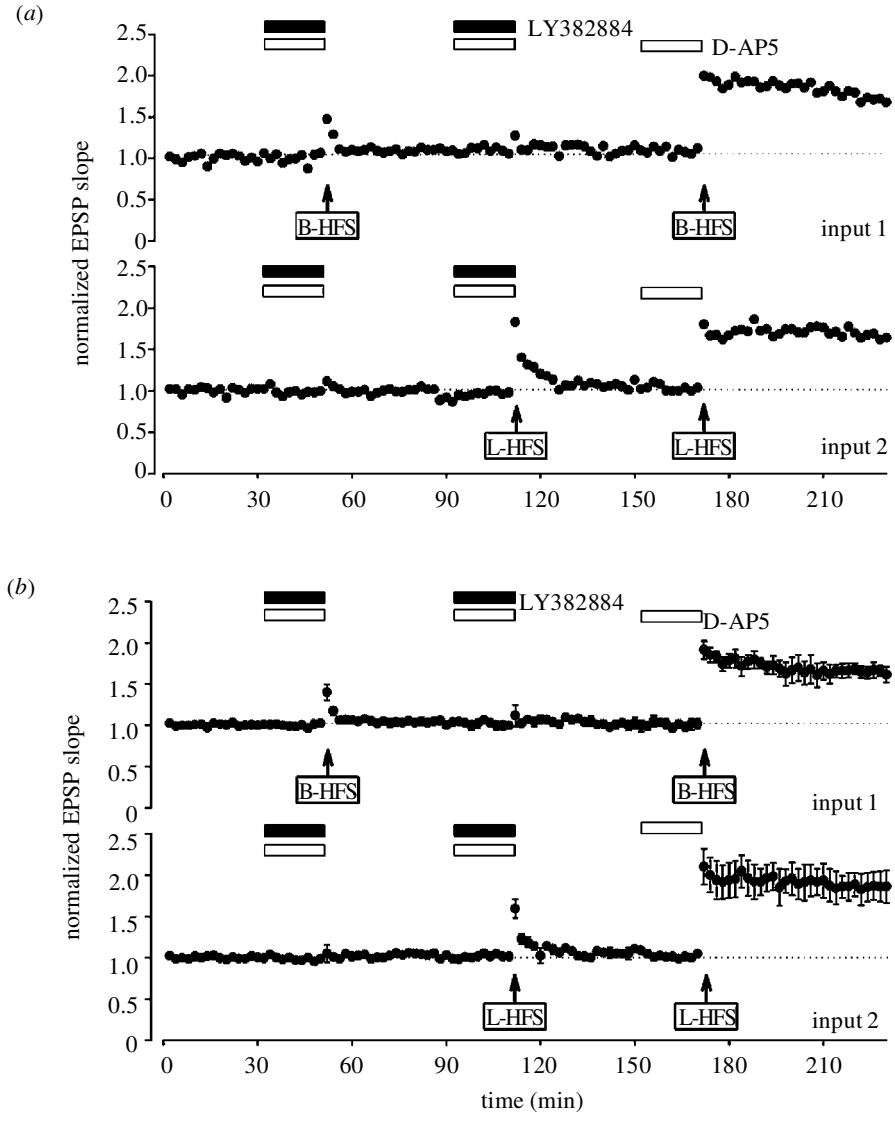

Figure 1. GLU_{K5}-containing kainate receptors are triggers for the induction of mossy fibre LTP. (*a*) A single experiment, plotting field EPSP slope (averages of four successive responses) versus time. Tetani were delivered at the times indicated by arrows, in the presence of D-AP5 (50 μM; duration of application indicated by bar) to ensure that only mossy fibre LTP was being investigated. Two independent mossy fibre inputs were studied simultaneously. In input 1, B-HFS, and in input 2, L-HFS were delivered. Note that LY382884 (10 μM) fully blocked the induction of LTP in a reversible manner in both inputs. (*b*) Pooled data (mean ± s.e. mean) from four similar experiments.

2. KAINATE RECEPTOR PHARMACOLOGY

Pharmacological experiments clearly identified kainate receptors as a distinct class of glutamate receptor (Davies *et al.* 1979; McLennan & Lodge 1979), which together with AMPA and NMDA receptors constitute the three classes of ionotropic glutamate receptors present in the CNS (Watkins & Evans 1981). Molecular cloning revealed five kainate receptor subunits (Bettler & Mulle 1995), which are named, according to IUPHAR nomenclature (Lodge & Dingledine 2000), GLU_{K5}, GLU_{K6}, GLU_{K7}, GLU_{K1} and GLU_{K2} (also known as GluR5 or iGlu5, GluR6 or iGlu6, GluR7 or iGlu7, KA-1 and KA-2, respectively). These subunits may exist as certain homomeric assemblies, although native receptors are most likely to be heteromeric assemblies. Kainate is a potent agonist at both AMPA and kainate receptors. However, its actions on kainate receptors can be studied by blocking AMPA receptors with selective antagonists such as GYKI53655. In addition, potent selective kainate receptor agonists have been described. The most widely used selective agonist is 2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (Clarke *et al.* 1997), which is very potent at GLU_{K5} receptors and only affects AMPA receptors and other kainate receptors in considerably higher concentrations.

Early, so-called, non-NMDA receptor antagonists such as kynurenic acid and the quinoxalinediones (e.g. CNQX and NBQX) antagonize both AMPA and kainate receptors to varying degrees. The first selectivity towards kainate receptors was obtained when a series of decahydroisoquinolines, synthesized by Paul Ornstein at Eli Lilly, was screened against AMPA and kainate receptor subunits expressed in HEK293 cells. These compounds had the expected AMPA receptor antagonist activity but surprisingly also antagonized homomeric GLU_{K5} receptors. The first compound, LY293558, was roughly equipotent at GLU_{K5} and AMPA-receptor subunits (Bleakman *et al.* 1996) but subsequent compounds, such as LY294486

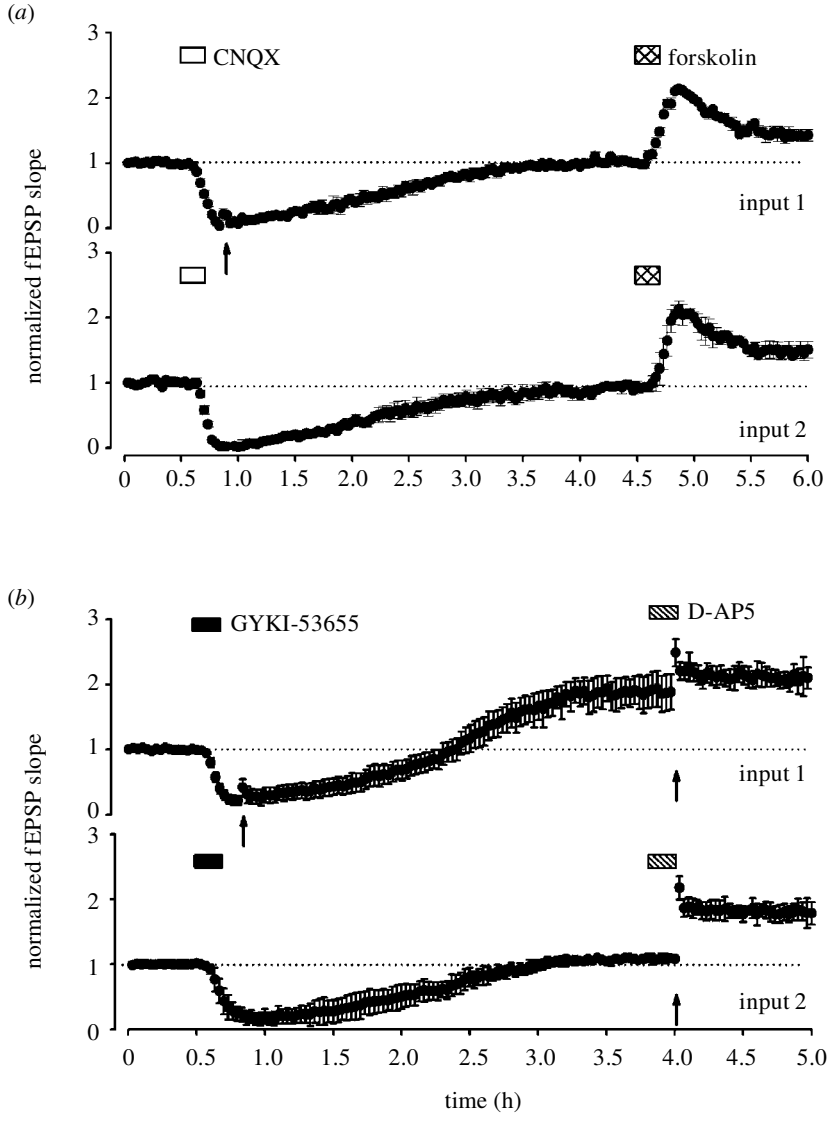

Figure 2. CNQX but not NBQX blocks the induction of mossy fibre LTP. (*a*) Pooled data from five two-input fEPSP experiments showing that CNQX (10 μM) blocked the induction of mossy fibre LTP, as assessed following recovery of AMPA receptor-mediated synaptic transmission. A single tetanus (100 Hz, 1 s, test intensity) was delivered to input 1 (at the time indicated by the arrow). Subsequent application of forskolin (50 μM) resulted in LTP in both inputs. (*b*) Pooled data for five equivalent experiments using GYKI53655 (30 μM). Note that the tetanus delivered to input 1 induced LTP, as assessed following recovery of AMPA receptor-mediated synaptic transmission. A second tetanus delivered to this input elicited very little additional LTP (due to saturation), whereas a tetanus delivered for the first time to input 2 elicited normal LTP.

(Clarke *et al.* 1997) and its active isomer LY377770 (O'Neill *et al.* 2000; Smolders *et al.* 2002) showed improved selectivity for GLU_{K5} receptors. The most selective GLU_{K5} receptor antagonist that is currently available is LY382884 (O'Neill *et al.* 1998; Bortolotto *et al.* 1999). This compound antagonizes GLU_{K5} receptors with a K_i value of 4.0 μM and is much less active on AMPA receptors. Like other decahydroisoquinolines, it is essentially inactive on other kainate receptor subunits. Importantly, but not surprisingly, it is roughly equipotent on homomeric GLU_{K5} receptors and heteromers containing the GLU_{K5} subunit (Bortolotto *et al.* 1999). In native tissue, LY382884 blocks a variety of effects of kainate receptor activation at a concentration (10 μM) that does not affect AMPA receptor-mediated synaptic transmission in the mossy fibre pathway (Bortolotto *et al.* 1999). It has been tested on a variety of other receptor systems and found to be inactive at this concentration (Lauri *et al.* 2001*b*). It is therefore a very useful compound with which to explore the functions of kainate receptors.

Recent studies in the spinal cord of mice have shown that LY382884 strongly inhibits kainate currents in wild-type and $GLU_{K6-/-}$ mice but is inactive in $GLU_{K5-/-}$ mice (Kerchner *et al.* 2002). This further confirms the selectivity of LY382884 for GLU_{K5} receptors. Interestingly, the current density in $GLU_{K5-/-}$ mice and wild-types was similar. This shows that GLU_{K5}-dependent functions in wild-type mice can be compensated for in $GLU_{K5-/-}$ mice. This result has important implications for the interpretation of experiments using kainate receptor knockout mice.

3. KAINATE RECEPTOR ANTAGONISTS BLOCK THE INDUCTION OF LTP AT MOSSY FIBRE SYNAPSES

The selective kainate receptor antagonist LY382884, applied at a concentration (10 μM) that did not affect

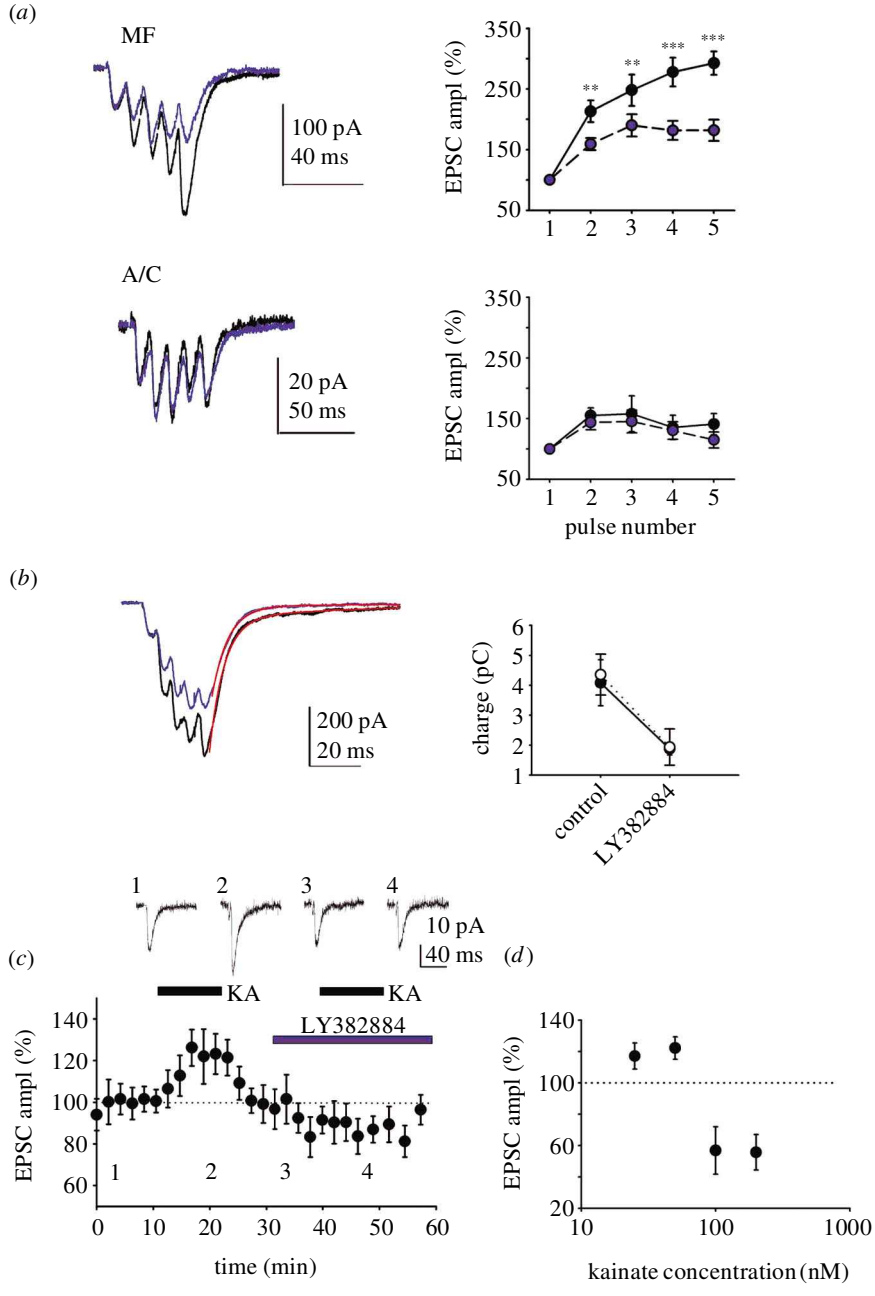

Figure 3. GLU_{K5}-containing kainate receptors mediate synaptic facilitation of AMPA receptor-mediated mossy fibre synaptic transmission. (*a*) AMPA receptor-mediated EPSCs in response to five shocks at 50 Hz under control conditions (black) and in the presence of LY382884 (blue) for mossy fibre (MF) and assoc./comm. (A/C) inputs. The graphs plot pooled data of EPSC amplitude, normalized with respect to the first EPSC in the train, for six neurons. (Data replotted from Lauri *et al.* (2001*a*).) (*b*) AMPA receptor-mediated mossy fibre EPSCs in response to five shocks at 100 Hz under control conditions (black) and in the presence of LY382884 (blue). Double exponential fits (red traces) are superimposed upon the EPSC decays. The graph plots the charge associated with the fast (black circles) (AMPA receptor-mediated EPSC) and slow (open circles) (kainate receptor-mediated EPSC) components. Note the parallel inhibition by LY382884. (*c*) Pooled data from four experiments to illustrate facilitation of AMPA receptor-mediated mossy fibre transmission by 50 nM kainate (KA) and its inhibition by 10 μM LY382884. The mossy fibre EPSCs were obtained at the times indicated (1–4). (Data replotted from Lauri *et al.* (2001*a*).) (*d*) Concentration-dependent effects of kainate on mossy fibre synaptic transmission. Each point is the mean of at least four experiments. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$.

mossy fibre synaptic transmission, completely blocked the induction of mossy fibre LTP in a fully reversible manner (Bortolotto *et al.* 1999). LY382884 had no effect on basal synaptic transmission, on pre-established LTP or on LTP induced by direct stimulation of adenylyl cyclase with forskolin. This shows that its action is specific for LTP induction. Furthermore, LY382884 had no effect on NMDA receptor-dependent LTP evoked in the same neurons by activation of assoc./comm. fibres. This provided the first, and to our minds compelling, evidence that kainate receptors are involved in the induction of mossy fibre LTP.

It has been suggested that different forms of mossy fibre LTP can coexist, and may have different expression mechanisms (Urban & Barrionuevo 1996). We have therefore investigated the sensitivity to LY382884 of additional induction protocols; B-HFS, which comprised 10 pulses

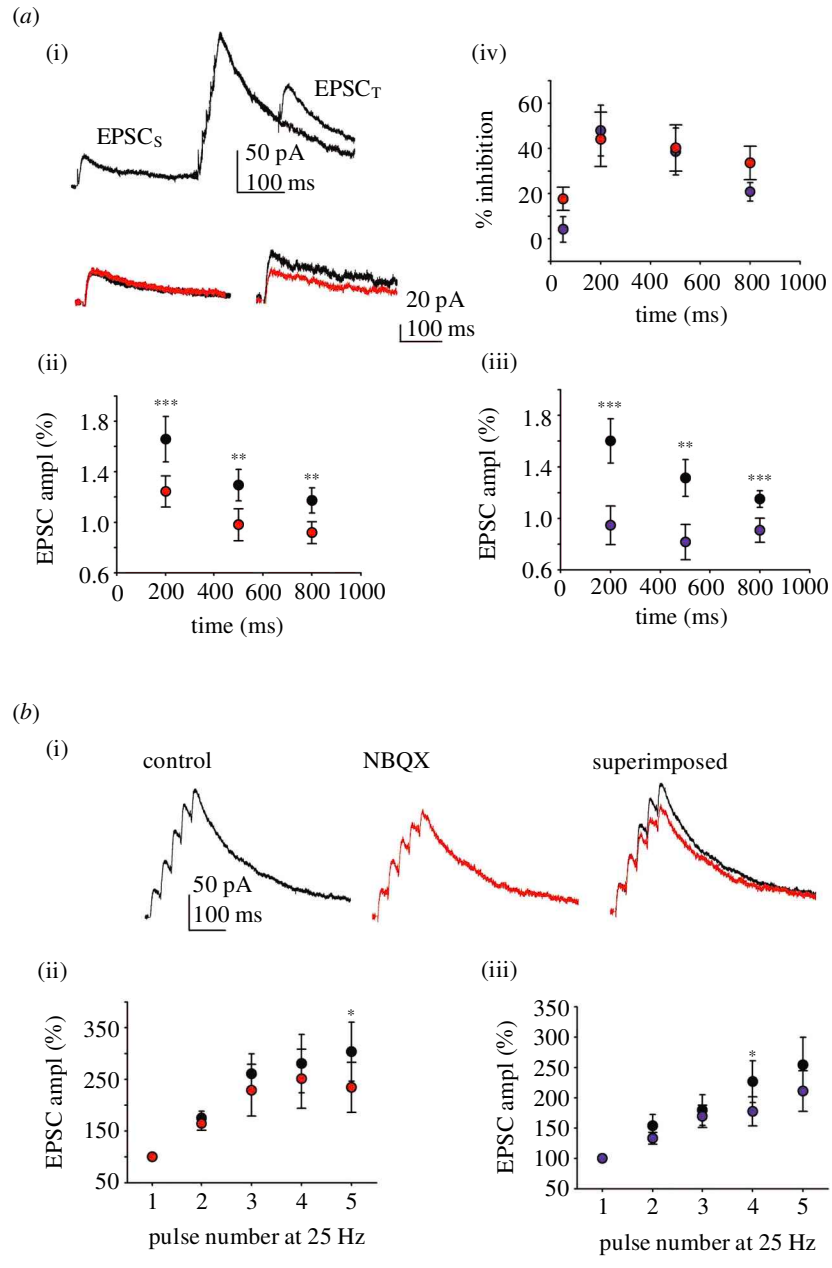

Figure 4. GLU$_{K5}$-containing kainate receptors mediate synaptic facilitation of NMDA receptor-mediated mossy fibre synaptic transmission. (*a*) (i) Upper recording shows NMDA receptor-mediated EPSCs (recorded at +40 mV) evoked by a single stimulus (EPSC$_S$) and five stimuli delivered at 100 Hz followed by a test pulse (EPSC$_T$) delivered 200 ms later. The traces are superimposed upon responses to the same stimulus protocol but without the test pulse. The lower recordings show single stimuli and test pulses under control conditions (black traces) and in the presence of NBQX (20 μM; red traces). Note the inhibition of synaptic facilitation by NBQX. (ii) The level of facilitation (EPSC$_T$/EPSC$_S$) plotted versus inter-pulse interval (time between first stimulus in 100 Hz train and test pulse) for 12 experiments. Control conditions, black; NBQX: red. (iii) Equivalent experiments using LY382884 (10 μM; $n = 10$). Control conditions, black; LY382884, blue. (iv) Plot of percentage inhibition of synaptic facilitation versus inter-pulse interval. The first points plot the peak response during the tetanus. Note the similarity between NBQX (red) and LY382884 (blue). (Data analysis expanded from Lauri *et al.* (2001*b*).) (*b*) Analysis during a high-frequency train. (i) NMDA receptor-mediated EPSCs (recorded at +40 mV) evoked by five shocks delivered at 25 Hz before (black) and in the presence of NBQX (20 μM; red) and their superimposition. Note the lack of effect of NBQX until later in the response. (ii) The graph plots the amplitude of each EPSC in the train, normalized to the first, for 12 NBQX experiments. Control conditions, black; NBQX, red. (iii) Equivalent data for eight LY382884 experiments. Control conditions, black; NBQX, blue. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$.

at 100 Hz repeated eight times at 5 s intervals, and L-HFS, which comprised 100 pulses at 100 Hz repeated three times at 10 s intervals (Urban & Barrionuevo 1996). As illustrated in figure 1, 10 μM LY382884 fully blocked the induction of mossy fibre LTP in a reversible manner when either protocol was used.

Since our conclusion that kainate receptors were triggers for the induction of LTP disagreed with the reports that kynurenate and CNQX do not block the induction of mossy fibre LTP (Ito & Sugiyama 1991; Castillo *et al.* 1994; Weisskopf & Nicoll 1995; Yeckel *et al.* 1999; but see Urban & Barrionuevo 1996) it became necessary to

repeat these experiments using these non-selective AMPA/kainate receptor antagonists. These experiments were complicated by the depression of AMPA receptor-mediated synaptic transmission; however, in all experiments a second non-tetanized input was used as a control to ensure stability of the recordings. Both kynurenate (10 mM) and CNQX (10 μM) reversibly blocked the induction of mossy fibre LTP, as determined after AMPA receptor-mediated synaptic transmission had recovered following washout of the antagonist (Bortolotto *et al.* 1999). Experiments reported in Bortolotto *et al.* (1999), but not previously illustrated, showing the block of mossy fibre LTP by 10 μM CNQX are presented in figure 2*a*. Significantly, the potency of these three structurally distinct compounds (LY382884, kynurenic acid and CNQX) as GLU_{K5} antagonists correlated with their potency at blocking the induction of mossy fibre LTP (Bortolotto *et al.* 1999). Conversely, selective inhibition of AMPA receptors, using GYKI53655, did not impair the induction of mossy fibre LTP, once again determined after AMPA receptor-mediated synaptic transmission had been restored. These experiments, noted in Bortolotto *et al.* (1999), are illustrated for the first time in figure 2*b*.

Our conclusion that kainate receptors are involved in the induction of mossy fibre LTP was challenged (Nicoll *et al.* 2000) because of the earlier failures to observe antagonism using kynurenic acid or CNQX. However, the group that disputed our findings subsequently presented data at the US Winter Conference on Brain Research (Steamboat Springs, January 2001) showing that in their hands CNQX blocked the induction of mossy fibre LTP, when a slightly different tetanus was delivered. Also, it has been shown that mossy fibre LTP is absent in certain kainate receptor knockout mice (Contractor *et al.* 2001). Therefore, an involvement of kainate receptors in mossy fibre LTP seems now to be generally accepted. What has yet to be fully resolved is the relative importance of the various kainate receptor subunits. In the study of Contractor *et al.* (2001) it was reported that LTP was reduced in $GLU_{K6-/-}$ mice but not affected in $GLU_{K5-/-}$ mice. Given the high degree of selectivity of LY382884 for GLU_{K5} versus other kainate-receptor subunits, we propose that the normal role of GLU_{K5} is compensated for in $GLU_{K5-/-}$ mice. This conclusion is supported by the compensation reported for kainate actions on spinal neurons (Kerchner *et al.* 2002) and for synaptic facilitation (see below). Whether the reduction in the magnitude of LTP observed in the $GLU_{K6-/-}$ mouse is due to the acute loss of this subunit or a developmental consequence of the absence of this receptor throughout development is not currently known. GLU_{K6}-subtype-selective antagonists would be useful to address this issue.

4. THE SYNAPTIC ACTIVATION OF KAINATE RECEPTORS AT MOSSY FIBRE SYNAPSES

High-frequency stimulation of the mossy fibre pathway elicits a postsynaptic kainate receptor-mediated EPSC (Castillo *et al.* 1997; Vignes & Collingridge 1997). This EPSC was identified by blocking AMPA receptor-mediated synaptic transmission using GYKI53655. The residual synaptic current is sensitive to CNQX (Castillo *et al.* 1997; Vignes & Collingridge 1997) and the more selective GLU_{K5} kainate receptor antagonists LY293558 and LY294486 (Vignes *et al.* 1998). This kainate receptor-mediated EPSC is not readily observed with single shock stimulation at mossy fibres but is rapidly recruited during the stimulus train, such that it is evident by the second stimulus. This enhancement during the train is due to the slow kinetics of the kainate receptor-mediated response, which promotes temporal summation, and the facilitation of glutamate release that occurs during repetitive stimulation (Salin *et al.* 1996). Unlike NMDA receptors, which also summate effectively during high-frequency stimulation (Herron *et al.* 1986), there is no voltage dependence to the synaptic kainate receptor-mediated response. The reason for the slow kinetics of synaptic kainate receptor-mediated currents is not known, but probably relates to intrinsic channel properties.

The recruitment of a kainate receptor-mediated EPSC is not the only occurrence during high-frequency stimulation of mossy fibres. Recordings made during the tetanus, used to induce mossy fibre LTP, showed that AMPA receptor-mediated synaptic transmission was rapidly enhanced and this enhancement was sustained throughout the high-frequency train (Bortolotto *et al.* 1999; Lauri *et al.* 2001*a*). This enhancement of the synaptic response was therefore very distinct from that seen during tetanic stimulation at CA1 synapses where AMPA receptor-mediated synaptic transmission was depressed but NMDA receptor-mediated synaptic transmission was greatly facilitated (Herron *et al.* 1986). The nature of the synaptic response during the tetanus at mossy fibres was most simply explained by a rapid, facilitatory autoreceptor mechanism resulting in enhanced glutamate release, and hence more AMPA receptor activation in response to every stimulus (following the initial one). Its sensitivity to LY382884 suggested that this putative autoreceptor was a kainate receptor. However, a synaptically elicited facilitatory autoreceptor had not been observed in the mammalian CNS before and, like any positive feedback process, could represent a highly unstable mechanism—particularly given that L-glutamate is potentially excitotoxic. We therefore set out to establish the existence (or otherwise) of such a mechanism.

5. THE SYNAPTIC ACTIVATION OF A FACILITATORY KAINATE AUTORECEPTOR

Whole-cell patch-clamp recordings confirmed the existence of a facilitatory kainate autoreceptor at mossy fibre synapses and enabled many of its properties to be established (Lauri *et al.* 2001*a*). These findings entered the public domain at the meeting of the Federation of European Neuroscience (Brighton, June 2000) and figure 3 shows data as presented at this meeting, some of which were subsequently published in Lauri *et al.* (2001*a*). Note the rapid facilitation of synaptic transmission at mossy fibre synapses but not assoc./comm. synapses and the selective effect of 10 μM LY382884 on mossy fibre responses (figure 3*a*). Note also that LY382884 had no effect on the first EPSC in the high-frequency train, consistent with its lack of effect on low-frequency AMPA receptor-mediated synaptic transmission, but antagonized the subsequent four EPSCs by *ca.* 50%. Figure 3*b* presents data from a train of five stimuli delivered at 100 Hz. Note again the lack of effect on the first EPSC

but substantial antagonism of subsequent EPSCs in the train. Thus, the onset latency was less than 10 ms, suggesting an ionotropic receptor mechanism. In this example, exponentials were fitted to the decay of the synaptic response—the decay comprises an early component mediated by AMPA receptors and a late component that is due to the synaptic activation of postsynaptic kainate receptors. The finding that both components are depressed equally is suggestive of a presynaptic locus of action of LY382884; namely inhibition of a facilitatory autoreceptor mechanism. Further evidence for a presynaptic action of LY382884 is the finding that the facilitation of mossy fibre transmission by low concentrations of kainate (Kehl *et al.* 1984; Lauri *et al.* 2001*a*; Schmitz *et al.* 2001) that presumably results from direct depolarization of presynaptic elements, is blocked by this antagonist (figure 3*c*).

Based on the extensive pharmacological characterization of LY382884 it seems extremely likely that its effect on synaptic facilitation is due to antagonism of GLU_{K5}-containing kainate receptors. This conclusion is supported by experiments using knockout mice (S. E. Lauri, J. T. R. Isaac and G. L. Collingridge, unpublished observations). We have found very pronounced synaptic facilitation, induced by 50 Hz stimulation, in both the $GLU_{K5-/-}$ and wild-type littermates that is not significantly different in magnitude. However, while LY382884 antagonizes synaptic facilitation in wild-type mice, in a similar manner to that in rats, it is inactive in $GLU_{K5-/-}$ mice. Thus, the $GLU_{K5-/-}$ mouse must compensate for the lack of GLU_{K5} receptors. What the consequences of this compensation are for synaptic function at mossy fibres remains to be fully explored.

Synaptic facilitation can also be observed by using the NMDA receptor-mediated component of synaptic transmission at mossy fibres as a monitor of synaptic glutamate release (Lauri *et al.* 2001*b*; Schmitz *et al.* 2001). This enables the non-selective AMPA/kainate receptor antagonists, such as CNQX and NBQX, to be used. As expected, these agents mimicked the effects of LY382884 on synaptic facilitation (figure 4). However, the use of the NMDA receptor-mediated synaptic component is limited in two respects. First, the level of facilitation is much less when compared with that observed using AMPA receptor-mediated synaptic transmission. Second, the effect of kainate receptor antagonists is less evident until later in the train (figure 4). Therefore, NMDA receptor-mediated EPSCs, compared with AMPA receptor-mediated EPSCs, are a poor reporter of synaptically released L-glutamate. These two differences can be explained by the higher affinity of NMDA receptors, compared with AMPA receptors, for L-glutamate resulting in greater occupancy of NMDA receptors during mossy fibre transmission.

In contrast to a facilitatory function for kainate receptor on mossy fibres, other work conducted in parallel to our own work concluded that L-glutamate released synaptically from either mossy fibre synapses or assoc./comm. synapses resulted in depression of mossy fibre transmission—i.e. an inhibitory auto and heteroreceptor function (Schmitz *et al.* 2000). In a reappraisal of their work, these authors subsequently reported bi-directional modifications of mossy fibre synaptic transmission depending on the number of pulses delivered at 200 Hz to assoc./comm. fibres (Schmitz *et al.* 2001). Thus, 3 pulses resulted in facilitation of an NMDA receptor-mediated EPSC while 10 pulses resulted in depression. This unusual sensitivity to stimulus number is specific to assoc./comm. influences since facilitation within mossy fibres persists throughout a long high-frequency train (e.g. 100 stimuli delivered at 100 Hz) when measured using AMPA receptor-mediated synaptic responses (Lauri *et al.* 2001*a*).

During the course of our experiments we asked whether L-glutamate released from one population of mossy fibres can regulate the release from another via activation of presynaptic kainate receptors in a heterosynaptic manner. To explore this possibility we stimulated two sets of mossy fibres and determined the influence of delivering 5 or 10 shocks to one input on NMDA receptor-mediated EPSCs at the other input. Heterosynaptic facilitation, antagonized by NBQX, was observed in each experiment (figure 5).

6. PRESYNAPTIC KAINATE RECEPTORS MEDIATE THE INDUCTION OF MOSSY FIBRE LTP

The finding that both pre- and postsynaptic kainate receptors are readily activated by synaptically released L-glutamate at mossy fibre synapses means that either or both could be involved in the induction of mossy fibre LTP. However, we found that a concentration of LY382884 (10 μM) that fully blocked the induction of mossy fibre LTP was able to completely inhibit kainate-mediated facilitation of mossy fibre synaptic transmission and inhibit synaptic facilitation without affecting postsynaptic kainate currents in CA3 neurons (Lauri *et al.* 2001*a*). Thus, LY382884 is a selective inhibitor of presynaptic kainate receptors at mossy fibre synapses. This therefore strongly suggests that presynaptic kainate receptors are involved in the induction of mossy fibre LTP. One piece of evidence that was cited against a role of GLU_{K5}-containing kainate receptors in the induction of LTP is the low levels of expression of GLU_{K5} message in the hippocampus (Nicoll *et al.* 2000). However, dentate granule cells, like CA3 pyramidal neurons, express detectable levels of GLU_{K5} message (Bahn *et al.* 1994) and presynaptic kainate receptors would, most probably, need only one GLU_{K5} subunit to confer sensitivity to GLU_{K5} antagonists.

The finding that kainate receptor-dependent mossy fibre LTP can be induced following selective blockade of AMPA receptors with GYKI53655 (Bortolotto *et al.* 1999) shows that activation of these receptors is not required. Although NMDA receptors can be readily activated synaptically by mossy fibre stimulation, NMDA receptors are not required for mossy fibre LTP (Harris & Cotman 1986). Indeed, mossy fibre LTP is often studied in the presence of an NMDA receptor antagonist to prevent the induction of LTP at assoc./comm. fibres that could be activated inadvertently. However, mGlu receptors may act as a parallel induction trigger since certain mGlu receptor antagonists, such as (*S*)-α-methyl-4-carboxyphenylglycine, can block the induction of mossy fibre LTP (Bashir *et al.* 1993; Yeckel *et al.* 1999; Contractor *et al.* 2001).

Although we have found that LY382884, kynurenic acid and CNQX readily block the induction of mossy fibre LTP, one needs to take account of previous reports that the latter two compounds do not affect mossy fibre LTP (Ito & Sugiyama 1991; Castillo *et al.* 1994; Weisskopf & Nicoll 1995; Yeckel *et al.* 1999; but see Urban & Barrion-

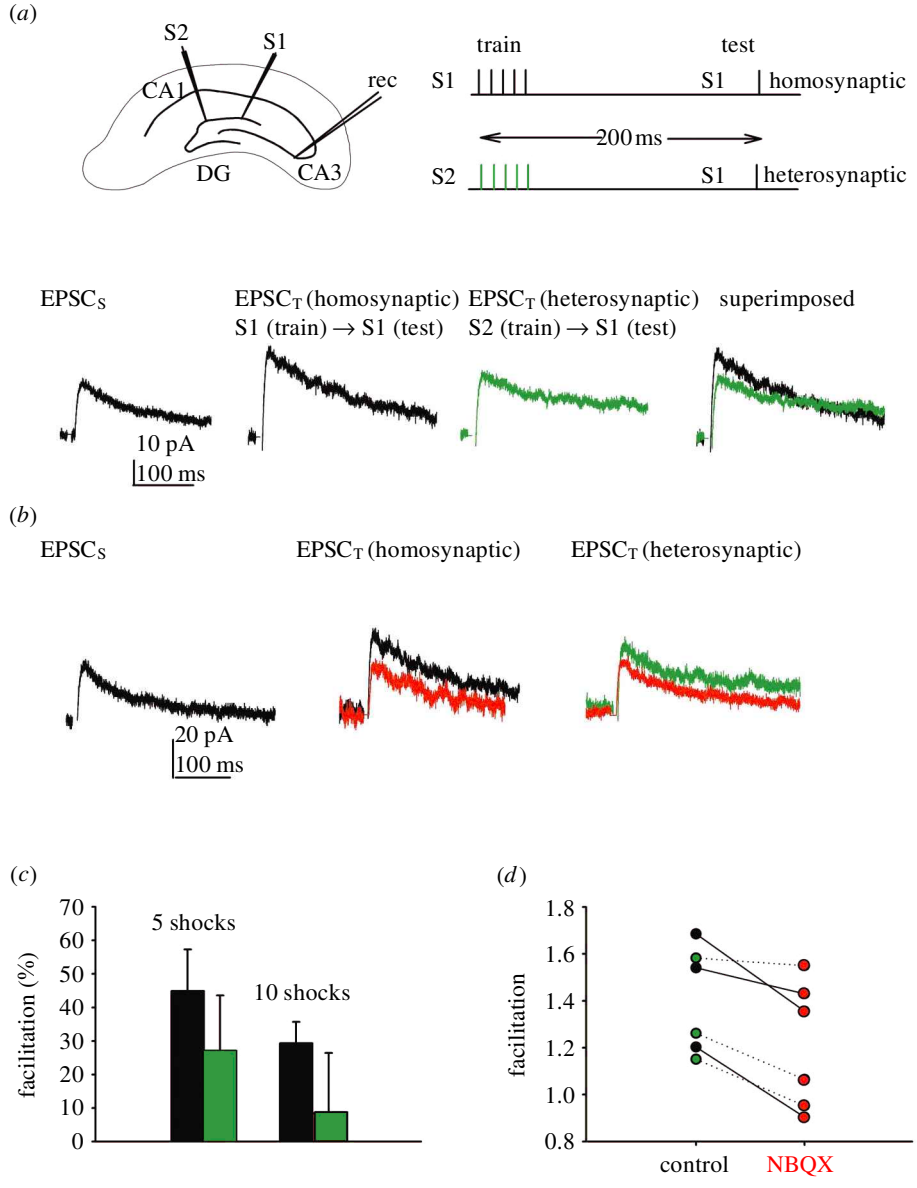

Figure 5. Kainate receptor-dependent heterosynaptic facilitation of mossy fibre synaptic transmission. (*a*) NMDA receptor-mediated EPSCs were evoked by stimulation of two inputs and a train (five shocks at 100 Hz) delivered 200 ms before a test pulse. The traces show, from left to right, $EPSC_S$ and $EPSC_T$ (homosynaptic), $EPSC_T$ (heterosynaptic) and the superimposition of the two $EPSC_T$s. (*b*) The traces show $EPSC_S$, $EPSC_T$ (homosynaptic) and $EPSC_T$ (heterosynaptic) and the effects of NBQX (red; 20 μM); control conditions are shown in black. (*c*) The graphs plot the level of homosynaptic (black) and heterosynaptic (green) facilitation for three experiments, in response to five shocks delivered at 100 Hz or 10 shocks delivered at 200 Hz. (*d*) The graphs plot the level of homosynaptic (black) and heterosynaptic (green) facilitation for three experiments, in response to five shocks delivered at 100 Hz, under control conditions and in the presence of NBQX (20 μM).

uevo 1996). One possibility is that in these earlier studies, mossy fibre LTP was not investigated due to contamination by inadvertent activation of other fibres. Another explanation is that it may be possible, under certain circumstances, to induce mossy fibre LTP without the need for the synaptic activation of kainate receptors. Future studies will explore this possibility.

7. THE RELATIONSHIP BETWEEN SYNAPTIC FACILITATION AND MOSSY FIBRE LTP

The observation that LY382884 inhibits synaptic facilitation and the induction of mossy fibre LTP could mean that the two functions are causally linked; or they could be two independent consequences of presynaptic kainate receptor activation. This is another area requiring further investigation. A surprising observation is that LY382884-sensitive synaptic facilitation is selectively occluded following the induction of mossy fibre LTP (Lauri *et al.* 2001*a*). Thus, following LTP induction, the level of synaptic facilitation is markedly reduced and the residual facilitation is insensitive to the actions of LY382884. This suggests that the kainate receptor-dependent component of synaptic facilitation shares mechanisms in common with the expression of mossy fibre LTP—in which case, studying the former may give clues to the latter.

8. CONCLUDING REMARKS

For the last 20 years of its 30-year lifetime, most interest has focused on the NMDA receptor-dependent form of LTP, such as that exhibited at Schaffer collateral-commissural fibres in the CA1 region, perforant path synapses in the dentate gyrus and at assoc./comm. synapses in the CA3 region of the hippocampus. Now that tools equivalent to NMDA receptor antagonists are available for studying mossy fibre LTP, in particular kainate receptor antagonists, it is likely that research in this form of LTP will intensify. Several immediate questions come to mind. These include the following. (i) What are the roles of the various kainate receptor subtypes in mossy fibre LTP? (ii) Under what conditions can the involvement of kainate receptors be bypassed? (iii) What is the physiological role of kainate receptor-dependent mossy fibre LTP? (iv) Do other forms of NMDA receptor-independent LTP in the brain involve kainate receptor dependency? Perhaps the most interesting question is whether the biophysical properties of kainate receptors confer important functional properties on kainate receptor-dependent LTP analogous to the role of NMDA receptors in NMDA receptor-dependent LTP.

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GLOSSARY

B-HFS: brief high-frequency stimulation
CNS: central nervous system
EPSC: excitatory postsynaptic current
EPSP: excitatory post-synaptic potential
L-HFS: long high-frequency stimulation
LTP: long-term potentiation
NBQX: 2,3-dihydroxy-6-nitro-7-sulphamoyl-benz(F) quinoxaline
NMDA: *N*-methyl-D-aspartate