REVIEW ARTICLE

Plasticity in the human central nervous system

S. F. Cooke and T. V. P. Bliss

Division of Neurophysiology, National Institute for Medical Research, London, UK

Correspondence to: Dr Sam Cooke, Division of Neurophysiology, National Institute for Medical Research,
Mill Hill, London NW7 1AA, UK
E-mail: scooke@nimr.mrc.ac.uk

Long-term potentiation (LTP) is a well-characterized form of synaptic plasticity that fulfils many of the criteria for a neural correlate of memory. LTP has been studied in a variety of animal models and, in rodents in particular, there is now a strong body of evidence demonstrating common underlying molecular mechanisms in LTP and memory. Results are beginning to emerge from studies of neural plasticity in humans. This review will summarize findings demonstrating that synaptic LTP can be induced in human CNS tissue and that rodent and human LTP probably share similar molecular mechanisms. We will also discuss the application of non-invasive stimulation techniques to awake human subjects to induce LTP-like long-lasting changes in localized neural activity. These techniques have potential therapeutic application in manipulating neural plasticity to treat a variety of conditions, including depression, Parkinson's disease, epilepsy and neuropathic pain.

Introduction

Long-term potentiation (LTP) of chemical synaptic transmission and the converse process of long-term depression (LTD) are the most widely studied physiological models of memory formation in the mammalian brain. LTP results from co-incident activity of pre- and post-synaptic elements, bringing about a facilitation of chemical transmission that lasts for hours *in vitro*, and that can persist for periods of weeks or months *in vivo* (Bliss and Gardner-Medwin, 1973; Abraham *et al.*, 2002). LTP was initially demonstrated at glutamatergic synapses between medial perforant path fibres, emanating from the entorhinal cortex, and granule cells in the dentate gyrus of the hippocampus of the anaesthetized rabbit (Bliss and Lømo, 1973). Subsequently, LTP has been studied in a variety of species, ranging from mice (Nosten-Bertrand *et al.*, 1996) to monkeys (Urban *et al.*, 1996), and at a number of different synapses throughout the central nervous system

(CNS), from the cerebral neocortex (Fox, 2002) to the spinal cord (Ji *et al.*, 2003). In this article, we show that there is now a convincing body of evidence to suggest that LTP and other forms of neural plasticity can occur in the human CNS. We also discuss the potential clinical applications of manipulating neural plasticity through non-invasive stimulation methods in humans.

To begin with, we provide a brief review of the animal literature that has informed our current understanding of the basic features and molecular mechanisms underlying LTP.

Long-term potentiation

Early experiments on anaesthetized animals used extracellular electrodes to monitor synchronous, synaptically evoked responses of large populations of cells in the tightly delineated

layers of the hippocampus—typically in the dentate gyrus. The introduction of the hippocampal slice preparation (Skrede and Westgaard, 1971), in which 400–500 μm thick transverse slices are kept alive for several hours in an oxygenated bath of artificial cerebrospinal fluid, has enabled easy access to the cornus ammonis cornus ammonis 3 (CA3) and cornus ammonis 1 (CA1) pyramidal subfields. The placement of recording and stimulating electrodes can be easily determined by eye in this preparation, drugs can be added to and washed out of the bathing medium and stable recordings of evoked potentials can be collected over many hours. It is for these reasons that the majority of work on LTP has been conducted *in vitro*, usually in the CA1 subfield of the hippocampus. From the beginning, experimenters have used high-frequency trains of electrical stimuli (tetani), delivered to Schaffer collateral/commissural fibres projecting from CA3 to CA1 pyramidal neurons, thereby ensuring sufficient synaptic input to induce action potentials post-synaptically. In later experiments, intracellular techniques were used to pair depolarization of a post-synaptic cell with simultaneous afferent stimulation, providing a demonstration at the single-cell level that coincidence between pre- and post-synaptic activity is essential for LTP induction (Gustafsson *et al.*, 1987; Markram *et al.*, 1997; Bi and Poo, 1998).

In addition to its longevity, LTP has other characteristics that make it an attractive candidate mechanism for the storage of information, characteristics that were predicted by the neuropsychologist Donald Hebb long before the discovery of LTP (Hebb, 1949). First, synaptic LTP is an input-specific process, such that a single pathway can be potentiated without effect on inactive neighbouring inputs to the same cell (Andersen *et al.*, 1980; Barrionuevo and Brown, 1983). Since cortical neurons can receive thousands of synaptic inputs, this feature could greatly enlarge the information encoding capacity of the brain; if pathway-specificity in fact reflects synapse-specificity, then the unit of information storage could be a single synapse. Second, the property of associativity ensures that a weak tetanus, which is not by itself capable of initiating LTP, can become potentiated through association with a strong tetanus (McNaughton *et al.*, 1978; Levy and Steward, 1979). This latter mechanism is of particular interest in relation to memory formation as it presents a means by which we can associate events or entities in the outside world—a defining feature of much animal learning from classical and operant conditioning up to higher-order cognitive processing.

Molecular mechanisms

The property of associativity relies upon a mechanism that detects coincident pre- and post-synaptic activity. At most glutamatergic synapses in the CNS the *N*-methyl-D-aspartate (NMDA) sub-class of glutamate receptor performs this function (Collingridge *et al.*, 1983) (*see* Fig. 1). Post-synaptically positioned NMDA receptors bind glutamate released into the synaptic cleft following the invasion of the pre-synaptic

terminal by an afferent action potential. This event alone does not open the NMDA receptor's intrinsic cation channel, however, since at near-resting membrane potentials the channel is blocked by magnesium ions (Nowak *et al.*, 1984). Only when the post-synaptic cell is sufficiently depolarized is the magnesium ion expelled from the cation channel, allowing an influx of sodium and calcium ions into the cell. It is this calcium influx that is thought to initiate LTP induction (Lynch *et al.*, 1983; Malenka *et al.*, 1988). Calcium-sensitive signalling mechanisms, such as the calcium/calmodulin-dependent kinase II (CaMKII) or the cyclic adenosine monophosphate (cAMP)-dependent pathways, are then activated. These molecules, in turn, initiate LTP expression mechanisms, either locally, where they phosphorylate receptors and alter the intrinsic properties of their ligand-gated ion channels, or by signalling to the cell nucleus via transcription factors to alter gene expression (Goelet *et al.*, 1986; Alberini *et al.*, 1995). Expression mechanisms may be both pre- and post-synaptic (Kauer *et al.*, 1988; Malgaroli and Tsien, 1992), with much current work focusing on changes in the intrinsic conductance of glutamate receptor channels (Derkach *et al.*, 1999; Lee *et al.*, 2003) or in the number inserted into the synaptic membrane (Takahashi *et al.*, 2003). Structural changes to the shape or even number of synapses may also enhance the efficacy of potentiated pathways (Engert and Bonhoeffer, 1999). Many of these mechanisms are still controversial and none is completely characterized.

Another important point is that the molecular mechanisms of LTP induction and maintenance vary somewhat from synapse to synapse. For instance, within the hippocampus, at the mossy fibre–CA3 pyramidal cell synapse, the NMDA receptor is not required for LTP induction (Harris and Cotman, 1986) and the site of LTP expression is primarily pre-synaptic (Weisskopf and Nicoll, 1995). In contrast, LTP induction is mediated by the NMDA receptor at both medial perforant path–dentate gyrus granule cell (Morris *et al.*, 1986; Errington *et al.* 1987) and Schaffer collateral–CA1 pyramidal cell synapses (Collingridge *et al.*, 1983). Moreover, there is a major post-synaptic component to LTP expression at both the latter connections (McNaughton, 1982; Manabe *et al.*, 1992; reviewed in Nicoll and Malenka, 1995). Even these two sets of synapses are dissociable in terms of signalling mechanisms, however, as CaMKII signalling is required at the latter but not the former (Zhang *et al.*, 2005; Cooke *et al.*, 2004). There is therefore no generalized picture for the molecular mechanisms supporting LTP induction and expression at glutamatergic synapses. It seems that the important properties of LTP, longevity, input specificity and associativity, can be implemented by a variety of receptors and signalling systems.

LTD is the converse process to LTP and results in a long-lasting decrease in synaptic efficacy. The standard protocol for inducing LTD uses long trains of low-frequency (1 Hz) stimulation (Dudek and Bear, 1992), or mismatching of pre- and post-synaptic action potentials (Markram *et al.*,
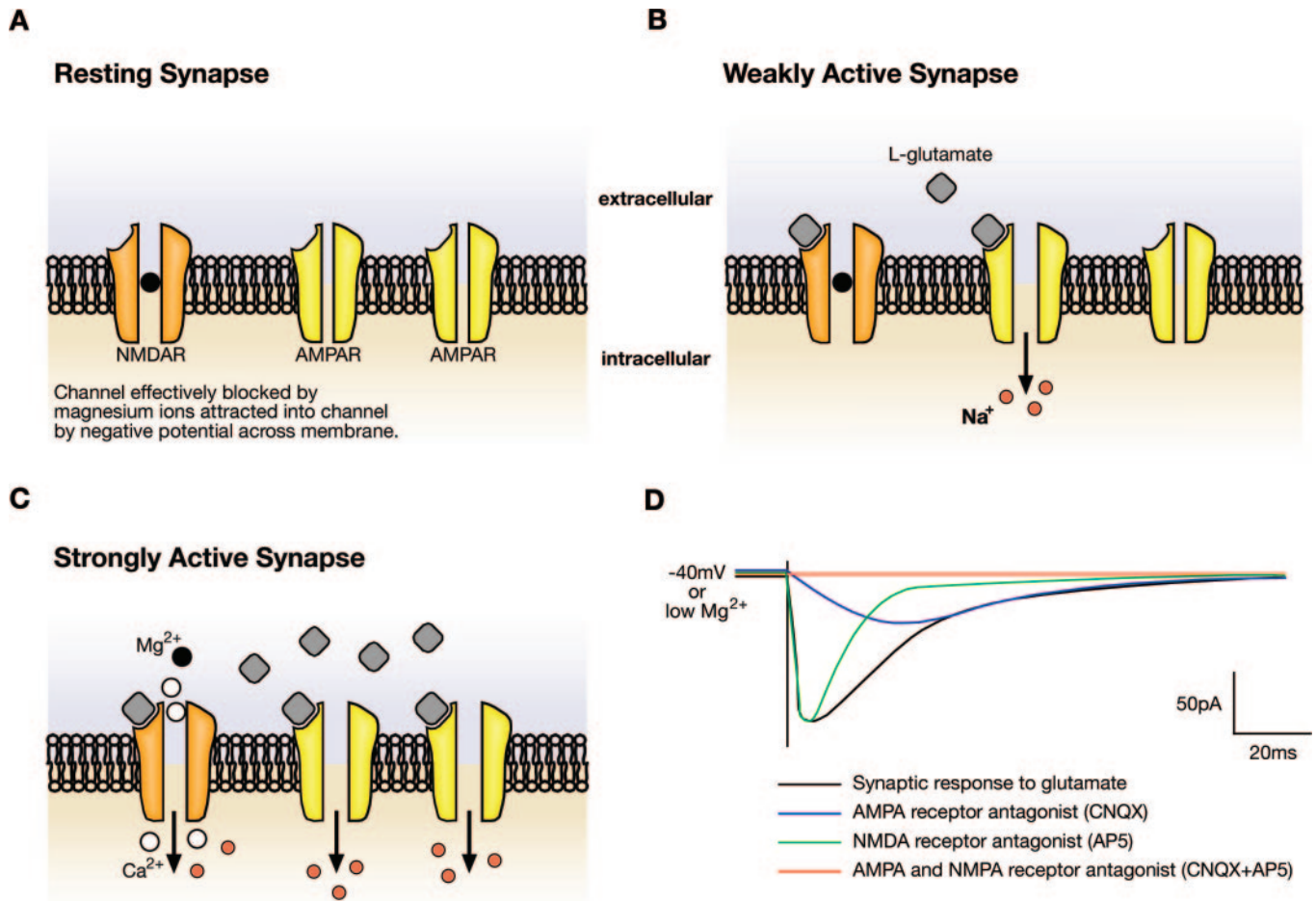
Fig. 1 The NMDA receptor as a coincidence detector. This figure depicts a glutamatergic postsynaptic membrane containing AMPA and NMDA subtypes of glutamate receptor. Under conditions of rest or low levels of input activity, the channel of the NMDA receptor is blocked by positively charged magnesium ions (Mg^{2+}) (A). Glutamate molecules released from the pre-synaptic terminal diffuse across the synaptic cleft and bind to both sub-types of receptor, opening AMPA receptor channels. The resulting inward current flow carried by Na^+ ions depolarises the post-synaptic membrane to produce an excitatory post-synaptic potential (EPSP). Low concentrations of released glutamate do not depolarise the postsynaptic membrane sufficiently to relieve the Mg^{2+}-block of the NMDA receptor channel (B). High concentrations of glutamate released at a strongly active synapse produce strong depolarisation of the post-synaptic membrane, resulting in the expulsion of magnesium ions from the NMDA receptor channel, and allowing influx of Na^+ and Ca^{2+} ions (C). Thus the NMDA receptor acts as a detector of coincident pre and post-synaptic activity. Panel D illustrates intracellular recordings of excitatory synaptic currents under conditions of low magnesium or membrane depolarisation (either of which result in unblocked NMDA receptor channels and ion permeation when glutamate is bound to the receptor; black trace). Application of antagonists of AMPA receptors (CNQX; blue trace) or NMDA receptors (AP5; green trace) results in selective abolition of fast or slow components of the EPSP, respectively.

1997). It seems likely that there are two mechanistically distinct forms of LTD: depotentiation, which refers to the reversal of LTP, and 'de novo' LTD, which refers to depression from an unpotentiated baseline. Some forms of LTD are dependent upon the NMDA receptor and are triggered by low concentrations of post-synaptic calcium (Nishiyama et al., 2000). Calcium-responsive phosphatases such as calcineurin and protein phosphatase 1 (PP1) are implicated as effector molecules in the mechanisms of LTD. These phosphatases dephosphorylate kinase targets such as glutamate receptors (Morishita et al., 2005) and the kinases themselves (Blitzer et al., 1998). LTD may serve as a homeostatic mechanism to ensure that CNS synapses are not saturated by learning. Alternatively, LTD may mediate learning in itself, forgetting or behavioural extinction.

LTP and memory in rodents

A large body of evidence has now been gathered demonstrating that LTP and memory are supported by similar molecular mechanisms. Blockade of the NMDA receptor with antagonists such as 2-amino-5-phosphonopentanoic acid (AP5) impairs learning by rodents in a variety of hippocampus-dependent memory tasks. For example, when AP5 is infused into the hippocampus, rats are impaired in their ability to form a spatial map of the position of a hidden platform in the Morris water-maze, and the drug also prevents the induction of hippocampal LTP in these animals (Morris et al., 1986; Abraham and Mason, 1988). Once a memory of the platform position has been acquired, AP5 infusions have no significant effect on the ability of
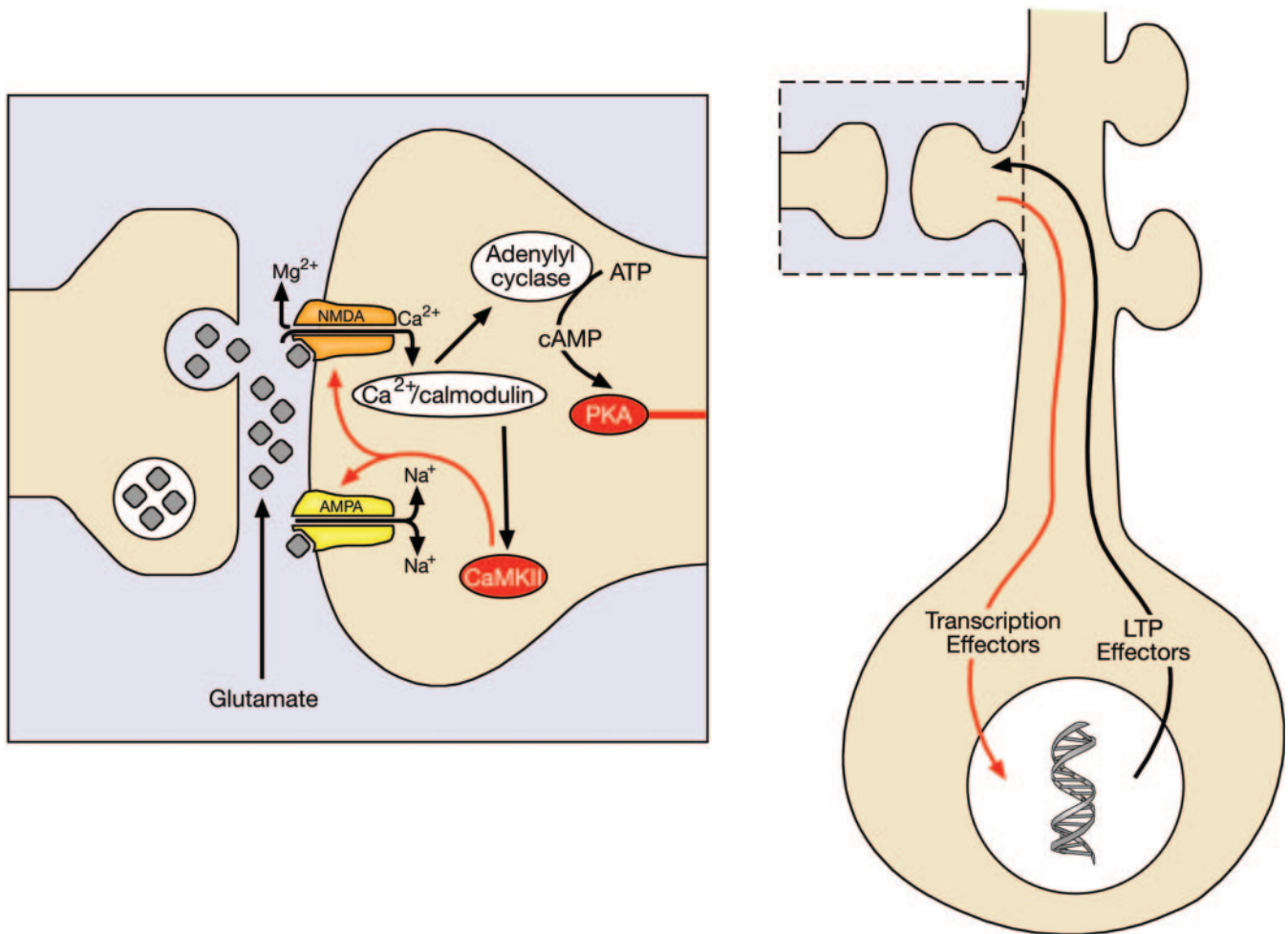
Fig. 2 Intracellular signalling mechanisms mediating NMDA receptor-dependent long-term potentiation (LTP). Localised signalling at the synapse is mediated by kinases activated by calcium (Ca^{2+}) influx through the NMDA receptor interacting with calmodulin (left-hand panel). Ca^{2+}/calmodulin activates Ca^{2+}/calmodulin-dependent kinase II (CaMKII) that, in turn, alters synaptic transmission through post-synaptic modifications of glutamate receptors, either via phosphorylation to alter the conductance state of existing channels or by facilitating the insertion of new receptors into the membrane. There is also evidence for pre-synaptic changes, culminating in increased transmitter release. Adenylyl cyclases are activated by Ca^{2+}/calmodulin, thereby activating the cAMP-dependent protein kinase (PKA). This kinase can initiate signalling to the cell nucleus, via a number of intermediary steps, resulting in changes in transcription and eventual expression of proteins involved in long-lasting changes that mediate persistent LTP (right-hand panel).

the animal to locate the platform, just as the drug has no effect on a potentiated response once LTP has been induced. It seems, therefore, that the NMDA receptor is required for the induction of LTP, but plays little role either in baseline transmission or in the expression of the potentiated response (Morris *et al.*, 1986).

Compelling evidence for the role of the NMDA receptor has come from a mutant mouse in which expression of the NR1 subunit of the NMDA receptor has been suppressed. This subunit is essential to the formation of a functional receptor, so the 'knockout' of this single gene in effect completely prevents the formation of functional NMDA receptors. Since NMDA receptors have vital roles early in the development of an organism, in addition to mediating synaptic plasticity in the adult, the NR1 knockout does not survive into post-natal life. In order to understand the role of the NMDA receptor in hippocampal LTP in the adult,

a 'conditional' knockout mouse has been generated, in which knockout of the NR1 gene is restricted to the CA1 subfield of the hippocampus (Tsien *et al.*, 1996). These mice survive well into adulthood, but fail to exhibit LTP at synapses in the CA1 subfield and also have specific spatial learning and memory deficits characteristic of hippocampal dysfunction. There is, therefore, strong correlative evidence to suggest a role for NMDA-receptor-dependent LTP in hippocampus-dependent learning and memory.

There are also common molecular features to the intracellular signalling mechanisms that mediate LTP and memory (*see* Fig. 2). Two of the major calcium-responsive signalling pathways that have been identified, CaMKII-dependent signalling and cAMP-dependent signalling, have been shown to participate in both LTP and learning and memory. The various isoforms of CaMKII are enzymes that respond to calcium when it is bound as a complex with the

calcium-binding protein calmodulin. The activated enzyme then has the ability to sustain its own activity through autophosphorylation (Miller and Kennedy, 1986). Given this property, and its synaptic localization, it has long been mooted that CaMKII could in this way act as a local, self-perpetuating memory molecule (Lisman and Goldring, 1988; Lisman et al., 2002). The targeted mutation of a single amino acid, threonine 286, in the alphaCaMKII protein, prevents the autophosphorylation that allows the kinase to act autonomously in the absence of calcium. Mice carrying this mutation have a profound deficit in hippocampus-dependent learning and memory and also completely fail to exhibit LTP induction in the hippocampal CA1 subfield under standard stimulation protocols (Giese et al., 1998; Cooke et al., 2004). This alphaCaMKII^{T286A} point mutant mouse demonstrates the importance of alphaCaMKII in hippocampus-dependent memory and some forms of NMDA-receptor-dependent LTP, and fits well with the model in which autonomously active kinase can sustain LTP for some period of time after calcium concentration has subsided back to a basal level.

The cAMP-dependent cascade is thought to mediate synapse to nucleus signalling and seems to initiate protein synthesis-dependent changes that take effect around an hour after LTP induction and that sustain both LTP and long-term memory in the long-term (Abel et al., 1997; Nguyen and Kandel, 1997). The calcium-sensitivity of this pathway relies upon calcium/calmodulin-initiated conversion of adenosine triphosphate (ATP) into cAMP by adenylyl cyclase. Elevation of cAMP activates the cAMP-dependent protein kinase (PKA). Application of forskolin, which increases adenylyl cyclase activity and, thereby, the concentration of cAMP, induces LTP in hippocampal slices without the requirement for an electrical tetanus, an effect that can be blocked with PKA inhibitors (Frey et al., 1993). Over-expression of a regulatory PKA subunit has the effect of greatly reducing the activity of this enzyme, preventing both long-lasting LTP and long-term memory, although, in the short term, neither is affected (Abel et al., 1997). Over-expression of adenylyl cyclase in a transgenic mouse, which increases available cAMP levels, has the reverse effect and enhances LTP and learning (Wang et al., 2004).

The next step in this signalling pathway is the mitogen-activated kinase (MAPK). Activation of this enzyme by PKA is increased after hippocampus-dependent learning in mice and application of a MAPK inhibitor blocks the maintenance of LTP (Rosenblum et al., 2000; Rosenblum et al., 2002; Waltereit and Weller, 2003; Sweatt, 2004) and long-term memory formation (Atkins et al., 1998; Blum et al., 1999; Bozon et al., 2003). This cascade leads, indirectly, to the phosphorylation and thereby activation of cAMP-responsive element binding protein (CREB) in the nucleus. CREB is a transcription factor that governs the expression of a variety of LTP/memory effector proteins. The importance of CREB was initially demonstrated in invertebrates (Dash et al., 1990; Yin et al., 1994, 1995). Since then, both mutant mice with a targeted disruption of CREB and transgenic mice expressing a repressor of CREB have been shown to have deficits in sustainable LTP and hippocampus-dependent long-term memory (Bourtchuladze et al., 1994; Bozon et al., 2003) and mice expressing an inhibitor of an endogenous CREB repressor have both enhanced LTP and long-term memory storage (Chen et al., 2003). This series of results suggests an important role for the cAMP-dependent signalling cascade in long-lasting LTP and memory in mammals.

Overall, the literature now suggests a strong correlation between the effects of molecular and pharmacological manipulation on hippocampal LTP, and hippocampus-dependent learning and memory. Examples of mutant mice that have normal LTP but deficient memory have been presented in the literature (Migaud et al., 1998; Fragkouli et al., 2005). However, these findings do little to damage the hypothesis that synaptic plasticity is a key process in learning and memory because there are many other factors that may affect learning and memory. Attention, sensory acuity and anxiety, for example, are clearly also important variables. Very few neuroscientists would make the claim that LTP is sufficient for learning or memory. However, taking a simplistic overview, the hypothesis that synaptic plasticity is necessary for learning and memory could be disproven by a single example of a mutant mouse in which LTP is abolished, but learning and memory are normal. At first glance, just such a mouse appeared on the scene with the publication of a paper describing a mutant lacking the GluR1 subunit of the α-amino-3-hydroxy-5-methylisoxazole-propionate (AMPA) receptor (Zamanillo et al., 1999). This knockout mouse performed as well as wild-type littermates in standard tests of hippocampus-dependent learning and memory, but LTP could not be induced in vitro in the CA1 subfield of the hippocampus using standard stimulation protocols. This, of course, is not the same as saying that LTP cannot under any circumstances be induced in vivo. Indeed, since the initial publication, it has been found that LTP can be induced at Schaffer collateral–CA1 synapses using a different and perhaps more physiologically realistic stimulation protocol (Hoffman et al., 2002). Moreover, deficits have now been reported in hippocampus-dependent learning and memory tasks in the GluR1 knockout mice (Reisel et al., 2005). All of these findings go to show that we will never entirely confirm or disprove a hypothesis that attempts to link LTP to behaviour by taking an essentially correlative approach (reviewed in Martin and Morris, 2002). Instead, by some as yet undetermined means we must test whether synaptic plasticity has an essential causal role in learning and memory. The most we can say at the moment is that synaptic plasticity, as modelled by LTP, is currently the favoured candidate mechanism for information storage within the CNS.

Memory mechanisms in humans

Associative memory is commonly separated into two major divisions: declarative memory, which encompasses the

recognition or recall of facts and episodes, and procedural memory, which refers to the retention of skills (Tulving, 1983). Declarative memory can be further divided into episodic memory, which relates to autobiographical information, and semantic memory, which pertains specifically to non-autobiographical facts and propositions. To a great extent these divisions reflect the underlying neurobiology because different neural substrates mediate declarative and procedural memory. The medial temporal lobe has been a site of major interest in understanding declarative memory processes and, in particular, episodic memory, since the publication of a case study of the patient H.M. (Scoville and Milner, 1957). H.M. was a 23-year-old man who was subjected to a bilateral medial temporal lobectomy in an attempt to control severe epilepsy. As a consequence he was unable to form new episodic memories, although further studies showed that he had no deficit in procedural learning and memory. Studies of declarative memory have subsequently focused on the medial temporal lobe, and, in particular, the hippocampus. Functional magnetic resonance imaging (fMRI) studies suggest that the medial temporal lobes are involved in spatial (Rosenbaum *et al.*, 2004), semantic (Bartha *et al.*, 2003) and recognition memory (Kirwan and Stark, 2004), all of which fall under the banner of declarative memory, and studies of patients with medial temporal lobe damage have revealed a causal role for these structures in the same types of task (Teng and Squire, 1999; Manns *et al.*, 2003*a*, *b*). The recognition of familiar and novel words is also believed to depend upon neural circuitry in the left medial temporal lobe (Bohbot *et al.*, 1998).

Evidence for the involvement of molecular memory mechanisms similar to those identified in rodents comes from the finding that learning a variant of the Rey verbal memory task is prevented by systemic application of the NMDA receptor blocker ketamine in human subjects (Grunwald *et al.*, 1999). Invasive recordings suggest that this may be due to an effect on neural plasticity. Measurements with implanted tungsten electrodes of event-related potentials (ERPs) in epileptic patients undergoing presurgery investigation reveal typical word-cued electrical responses, known as AMTL-N400s, in the medial temporal lobe, peaking at ∼400 ms after presentation of the word (Elger *et al.*, 1997). These responses normally undergo characteristic changes during learning in the Rey verbal test variant (Heit *et al.*, 1990; Nobre and McCarthy, 1995). Interestingly, these changes in ERP are not sustained if words are presented and available to working memory but prevented from entering long-term memory by distracting patients during the key post-training consolidation period (Fernàndez *et al.*, 1999). Memory-related changes in ERPs can be observed in medial temporal lobe structures, such as the hippocampus and rhinal cortex, but not in Wernicke's area, another region of cortex that plays an essential role in word processing and in which words elicit similar ERPs (Fernàndez *et al.*, 1999). These findings are consistent with medial temporal lobe structures, but not

Wernicke's area, being sites of storage for word-related memories. Significantly, the application of NMDA receptor antagonist ketamine prevents learning-related alteration of AMTL-N400s (Grunwald *et al.*, 1999), strengthening the correlation between changes in medial temporal lobe ERPs and learning, and demonstrating that both are NMDA-receptor-dependent.

Further evidence for the involvement of molecular signalling pathways in human memory that are similar to those identified in rodents comes from recent attempts by the pharmaceutical industry to develop drugs to enhance memory. Many of this group of chemically unrelated substances, collectively known as nootropics, have been shown to enhance LTP in rodents. The most promising candidate nootropics for clinical use include modulators of AMPA receptors, NMDA receptors and the cAMP-dependent signalling cascade. AMPA receptor modulators, including the much-publicized AMPAkines, enhance LTP induction by prolonging the depolarization produced by synaptically released glutamate and thus facilitating activation of the NMDA receptor (Arai *et al.*, 2004). One of these substances, CX-516, seems to have beneficial effects on learning and memory in humans and has reached phase II clinical trials for the treatment of Alzheimer's disease, dementia and schizophrenia (Goff *et al.*, 2001). An alternative enhancement strategy directly targets the NMDA receptor. Memantine acts to enhance memory, in a somewhat contradictory fashion, by weakly antagonizing the NMDA receptor (Parsons *et al.*, 1993). This seems to have beneficial effects on cognition in Alzheimer's disease (Lipton, 2005). NMDA-antagonism by memantine is thought to exert a nootropic effect in the long term as a result of protection from glutamate-induced excitotoxicity. It is important to note that memantine does not enhance memory through a direct modulation of LTP itself, although it does rescue deficits in LTP induced by excitotoxicity (Frankiewicz and Parsons, 1999). Another major strand of commercial and clinical investigation into nootropics has focused on the cAMP-dependent signalling pathway. Rolipram, an inhibitor of phosphodiesterases that increases the availability of cAMP and thereby increases the activity of PKA, enhances LTP and memory in rodents (Barad *et al.*, 1998), most notably recovering some memory deficits in a mouse model of Alzheimer's disease (Gong *et al.*, 2004). Rolipram has been considered a potential candidate for clinical use.

LTP in humans

Investigations into LTP in humans are obviously limited. A rare opportunity for experiments comparable with those conducted in animal models has been provided by excision of hippocampal tissue from individuals undergoing surgery as a treatment for temporal lobe epilepsy (*see* Fig. 3). Careful treatment of this tissue after removal from the brain has enabled investigators to test some of the molecular features of LTP in the temporal cortex (Chen *et al.*, 1996) and, more
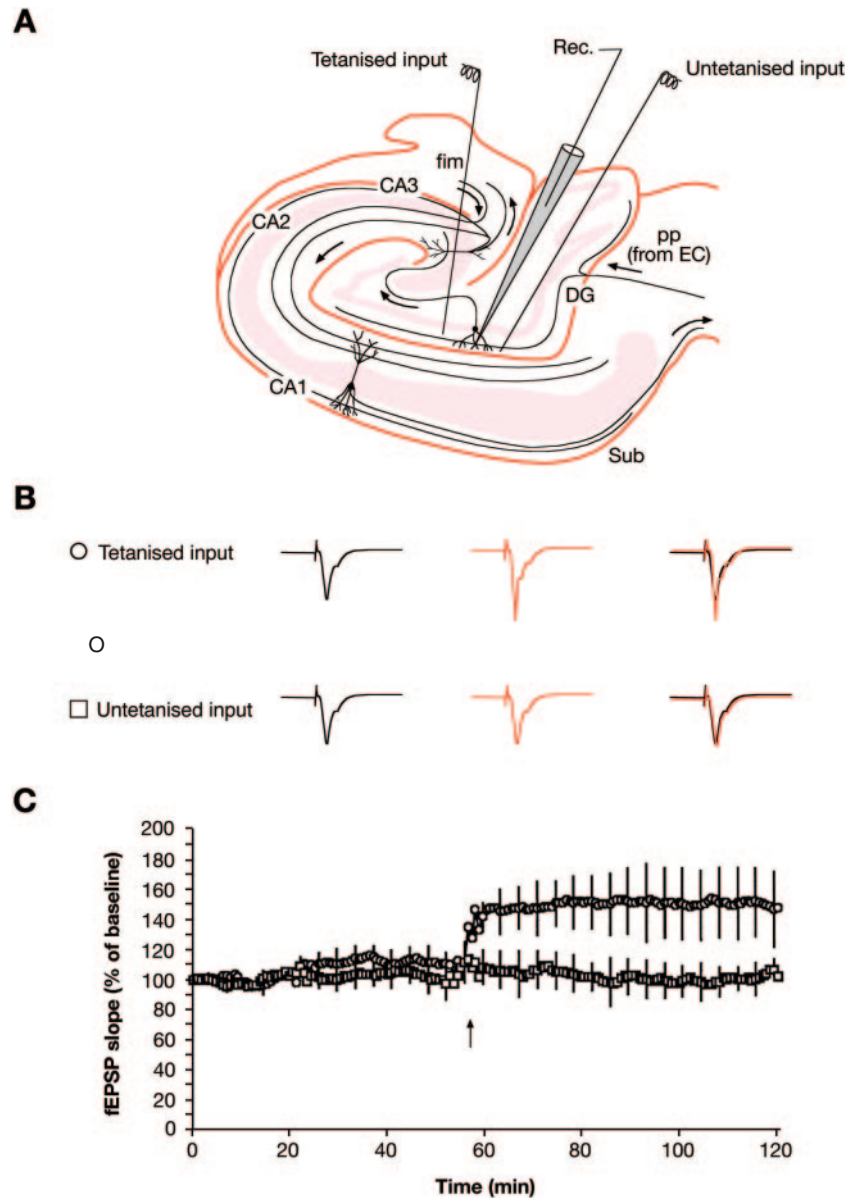
Fig. 3 Induction of long-term potentiation (LTP) in the human hippocampus. (A) Diagram of a hippocampal slice prepared from excised human temporal lobe. Granule and pyramidal cell fields are shaded in pink. The schematic shows the excitatory tri-synaptic loop comprising (i) perforant path (PP) fibres originating in the entorhinal cortex (EC) terminating on granule cells in the dentate gyrus (DG), (ii) granule cells in turn projecting, via their axons, the mossy fibres, to the CA3 subfield of the hippocampus. Here the mossy fibres terminate on proximal apical dendrites of pyramidal cells. The latter in turn, project via the Schaffer collaterals to subfield CA1, where they terminate upon pyramidal neurons, the axons of which project to the subiculum (Sub). A recording electrode is shown in the dendritic field of granule cells to monitor field potentials evoked by two electrodes placed on either side to activate non-overlapping populations of PP axons. Evoked synaptic field potentials are shown in panel B. Responses prior to the delivery of a high-frequency train through one of the two stimulating electrodes are shown in black. Responses after the tetanus are shown in red. The other stimulating electrode activates a non-tetanised control pathway. Low frequency stimuli are delivered to both pathways throughout the experiment. A plot of the slope of the population EPSP against time shows a large and maintained increase in synaptic efficacy in the tetanised pathway but not in the control pathway following delivery of the high frequency train (arrow) (C). Adapted, with permission, from Beck et al. (2000).

recently, at human perforant path–granule cell synapses in the dentate gyrus (Beck *et al.*, 2000). Substantial LTP can be induced in acute slices prepared from excised hippocampal tissue by brief tetanic stimulation of perforant path fibres. Potentiation of synaptic responses can be sustained for at least 2 h. Application of APS during the tetanus prevents the induction of LTP, demonstrating a requirement for the NMDA receptor, and sustained potentiation of synaptic responses results from bath application of forskolin, suggesting the involvement of the cAMP-dependent signalling pathway in LTP in humans.

Patients contributing tissue to these studies fall into two groups: those with an epileptic focus in the hippocampus and those with a focus elsewhere in the temporal lobe.

LTP can be readily induced in hippocampal tissue taken from patients with extra-hippocampal epileptic foci. The degree of LTP induced by tetanic stimulation in tissue taken from patients with hippocampal epileptic foci, however, is far more modest, and potentiation cannot be induced using forskolin. A possible reason for these observations is that synapses in epileptic tissue have become potentiated through epileptic activity, and are near saturation. A separate study found that expression of CaMKII is elevated in dentate granule cells of patients with hippocampal epileptic foci, perhaps reflecting a compensatory alteration of CaMKII signalling (Lie *et al.*, 1998). Finally, patients with hippocampal foci perform worse on the Rey verbal memory task than individuals with neo-cortical temporal lobe epileptic foci (Helmstaedter *et al.*, 1997). This series of results from human subjects comprises a set of correlations between synaptic LTP, declarative memory, the NMDA receptor and intracellular signalling mechanisms that have previously been identified in animal models.

Non-invasive stimulation in awake humans

Technical advances have presented the possibility of delivering tetanic stimulation to awake human subjects. This can be achieved using repetitive transcranial magnetic stimulation (rTMS), in which the cerebral cortex of an awake human subject can be stimulated non-invasively with a remote hand-held apparatus. Interventional paired associative stimulation (IPAS), which pairs TMS with electrical stimulation of peripheral nerves that provide input to the same cortical region, can be used in a similar manner. The risks of inducing seizure or long-lasting pathologies have had to be carefully evaluated before proceeding with experiments using remote stimulation with the high frequencies necessary for inducing LTP (Wassermann *et al.*, 1996). Experiments using these technologies have not focused on the medial temporal lobe for two major reasons. First, the hippocampus and surrounding structures lie deeper than 2 cm below the surface of the skull in humans, the current limiting distance for application of TMS (Bohning *et al.*, 1997), and second, there is no well-defined behavioural output to use as a positive control of successful remote stimulation. For these reasons many TMS studies have been conducted in the motor cortex where remote stimulation can be used to elicit limb movements (Pridmore *et al.*, 1998), typically in the hand. This positive control allows investigators to establish a motor threshold, which varies greatly from individual to individual, and set experimental stimulation intensity accordingly. In addition, monitoring motor output allows for the observation of long-term behavioural consequences of higher-frequency remote stimulation.

Transcranial magnetic stimulation

TMS stimulation of motor cortex in humans using frequencies of 1–20 Hz produces effects on motor-evoked potentials that vary from individual to individual. Generally, 1 Hz stimulation reduces neural activity and anything over 5 Hz increases activity and motor output. In both cases the effects of such stimulation appear to be transient, lasting around half an hour at most (Hallett, 2000). Interestingly, application of this low-frequency TMS to area M1 in the motor cortex can be used to block consolidation of motor skill acquisition in normal human subjects without interfering with motor performance itself (Muellbacher *et al.*, 2002). Although changes in evoked potentials persist after the higher-frequency (5 Hz) trains of stimuli, the effect is not consistent and never lasts long enough to be comparable with LTP (Maeda *et al.*, 2000). Higher-frequency tetani (50 Hz) have now been delivered and shown to be safe in normal individuals, provided the intensity of the stimulation is reduced to below motor threshold, although even this mode of stimulation does not produce changes that persist for longer than hundreds of milliseconds (Huang and Rothwell, 2004).

LTP is often induced in animals using repeated trains of high-frequency stimulation spaced at a frequency that mimics a spontaneous 5-7 Hz neural rhythm, the theta wave. Tetani of this sort via TMS can induce long-lasting changes in motor cortical output (Huang *et al.*, 2005). Again the frequency of stimulation never exceeds 50 Hz in this sort of experiment [animal investigators may use frequencies as high as 400 Hz (Davis *et al.*, 1997)], and the stimulation intensity must be set well below motor threshold during the tetanus. Nevertheless, the amplitude of motor-evoked potentials in the hand as a result of super-threshold baseline stimulation can increase by ~50% for at least 20 min after application of several theta burst-like tetani spaced 10 s apart. This finding strongly suggests that remote stimulation can be used to induce a long-lasting change in motor cortical output. It has yet to be demonstrated, however, that the site of such change is the synapse.

Interventional paired associative stimulation

As described above, an alternative means of inducing LTP that does not require the application of a high-frequency tetanus, is to pair pre- and post-synaptic action potentials (Wigstrom *et al.*, 1987; Markram *et al.*, 1997; Bi and Poo, 1998). Pairing of this sort can potentially be modelled in humans by combining low-frequency TMS to the cortex whilst simultaneously stimulating a peripheral nerve, an approach known as IPAS (*see* Fig. 4). For example, peripheral stimulation of the right median nerve can be followed by TMS directed at the hand representation area in contralateral primary motor cortex (M1), at a latency determined by the time-lag in evoking an M1 cortical potential via activation of somatosensory cortex (Stefan *et al.*, 2000). Motor-evoked potentials can again be used as an index of the resultant increase in motor cortical output, here in the abductor pollicis brevis muscle in the thumb. One benefit
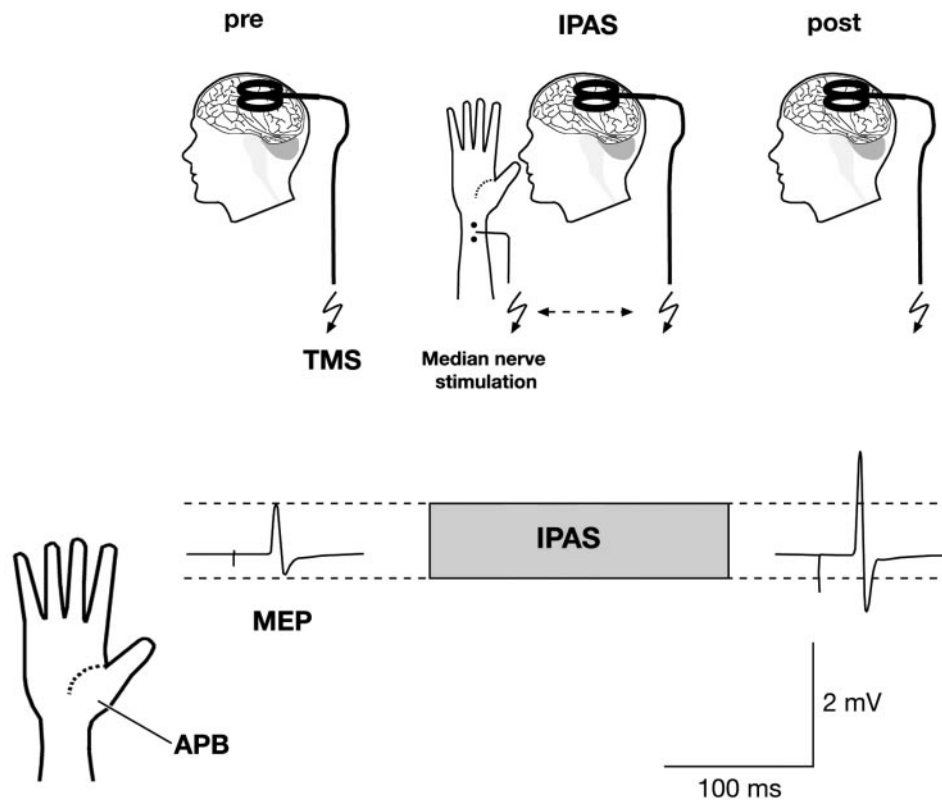
Fig. 4 Interventional paired associative stimulation (IPAS). Transcranial magnetic stimulation (TMS) is delivered to the hand representation area in region M1 of the motor cortex with single test pulses at an intensity set to elicit a motor evoked potential (MEP) in the abductor pollicis brevis (APB) muscle of the thumb. Electrical stimuli to the median nerve are paired with TMS (e.g. 90 pairs with an inter-stimulus interval of 25msec). After pairing, test TMS pulses elicit an enhanced MEP. The potentiation of the MEP can last for at least an hour. MEPs represent averages of 20 samples. Adapted, with permission, from Stefan et al. (2000).

of using this approach compared with high-frequency TMS is that any risk of seizure is greatly reduced. Another is that it is more physiologically realistic and enables the testing of one of the key requirements for LTP—coincident pre- and post-synaptic activity. While coincident pre- and post-synaptic stimulation in the cortex, using peripheral stimulation preceding TMS stimulation, results in an increase in cortical excitability lasting for at least an hour (Stefan *et al.*, 2000), mis-timing of peripheral and TMS stimulation, by shortening the interval between the two, results in a depression of cortical excitability lasting for an hour and a half (Wolters *et al.*, 2003). Both of these effects can be blocked by the NMDA receptor antagonist dextromethorphan. This finding is consistent with the involvement of LTP/LTD-like processes. Moreover, the plasticity is limited to only those cells receiving stimulation in the cortex due to both peripheral stimulation and direct TMS, as demonstrated by the fact that there is no potentiation of motor-evoked responses in muscles controlled by neighbouring regions of motor cortex, such as the biceps brachii, which receive TMS stimulation but not peripherally induced stimulation. This experiment establishes that the potentiating effect is restricted to cells receiving paired input. Recent experiments reveal that motor learning prior to IPAS stimulation can prevent induction of the

LTP-like plasticity in motor cortex for a period of 6 h (Stefan *et al.*, 2005). Again, this finding suggests that the early motor learning may have saturated plasticity, thereby occluding further change. At the same time, the induction of LTD-like plasticity during this same period is facilitated (Ziemann *et al.*, 2004), consistent with the idea that motor learning increases output from M1 through an LTP-like process, thereby allowing a greater scope for a reduction in the motor output, through depotentiation.

Auditory and photic stimulation

An alternative to TMS has recently been used to induce long-lasting changes in neuronal excitability in human subjects, this time in the auditory (Clapp *et al.*, 2005) and visual cortices (Teyler *et al.*, 2005). ERPs can be recorded in either area using scalp electrodes to monitor responses to auditory or visual stimuli. In these experiments, long-lasting enhancement of the amplitude of a component of either auditory-evoked or visual-evoked responses is achieved using a 13 Hz auditory tetanus, comprising a sequence of tone pips, or a photic tetanus generated on a computer screen, which comprises a series of chequerboard stimuli delivered at a frequency of 9 Hz. Either of these tetani is

sufficient to increase the amplitude of a component of ERP in the respective area of cortex for at least 50 min afterwards. Moreover, in the latter case, delivery of lower-frequency visual stimuli (1 Hz) reduces the amplitude back to baseline levels, suggesting a depotentiation-like process.

The authors of these studies argue that the selective alteration of a single component of the ERP, which consists of electrical fields generated by a large number of neurons, constitutes a form of synaptic plasticity. This interpretation cannot be validated without more refined analysis, which, with the limits of current technology, is not yet possible. Nonetheless, it is a fascinating finding that a sensory tetanus alone can be used to induce long-lasting effects on neuronal responses in cerebral cortex. The finding complements animal studies in which LTP is induced at synapses made by fibres from projection neurons in the lateral geniculate nucleus on layer IV cells in the visual cortex pathway of rats (Heynen and Bear, 2001). Here LTP is induced by tetanic electrical stimulation, but subsequent to the tetanus, responses in primary visual cortex evoked by visual stimuli, such as light flashes and patterned gratings, are enhanced. The authors demonstrate that the potentiation is NMDA-receptor-dependent, using the NMDA receptor antagonist CPP [($\pm$)-3-(2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid] and indicate that the site of plasticity is synaptic, as revealed using current source density analysis (CSD; for an explanation of CSD methods, *see* Mitzdorf, 1985).

Regardless of the means of stimulation—whether TMS, IPAS, or photic or acoustic tetani—the end result is a long-lasting increase in cortical responsiveness. As yet, however, investigators have not been able to establish the exact nature of the underlying neural plasticity. Possibilities include changes in synaptic efficacy or in the threshold for action potential generation in the excitatory output cells of the cortex, or changes of similar sorts in intrinsic inhibitory networks. It is possible to observe alteration of activity in the neocortex using remote recording technology with electroencephalogram scalp electrodes (Clapp *et al.*, 2005; Halder *et al.*, 2005; Teyler *et al.*, 2005), but non-invasive remote recording has poor spatial resolution and does not currently allow for discrimination between synaptic events and action potentials. EPSP-spike (E–S) potentiation, another form of neural plasticity in which the probability of an action potential being generated by a given synaptic input is increased, may well play a significant role in learning and memory (Giese *et al.*, 2001). However, E–S potentiation is unlikely to provide the same capacity for information storage as potentiation of chemical transmission at individual synapses, because changes in the mechanism of action potential generation should, in theory, have an equivalent effect on many inputs to the same cell. Curiously, there is some evidence for a degree of input specificity in E–S plasticity (Douadal *et al.*, 2002). However, it is likely that the specificity is limited to small populations of synapses rather than individual synapses.

At this point it is important to stress that the only direct evidence for synaptic plasticity in the human CNS comes from the experiments described earlier on excised human tissue (Chen *et al.*, 1996; Beck *et al.*, 2000). All those studies that we have described so far using remote means to induce changes in neuronal excitability and functional output of the human CNS in awake subjects are consistent with the induction of LTP or LTD at synapses. However, in order to demonstrate in these cases that change occurs in the efficacy of synaptic transmission rather than in the excitability of the cell, or in the balance of excitation and inhibition in the network in which the cell is embedded, it will be necessary to conduct experiments in which synaptic responses are monitored to activation of two clearly defined, and separately stimulated, input pathways. If change is synaptic it should be possible to potentiate or depress responses to one pathway without interfering with the other (*see* Fig. 3). Current approaches to non-invasive recording and stimulation have not yet allowed such observations to be made.

Neural plasticity and therapy

Regardless of whether it is synaptic efficacy that is altered for long periods, or some other long-lasting form of neural plasticity, the net effect of the stimulation protocols described above is an increase in output from the neocortex. Repetitive stimulation of the brain can exert long-lasting functional effects, as demonstrated by the increased muscle activity in the hand in response to TMS directed at primary motor cortex. Treatment could potentially be provided for neurological disorders that arise from a reduction in the output of particular regions of the brain, as in Parkinson's disease and depression, using remote stimulation to induce long-lasting increases in excitatory drive. Currently available therapies using electrical stimulation rely upon invasive surgery. A non-invasive method of achieving the same end would obviously be preferable.

Depression

Electroconvulsive therapy (ECT) has long been used to treat depression in cases in which other treatments fail (Potter and Rudorfer, 1993). This is an extreme measure that, although effective in some cases, can also result in memory loss and other cognitive deficits (Frasca *et al.*, 2003). A major benefit of using rTMS as a therapeutic treatment over ECT is that anaesthesia is not required. A number of studies have shown significant anti-depressant effects of rTMS (between 1 and 20 Hz), delivered to the prefrontal cortex, in patients with medication-resistant depression, as assessed using objective scales (George *et al.*, 2000; Fitzgerald *et al.*, 2003; Nahas *et al.*, 2004), although effects are variable. It has been suggested that the use of theta burst rTMS, as recently demonstrated by Huang *et al.* (2005), may produce more consistent results because the effects appear to be longer-lasting than low-frequency stimulation (Paulus, 2005). A

major concern with this method is the possibility that mania may result from increased activity in the same prefrontal areas targeted with rTMS (Kaptsan *et al.*, 2003). Nonetheless, treatment of depression with rTMS is a promising avenue of clinical research.

Parkinson's disease

The use of rTMS to treat Parkinson's disease may be of less obvious therapeutic value. It is well known that the primary site of degeneration in this disease is a deep-lying midbrain structure—the substantia nigra. This is not accessible to remote stimulation with TMS. However, it is possible that some of the secondary effects of reduced nigral output, such as disrupted motor cortical activity, may be open to manipulation with non-invasive stimulation over the scalp. Basal ganglia dysfunction resulting in reduced nigral output results in characteristic synchronized activity in the motor cortex that is believed to contribute to akinesia and limb rigidity (Goldberg *et al.*, 2002). It has been shown that high-frequency stimulation targeted at the M1 area of the motor cortex can induce recovery from Parkinson's-like motor deficits in baboons treated with the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP selectively kills dopaminergic neurons in the substantia nigra, thereby affecting basal ganglia function and initiating the parkinsonian symptoms of akinesia, bradykinesia, tremor and rigidity. Curiously, given the fact that motor cortical activity is not reduced by MPTP-induced pathology, but is instead simply highly synchronized, delivery of high-frequency (130 Hz) stimulation of the motor cortex, which presumably boosts motor cortical activity if it induces LTP, results in a significant long-lasting functional recovery from symptoms of akinesia and bradykinesia in MPTP-treated baboons (Drouot *et al.*, 2004). The rationale behind this approach is somewhat counter-intuitive but the technique seems to produce results. Recently developed invasive therapies are beginning to yield some success in the treatment of Parkinson's disease (Houeto *et al.*, 2002; Krack *et al.*, 2003). In order to mimic the output of the substantia nigra, electrodes are implanted into the sub-thalamic nucleus or internal segment of the globus pallidus, basal ganglia components that lie downstream of the substantia nigra. However, this is a very difficult surgical procedure and involves significant risk. Non-invasive therapy may be preferable, even if it requires multiple treatments, so further research into the effects and side-effects of non-invasive motor cortex stimulation in Parkinson's models may yet lead to significant amelioration of symptoms.

Epilepsy

A final potential application for TMS is in the treatment of intractable epilepsy, a probable contributory factor to which is increased efficacy of glutamatergic synaptic transmis-

sion or reduced inhibition at a neuronal population level. It is possible that neuronal hyperexcitability in epilepsy could be reduced by induction of LTD. LTD may either de-potentiate over-potentiated synapses or compensate for other causes of hyperexcitability. Application of low-frequency rTMS (0.3 Hz) to epileptic foci in severely epileptic patients has been shown to have beneficial effects (Tergau *et al.*, 1999), reducing the number of seizures for a period of a month after stimulation. Further development of this approach seems warranted, given the demonstrations that it can be used to induce an LTD-like phenomenon in the neocortex (Chen *et al.*, 1997; Ziemann *et al.*, 2004).

Hyperalgesia

LTD induction may also have therapeutic value in the treatment of chronic neuropathic pain. Hyperalgesia of this sort can be modelled in rodents by injecting formalin subcutaneously into a paw. Alterations of central circuitry within the spinal cord occur that, in turn, mediate a long-lasting hypersensitivity to cutaneous stimulation around the conditioning site (Woolf *et al.*, 1983). This model has enabled the identification of cell types and signalling pathways involved in long-lasting central sensitization (reviewed in Han, 2003), and has also suggested the involvement of synaptic LTP in the induction of hyperalgesia (reviewed in Ji *et al.*, 2003). Synapses between primary afferent peptidergic nociceptive fibres, which release substance P as a neurotransmitter, and projection neurons from lamina I of the dorsal horn of the spinal cord expressing the neurokinin 1 (NK1) receptor, which binds substance P, can display LTP in response to high-frequency stimulation. Neighbouring cells that receive nociceptive input but do not express the NK1 receptor do not exhibit LTP. The NK1 receptor is required for LTP at these synapses, as is the NMDA receptor and a rise in free intracellular Ca^{2+} (Liu and Sandkühler, 1997; Ikeda *et al.*, 2003). Similar LTP occurs in rats using noxious stimulation itself, or following nerve injury (Sandkühler and Liu, 1998). If chronic neuropathic pain is mediated by LTP at a limited population of synapses then a logical approach to treatment would be to attempt to induce LTD at these same synapses. A recent study has used transcutaneous electrical nerve stimulation (TENS) to deliver high- and low-frequency tetani in human subjects in order to induce long-term hyper- and hypoalgesia, respectively, in response to mechanical stimulation of surrounding skin (Klein *et al.*, 2004). Here ratings of pain levels by the subjects serve as an index of the degree of sensitization or analgesia. Although pain was reported to increase acutely during both high- and low-frequency stimulation, reported pain levels were persistently increased after high-frequency stimulation and decreased after low-frequency stimulation. LTD induced by low-frequency TENS, therefore, presents a potential avenue for therapy in neuropathic pain.

Conclusion

Thirty years of research into LTP has yielded a huge amount of data on the properties of longevity, input specificity and associativity, on the molecular mechanisms that support both short-lasting and persistent LTP, and on the correlation between LTP and learning and memory (reviewed in Bliss *et al.*, 2003, a themed issue of the *Philosophical Transactions of the Royal Society of London*). We are not yet in a position to conclude definitively that LTP provides a mechanism for the neural basis of learning and memory but it is certainly a compelling physiological model of these processes. Animal studies during the past three decades have covered a wide range of preparations, from dissociated cell cultures to awake, freely moving animals, but only recently has progress been made in the study of LTP in humans. Synaptic LTP can be induced in hippocampal tissue excised from human patients, and this plasticity, unsurprisingly, shares molecular mechanisms with animal models. Moreover, deficits in LTP are correlated with deficits in hippocampus-dependent memory in humans. Progress in remote stimulation technology is now making it possible to consider treatments based on the induction of long-lasting changes in cortical output using stimulation protocols similar to those that have been used to induce synaptic plasticity in animals. These LTP- and LTD-like effects may be of therapeutic value, and offer a potentially more targeted treatment for depression than ECT. Similar treatments may also be beneficial for other neurological disorders such as Parkinson's disease and epilepsy.

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