British Journal of Pharmacology (2008) 153, S428–S437

0007–1188/08 $30.00
www.brjpharmacol.org

LifeSciences2007

REVIEW

The role of GSK-3 in synaptic plasticity

S Peineau, C Bradley, C Taghibiglou, A Doherty, ZA Bortolotto, YT Wang and GL Collingridge

Department of Anatomy, MRC Centre for Synaptic Plasticity, School of Medical sciences, University Walk, Bristol, UK

Glycogen synthase kinase-3 (GSK-3), an important component of the glycogen metabolism pathway, is highly expressed in the CNS. It has been implicated in major neurological disorders including Alzheimer's disease, schizophrenia and bipolar disorders. Despite its central role in these conditions it was not known until recently whether GSK-3 has neuronal-specific functions under normal conditions. However recent work has shown that GSK-3 is involved in the regulation of, and cross-talk between, two major forms of synaptic plasticity, N-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) and NMDAR-dependent long-term depression (LTD). The present article summarizes this recent work and discusses its potential relevance to the treatment of neurological disorders.
British Journal of Pharmacology (2008) **153,** S428–S437; doi:10.1038/bjp.2008.2

Keywords: glycogen synthase kinase; long-term potentiation; long-term depression; PI3K; Akt; PP1; NMDA receptor; AMPA receptor; metaplasticity; hippocampus

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors; DP, depotentiation; GSK-3, glycogen synthase kinase-3; LTD, long-term depression; LTP, long-term potentiation; NMDAR, *N*-methyl-D-aspartate receptor; PI3K, phosphatidylinositol 3-kinase; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A

Introduction

Glycogen synthase kinase-3 (GSK-3) is a multifunctional serine/threonine (ser/thr) kinase that was originally identified as a regulator of glycogen metabolism (Embi *et al*., 1980). Since then, it has been shown to be ubiquitously expressed in eukaryotes (see Ali *et al*., 2001), where it plays a fundamental role in a wide variety of functions, including the division, proliferation, differentiation and adhesion of cells (Frame and Cohen, 2001; Grimes and Jope, 2001). GSK-3 dysfunction is implicated in major diseases including cancer and diabetes (Frame and Cohen, 2001).

There are two isoforms of GSK-3 in mammals that are encoded by different genes (GSK-3α and GSK-3β) (Woodgett, 1990), the latter of which has two splice variants. These proteins are highly homologous in their kinase domains but differ in other regions, in particular the α isoform possesses an extended glycine-rich N-terminal tail. Both enzymes are highly regulated by phosphorylation. For example, in GSK-3β, phosphorylation of tyr216 is required for basal activity and high levels of phosphorylation of this residue result in GSK-3β being active in resting cells (Hughes *et al*., 1993). A second level of regulation by phosphorylation of ser9, by a variety of kinases, leads to inactivation of GSK-3β, overriding the activation induced by phosphorylation of tyr216 (Bhat *et al*., 2000). Conversely, dephosphorylation of ser9, by ser/thr protein phosphatases such as protein phosphatase 1 (PP1) and protein phosphatase 2A, results in the disinhibition of its activity (Figure 1; GSK-3α is similarly regulated via tyr279 and ser21). For example, in glycogen metabolism, insulin stimulates PI3K (phosphatidylinositol 3-kinase), which leads to activation of Akt (also known as protein kinase B). This then results in phosphorylation of ser9 of GSK-3β to inhibit its activity, allowing for dephosphorylation of glycogen synthase and the stimulation of glycogen synthesis (Frame and Cohen, 2001; Doble and Woodgett, 2003). In addition to Akt, PKA has been shown to phosphorylate both α and β subtypes of GSK-3 (Fang *et al*., 2000) whereas PKC has been shown to phosphorylate GSK-3β (Goode *et al*., 1992; Fang *et al*., 2000). Other regulators of GSK-3β include the mammalian target of rapamycin pathway and mitogen-activated protein kinase cascades (Frame and Cohen, 2001).

Numerous potential substrates for GSK-3β have been identified, including several different transcription factors, metabolic enzymes, proteins that bind to microtubules and components of the machinery involved in cell division and cell adhesion (Frame and Cohen, 2001; Doble and Woodgett, 2003). Some of the substrates that are relevant to neuronal function are shown schematically in Figure 1.

Correspondence: Professor GL Collingridge, Department of Anatomy, MRC Centre for Synaptic Plasticity, School of Medical sciences, University Walk, Bristol BS8 1TD, UK.
E-mail: g.l.collingridge@bris.ac.uk
Received 6 August 2007; revised 29 October 2007; accepted 30 October 2007

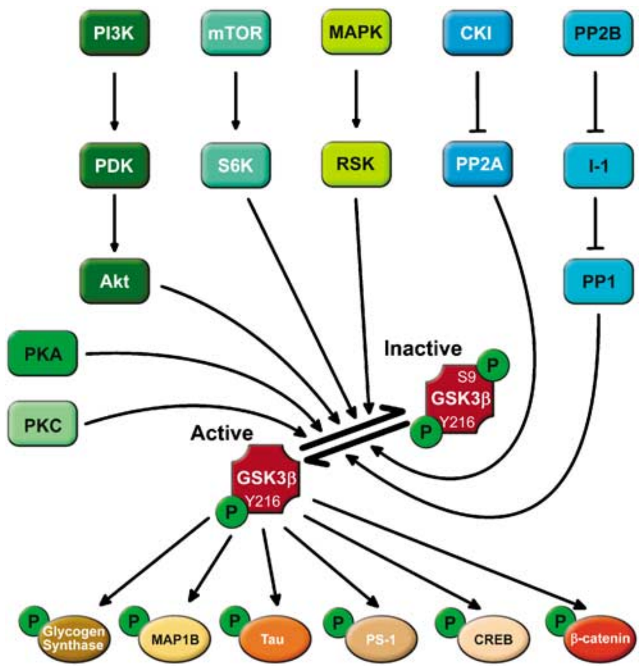

Figure 1 Example of signalling pathways upstream and downstream of GSK-3β. Under resting conditions, GSK-3β is basally activated by phosphorylation at tyr216. Various ser/thr kinase cascades result in phosphorylation of ser9 of GSK-3β, which results in inhibition of its activity. Conversely, dephosphorylation of this residue results in disinhibtion of the enzyme. GSK-3β phosphorylates a wide range of substrates. A selection of such substrates that relate to neuronal function is shown. CREB, cAMP responsive element-binding protein; CK1, casein kinase 1; I-1, inhibitor 1; MAP1B, microtubule-associated protein 1B; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PDK, phosphoinositide-dependent protein kinase; PI3K, phosphatidylinositol 3-kinase; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PP2B, protein phosphatase 2B; PS-1, presenilin 1; RSK, p90 ribosomal S6 kinase; S6K, p70 ribosomal S6 kinase-1.

Involvement of GSK-3 in neurological and psychiatric disorders

Although both isoforms of GSK-3 are implicated in neurological and psychiatric disorders, most investigations have focussed on the β isoform. GSK-3β is highly enriched in the brain (Woodgett, 1990; Takahashi *et al*., 1994; Leroy and Brion, 1999) (Figure 2) where it has been implicated in various disorders including Alzheimer's disease (Anderton, 1999; Grimes and Jope, 2001; Alvarez *et al*., 2002; Eldar-Finkelman, 2002; Bhat *et al*., 2004), schizophrenia (Beasley *et al*., 2001; Eldar-Finkelman, 2002; Kozlovsky *et al*., 2002) and bipolar disorders (Klein and Melton, 1996; Grimes and Jope, 2001; Eldar-Finkelman, 2002). Therefore, GSK-3β is a prime drug target for a variety of CNS therapies. Of particular relevance to neurological disorders, GSK-3β (also known as tau kinase 1) has been shown to bind to and phosphorylate both presenilin-1 and tau; proteins implicated in the aetiology of Alzheimer's disease (Hanger *et al*., 1992; Kirschenbaum *et al*., 2001; Avila *et al*., 2004). Indeed, GSK-3β is probably the critical kinase for tau hyperphosphorylation (Plattner *et al*., 2006). Lithium has long been used to treat bipolar disorders (Gould and Manji, 2005) and has been shown to be a competitive inhibitor of GSK-3 with respect to magnesium, a property not found in other group I metal ions (Ryves and Harwood, 2001). This may account for its ability to act as a mood-stabilizing drug (Klein and Melton, 1996), though other actions of lithium, such as its well-known ability to inhibit inositol-1,4 bisphosphate 1-phosphatase and inositol-1(or 4)-monophosphatase, could also explain or contribute to its therapeutic effects (see Harwood, 2005).

Mechanisms of synaptic plasticity in CNS

It is generally accepted that most information is stored at synapses in the form of alterations in synaptic efficiency. In particular, two forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD), have been extensively investigated in the pursuit of understanding the molecular and cellular basis of learning and memory (Bliss and Collingridge, 1993; Bear and Abraham, 1996). Most information has been derived from studies in the hippocampus, a brain region that is critically involved in learning and memory. However, mechanisms discovered in the hippocampus appear to be utilized widely in the brain for other forms of synaptic plasticity. The remainder of this article refers to work performed in the hippocampus and this section provides a brief overview of hippocampal synaptic plasticity. For a comprehensive account of this field, see Bliss *et al*. (2007).

The vast majority of synapses that exhibit LTP and LTD are glutamatergic. L-glutamate acts on four main classes of glutamate receptors: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs), kainate receptors, *N*-methyl-D-aspartate receptors (NMDARs) and metabotropic glutamate receptors (mGluRs), all of which are important for various aspects of synaptic plasticity. The most extensively studied forms of both LTP and LTD are triggered by the synaptic activation of the NMDA receptor (Collingridge *et al*., 1983; Dudek and Bear, 1992; Mulkey and Malenka, 1992). However, there are also NMDAR-independent forms of both LTP and LTD. For example, LTP at mossy fibre synapses that connect dentate granule cells to CA3 neurons involves activation of kainate receptors (Bortolotto *et al*., 1999a) rather than NMDARs (Harris and Cotman, 1986). In addition, some forms of LTD require the activation of mGluRs rather than NMDARs (Bortolotto *et al*., 1999b). In this context, it is important to note that there are mechanistically two distinct types of long-lasting synaptic depression. A long-lasting depression of baseline transmission, which is commonly referred to as LTD (or sometimes as *de novo* LTD) and a reversal of pre-established LTP, which is usually referred to as depotentiation (DP). Both forms of synaptic plasticity are similar in that they are long-lasting depressions of synaptic efficiency but they are different in the sense that they depend upon the pre-existing state of synaptic efficiency (baseline vs potentiated). With respect to both LTD and DP there are two forms, one which requires the activation of NMDARs (Fujii *et al*., 1991; Dudek and Bear, 1992) and another that requires the activation of mGluRs (Bashir *et al*., 1993; Bolshakov and Siegelbaum, 1994). Precisely what determines whether NMDAR and mGluR-dependent forms of long-lasting depression are induced is not fully understood.

All forms of synaptic plasticity are expressed as long-term alterations in the efficiency of synaptic transmission. The

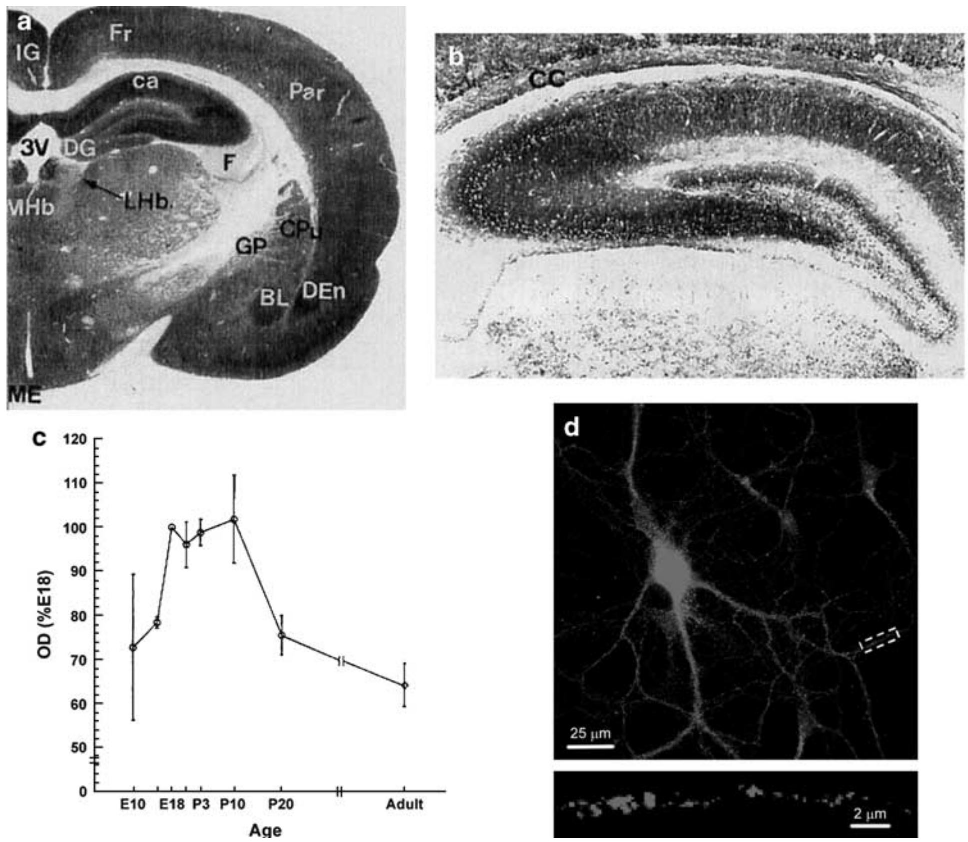

Figure 2 GSK-3β is widely distributed in CNS. (**a**) Coronal section of rat brain showing the widespread distribution of GSK-3β. (**b**) Distribution within the hippocampus. (**c**) Developmental regulation of GSK-3β expression showing a peak around the first 2 weeks of life. (**d**) Immunohistochemical analysis of the distribution of GSK-3β in cultured hippocampal neurons. Data (**a–c**) modified from Leroy and Brion (1999) and (**d**) Peineau *et al.* (2007). GSK-3β, glycogen synthase kinase-3β.

synaptic response evoked by low-frequency synaptic stimulation under standard experimental conditions is mediated primarily by the activation of AMPARs (Andreasen *et al*., 1989; Davies and Collingridge, 1989). Therefore, in most studies of synaptic plasticity, it is alterations in the efficiency of AMPAR-mediated synaptic transmission that is studied. However, long-term alterations in the efficiency of synaptic transmission mediated by other classes of glutamate receptor, in particular the NMDAR (Bashir *et al*., 1991), are also prevalent.

GSK-3β is highly expressed in the hippocampus

Glycogen synthase kinase-3β is widely expressed throughout the rat CNS (Leroy and Brion, 1999), with particularly high levels of expression in the hippocampus (Figures 2a and b). It is expressed throughout embryonic development and into adulthood, but with a developmental peak between birth and the second week of life (Figure 2c). In cultured hippocampal neurons, it is expressed throughout the cell, including dendritic spines (Figure 2d). In fractionation studies, GSK-3β is readily detected within the synaptosomal fraction (Hooper *et al*., 2007; Peineau *et al*., 2007).

GSK-3β is involved in LTD

The presence of GSK-3β within dendrites and dendritic spines suggests that it may have a role in synaptic function in addition to its role in other neuronal functions such as in the determination of neuronal polarity during development (Jiang *et al*., 2005; Yoshimura *et al*., 2005) and in gene regulation (Graef *et al*., 1999). A variety of inhibitors have been developed that inhibit GSK-3β (as well as GSK-3α). When applied to hippocampal slices obtained from 2-week-old rats, inhibition of GSK-3 had no apparent effect on AMPAR-mediated synaptic transmission, as studied at the monosynaptic connection between CA3 and CA1 pyramidal neurons. The activity of GSK-3 is, therefore, probably not required for low-frequency transmission at these synapses.

We have, however, recently obtained evidence for a role of GSK-3β in NMDAR-dependent LTD at CA3–CA1 synapses of 2-week-old rats (Peineau *et al*., 2007). We found that a variety of inhibitors of GSK-3 were able to prevent the induction of LTD when loaded into the recorded neuron using a patch pipette (Figure 3). The structurally unrelated inhibitors, SB415286, lithium and kenpaullone, prevented the induction of LTD over the appropriate concentration range at which they inhibit GSK-3. In contrast, an inhibitor of the closely related cyclin-dependent kinases (for example, CDK5), roscovitine, had no effect. The effect of GSK-3 inhibition was selective for LTD. In field potential recording experiments, we found that at a time when LTD was blocked, neither LTP nor DP was affected. These extracellular experiments required long periods of perfusion with SB415286 to be effective, presumably due to slow penetration of the

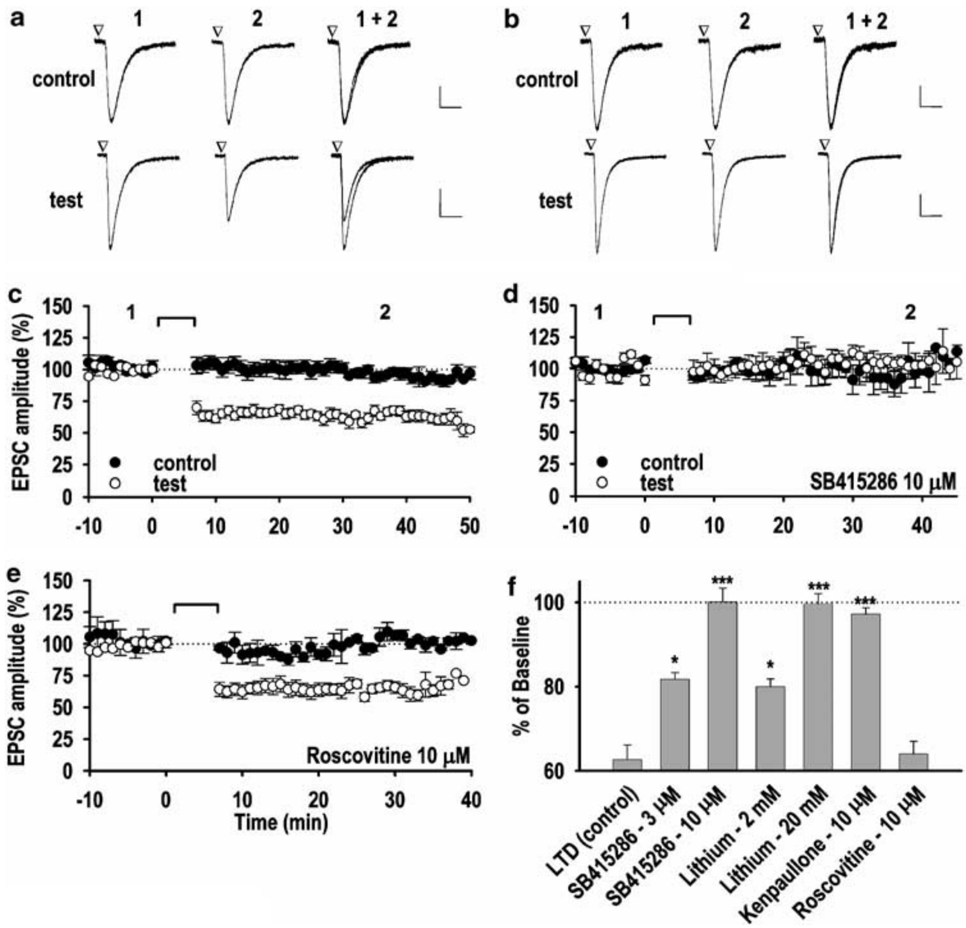

Figure 3 GSK-3 inhibitors block the induction of LTD. (**a**) Excitatory post-synaptic currents (EPSCs) obtained before and following the induction of LTD are illustrated for the control and test inputs of a control experiment. (**b**) Equivalent recordings from an experiment in which 10 μM SB415286 was added to the patch pipette solution. The calibration bars for the traces in panels a and b depict 40 pA and 50 ms. The numbers indicate the time of the recordings shown in (**c** and **d**). (**c**) Pooled data (mean ± s.e.) from control experiments illustrating input-specific LTD. (**d**) Effects of 10 μM SB415286. (**e**) Effects of 10 μM roscovitine. (**f**) Summary graphs illustrating the effects of various inhibitors on LTD quantified 20 min following induction. Modified from Peineau *et al*. (2007). GSK-3β, glycogen synthase kinase-3β; LTD, long-term depression. **P*<0.05, ****P*<0.01.

compounds within brain slices. Whether LTP or DP would have been affected with even longer incubation times is not known. However, it can be concluded that LTD is preferentially affected by inhibition of GSK-3. In these experiments, two different protocols were used to induce LTD (pairing depolarization to −40 mV with the delivery of 300 pulses at 0.75 Hz) and extracellular low-frequency stimulation (LFS; 900 stimuli delivered at 1 Hz). Whether the requirement for GSK-3 can be negated using different induction protocols is not known. Also, it is not clear whether GSK-3 activity is a general requirement for NMDAR-dependent LTD throughout CNS and at different stages during development. In addition, whether GSK-3 activity is involved in NMDAR-independent forms of LTD (such as those triggered by the activation of mGluRs) also remains to be determined.

A commonly described feature of GSK-3β is that it is constitutively active under resting conditions. Conceivably, this ‘basal activity’ might be sufficient to permit the induction of LTD. Alternatively, its activity may be regulated during the induction of LTD. To determine whether it is regulated during LTD, we measured the activity of GSK-3β in the CA1 dendritic region of hippocampal slices following the delivery of LFS. The LTD-induction protocol increased the activity of GSK-3β in the CA1 region of hippocampal slices, as assessed by determining the phosphorylation status of ser9 (Figure 4) and by performing a kinase activity assay. Collectively, these data support a model whereby GSK-3β is activated during LTD and is required for LTD to be induced. Whether the activity of GSK-3α is also regulated during LTD is not known.

It is established that the induction of LTD involves a protein phosphatase cascade; Ca^{2+} entering via NMDARs triggers the calcium/calmodulin-sensitive enzyme calcineurin (PP2B). This dephosphorylates inhibitor-1, which leads to activation of PP1 (Mulkey *et al*., 1993, 1994). PP1 is also a known activator of GSK-3β via dephosphorylation of ser9 (Morfini *et al*., 2004; Lee *et al*., 2005; Szatmari *et al*., 2005). Therefore, one way in which GSK-3β may be activated during LTD is via this protein phosphatase cascade. Consistent with this possibility, the PP1 inhibitor okadaic acid prevented the LTD-associated decrease in ser9 phosphorylation (Figure 4b). Okadaic acid also increased the basal phosphorylation of GSK-3β, which suggests that PP1 provides a tonic level of activation of GSK-3β under basal conditions, which could

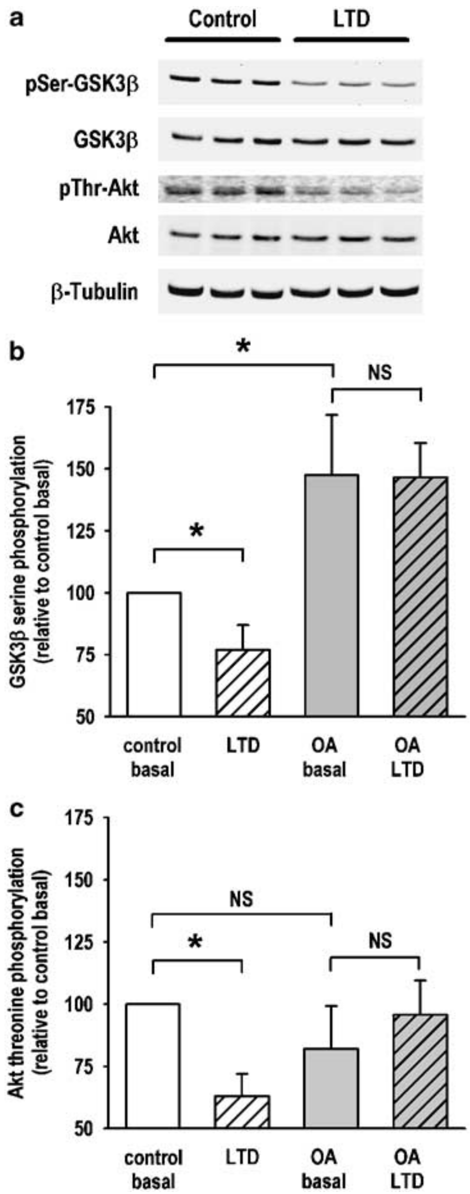

Figure 4 GSK-3β activity is regulated during LTD. (**a**) LTD is associated with an increase in GSK-3β activity (decrease in ser9 phosphorylation) and a decrease in Akt activity (decrease in thr308 phosphorylation). (**b**) Quantification of these experiments. Note that LTD is associated with activation of GSK-3β and that inhibition of PP1 by okadaic acid prevents this effect and also inhibits basal GSK-3β activity. (**c**) Equivalent data for Akt experiments. Modified from Peineau *et al*. (2007). GSK-3β, glycogen synthase kinase-3β; LTD, long-term depression; PP1, protein phosphatase 1. *$P<0.05$.

account for its known 'constitutive activity'. In addition to direct dephosphorylation of GSK-3β, PP1 could also activate GSK-3β by inhibiting kinases that phosphorylate this residue. A major pathway for inhibition of GSK-3β is the PI3K/Akt pathway. During LTD, there is dephosphorylation of Akt, which corresponds to its inhibition. This dephosphorylation is also inhibited by okadaic acid, without alterations in basal activity (Figure 4c). Therefore, during LTD, the activation of PP1 could lead to activation of GSK-3β both by direct dephosphorylation of ser9 and by inhibition of Akt (see Figure 8). Whether during the induction of LTD, these are the only targets of PP1 or whether PP1 dephosphorylates other substrates required for the process is not known.

LTP regulates the activity of GSK-3β

Two independent studies have shown that following the induction of LTP there is inhibition of GSK-3β (Hooper *et al*., 2007; Peineau *et al*., 2007). This has been demonstrated following the induction of LTP *in vivo* in both dentate gyrus and area CA1 in hippocampal slices (Figure 5a). The inhibition of activity, assessed as an increase in phosphorylation of ser9, was prominent 10–20 min after the induction of LTP and lasted for at least an hour. This link between LTP and GSK-3β raises two questions. First, what influence GSK-3β has on LTP and second, what role the LTP-induced regulation of GSK-3β activity plays. With respect to the first issue, it was shown that in a transgenic animal that overexpressed GSK-3β, there was a pronounced inhibition of LTP (Figure 5b), which could account for the learning deficits observed in these mice (Hernandez *et al*., 2002). This deficit was restored by treatment with lithium, suggesting that it was the overexpression of GSK-3β that was responsible for the effect rather than some developmental alteration (Hooper *et al*., 2007). Could GSK-3β, given that it is 'constitutively active', be providing a tonic inhibition of LTP? In which case, GSK-3β inhibitors would be expected to enhance LTP. Quantitative comparisons of the effects of a range of GSK-3β inhibitors on LTP will be required to address this issue.

A role for GSK-3β in metaplasticity

Metaplasticity is the plasticity of synaptic plasticity (Abraham and Bear, 1996). An example is the situation where the generation of one form of synaptic plasticity modifies the ability of the synapses to undergo another form of synaptic plasticity. Metaplasticity can take on many configurations, but little is known about the underlying mechanisms.

Given that LTP inhibits GSK-3β and that the activation of GSK-3β is required for LTD, these observations suggest that LTP might inhibit LTD, via the regulation of the activity of this enzyme. However, despite intense investigation of LTP and LTD for many years, a direct inhibition of LTD by LTP had not been reported. Indeed, the contrary is often observed that the induction of LTP facilitates the generation of long-lasting synaptic depression, by enabling the production of DP. We reasoned that the coexistence of DP might be masking the interaction between LTP and LTD. We therefore devised two ways of studying the interaction of LTP and LTD in the absence of DP (Figure 6) (Peineau *et al*., 2007). In the first set of experiments, we utilized the well-established phenomenon of 'washout'. This is a phenomenon whereby soluble factors required for LTP are lost during dialysis with whole-cell solution; LTD is unaffected by this process. We made whole-cell recordings and delivered a pairing protocol that would be sufficient to induce LTP had it been delivered before washout. Due to the washout of soluble factors required for LTP, no potentiation was observed (and hence no DP could be induced). However, the pairing protocol was

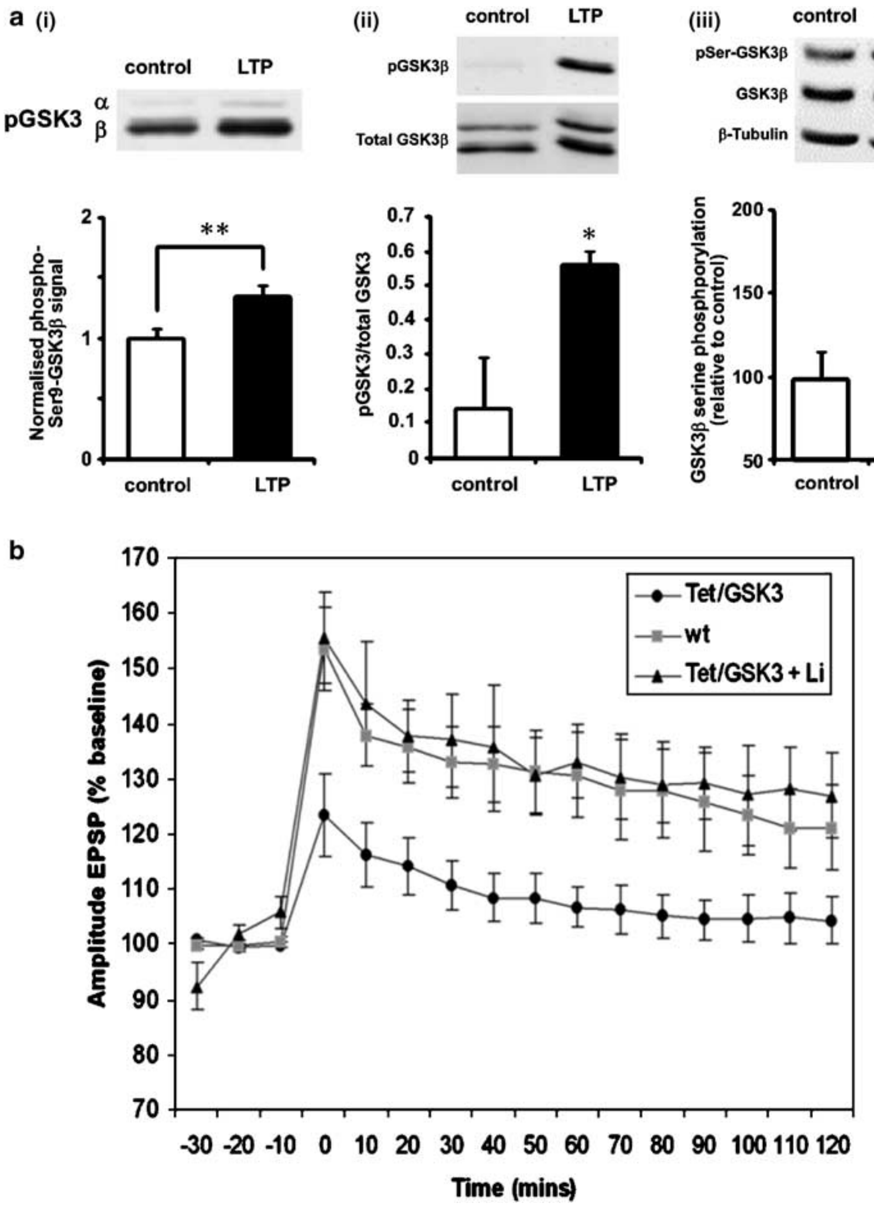

Figure 5 GSK-3β is regulated during LTP. (**a**) LTP is associated with a decrease in GSK-3β activity (increase in ser9 phosphorylation). Experiments were performed in (i) dentate gyrus *in vivo* (ii) CA1 *in vivo* and (iii) CA1 *in vitro*. (**b**) Overexpression of GSK-3β inhibits the induction of LTP. The LTP deficit is normalized by treatment with lithium. Panels a (i and ii) and b are from Hooper *et al*. (2007), and panel a (iii) is from Peineau *et al*., (2007). GSK-3β, glycogen synthase kinase-3β; LTD, long-term depression. *$P<0.05$, **$P<0.01$.

able to completely prevent the induction of LTD (Figure 6a). This inhibitory effect lasted for approximately 1 h and required the synaptic activation of NMDARs. In the second set of experiments, we made field potential recordings and induced LTP using a tetanus. These experiments were performed in the presence of the broad-spectrum mGluR antagonist, LY341495, which we have shown previously blocks the induction of DP (Fitzjohn *et al*., 1998). When we delivered a standard protocol for inducing NMDAR-dependent LTD, we observed no synaptic depression shortly after the induction of LTP (Figure 6b), but a full reversal of LTP was observed if the stimuli were delivered 1 h after the induction of LTP. This synaptic depression was fully dependent on the synaptic activation of NMDARs (this synaptic depression could be considered a form of NMDAR-dependent DP or NMDAR-dependent LTD superimposed upon LTP; either way it is mechanistically distinct from the mGluR-dependent form of DP, which is readily induced immediately following the induction of LTP).

So how could LTP inhibit LTD? As mentioned earlier, a major regulator of GSK-3β is via the PI3K-Akt pathway. It is known that during the induction of LTP there is activation of PI3K (Man *et al*., 2003). We reasoned, therefore, that LTP could inhibit LTD via this pathway. To test this hypothesis directly we examined the ability of PI3K inhibitors to block the inhibition of LTD by the LTP stimulus. In both protocols, the PI3K inhibitor LY294002 completely prevented the inhibition of LTD by the LTP stimulus (Figures 6c and d). Using the whole-cell protocol we additionally confirmed the effects using a second PI3K inhibitor, wortmannin, and also

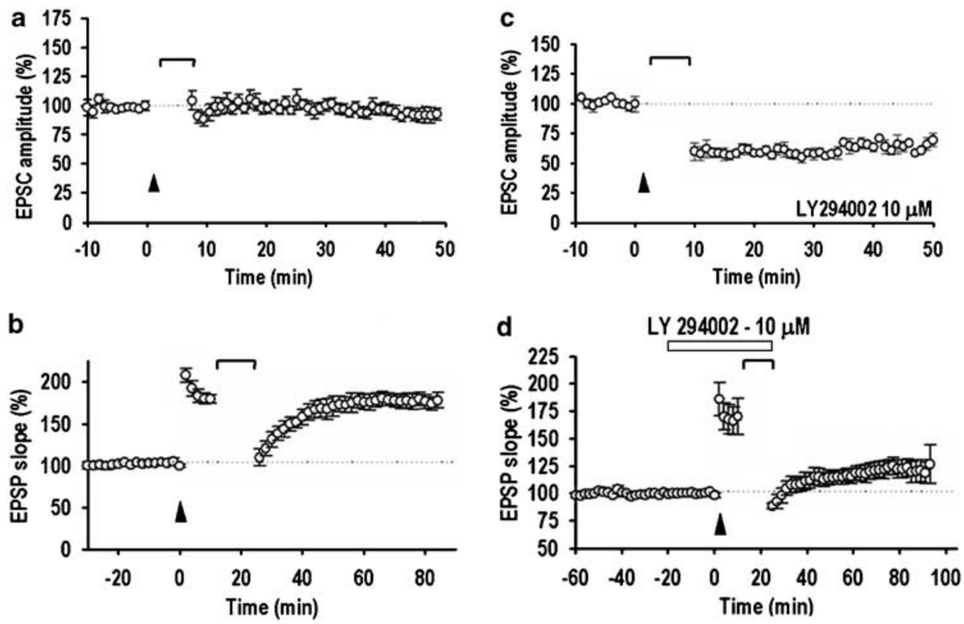

Figure 6 A role for GSK-3 in metaplasticity. (**a**) Whole-cell recording experiments showing that a conditioning stimulus (60 pulses, 0.5 Hz, 0 mV; arrowhead) completely blocks the induction of LTD. (**b**) Field potential recording experiments showing that the induction of LTP (arrowhead) blocks the induction of LTD. (**c** and **d**) These effects are prevented by treatment with LY294002 (10 μM). In the experiments illustrated in (**c** and **d**), the mGluR antagonist LY341495 was present to block DP. Note that by using a strong LTP induction protocol (4 bursts of 100 pulses at 100 Hz, delivered at 30 s intervals), LY294002 did not affect the induction of LTP. Adapted from Peineau *et al.* (2007). DP, depotentiation; GSK-3β, glycogen synthase kinase-3β; LTD, long-term depression; LTP, long-term potentiation; mGluR, metabotropic glutamate receptor.

demonstrated a role for Akt, using a variety of strategies. Thus, GSK-3β plays a central role in a form of metaplasticity where it is regulated via the PI3K-Akt pathway.

Molecular mechanisms

A key issue for the future is to determine how GSK-3β regulates the induction of LTD. Recently it has been shown that inhibition of GSK-3 activity results in a rapid internalization of NMDARs (Chen *et al.*, 2007). Thus, LTP might inhibit LTD by regulating the levels of the receptor that triggers the induction process. The prediction would be that LTP leads to a rapid internalization of NMDARs followed by a recovery in the synaptic population of NMDARs over the time course of an hour or so. Most studies of LTP that have monitored NMDAR-mediated synaptic transmission have reported LTP rather than a transient depression. Some studies have reported no change in synaptic transmission, perhaps reflecting a balance between these two opposing effects. Clearly, future work is needed to establish the extent to which the regulation of NMDARs by GSK-3β accounts for its involvement in synaptic plasticity.

The inhibition of NMDARs by GSK-3 antagonists is unlikely to account for their ability to inhibit LTD for several reasons. First, the effects observed on NMDARs were relatively small (typically around 20% inhibition). Second, whilst we observed a complete block of LTD there was sufficient NMDAR activation for LTP to be induced (Peineau *et al.*, 2007). Third, lithium was able to fully block LTD even when applied after the induction of LTD (Peineau *et al.*, 2007). Thus, whilst inhibition of NMDAR function may contribute to the effects it cannot be the sole mechanism.

We have observed that GSK-3β forms part of a complex with AMPARs (Figure 7a) and that the activity of this AMPAR-associated GSK-3β is regulated by LTP (Figure 7b) (Peineau *et al.*, 2007). This suggests that GSK-3β may be directly involved in the LTD process *per se*. For example, its activation may be required for the internalization of AMPARs during the LTD process, as shown schematically in Figure 8. At the present time, the downstream effectors of GSK-3β that are involved in the LTD process are unknown. There are, however, a number of interesting candidates, including tau, presenilin-1 and β-catenin (Figure 1) that may be involved in the late phase of LTD, where protein synthesis may be required (Manahan-Vaughan *et al.*, 2000).

Implications for the development of new treatments for neurological diseases

It has long been thought that alterations in synaptic transmission and plasticity are involved in the development and expression of various neurological disorders. It is now becoming clear that the hippocampus plays important roles in neuropsychiatric disorders such as bipolar disorder (Frey *et al.*, 2007). For example, recent work has shown altered glutamate receptor expression in the hippocampus and surrounding cortices in post-mortem brains from patients suffering from this condition (Beneyto *et al.*, 2007), whereas chronic exposure to lithium has been shown to decrease the surface expression of GluR1 and GluR2 AMPA receptor subunits in hippocampal cultures (Du *et al.*, 2004, 2007). Similar results have been demonstrated for the use of valporate, an antimanic drug used in the treatment of

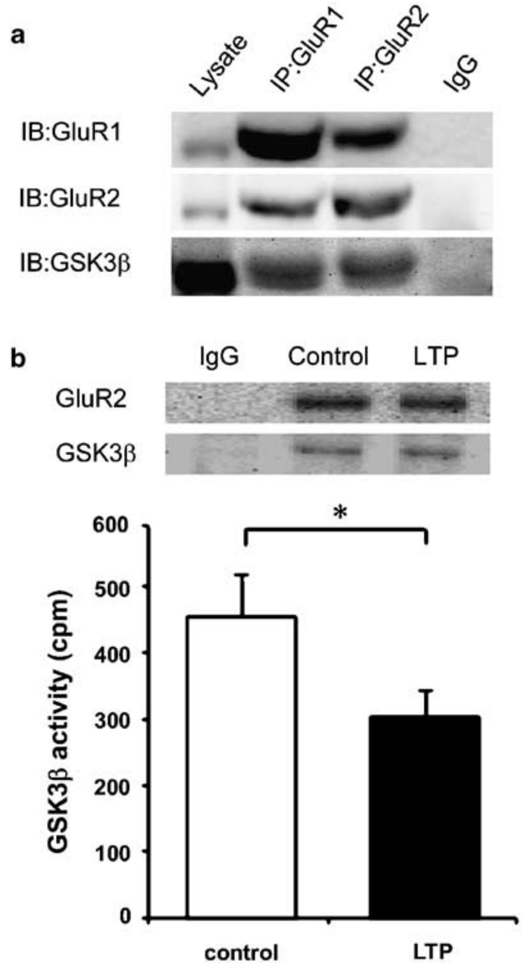

Figure 7 GSK-3β is associated with AMPARs. (**a**) Immunoprecipitation of either GluR1 or GluR2 coimmunoprecipitates GSK-3β. (**b**) A chemical LTP protocol that causes the insertion of AMPARs results in a decrease in AMPAR-associated GSK-3β activity. Top: representative western blot showing equal immunoprecipitation of GluR2 and coimmunoprecipitated GSK-3β in unstimulated controls and LTP-induced lysates used in the subsequent kinase reactions. Bottom: quantification of GSK-3β kinase activity after LTP induction and AMPA receptor immunoprecipitation. Adapted from Peineau *et al.* (2007). AMPARs, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors; GSK-3β, glycogen synthase kinase-3β; LTP, long-term potentiation; GluR, glutamate receptor. $P<0.05$.

bipolar disorder that also blocks GSK-3 signalling (Du *et al.*, 2004, 2007).

Given the role of GSK-3 in tau hyperphosphorylation and its emerging role in other CNS disorders, there is currently great interest in developing therapeutically useful GSK-3 antagonists for disease intervention. Indeed, the recent battery of small molecule GSK-3 inhibitors, as well as the more established lithium, is showing positive results for the possible therapeutic benefits of blocking GSK-3 activity in such diseases as Alzheimer's (SB216763, CHIR98014, Alsterpaullone; Selenica *et al.*, 2007), amyotrophic lateral sclerosis (GSK inhibitor VIII; Koh *et al.*, 2007), hippocampal epileptic neurodegeneration (lithium; Busceti *et al.*, 2007) and polyglutamine disorders such as Huntington's disease (lithium; Wood and Morton, 2003) and spinocerebellar ataxia type 1 (lithium; Watase *et al.*, 2007).

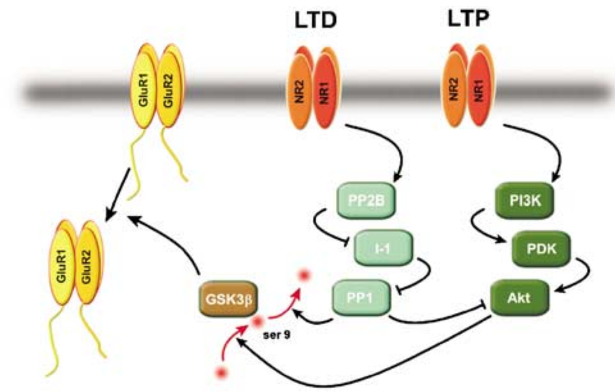

Figure 8 A schematic to illustrate how GSK-3β may be involved in the induction of LTD and how LTP may inhibit LTD via the inhibition of this enzyme. GSK-3β, glycogen synthase kinase-3β; LTD, long-term depression; LTP, long-term potentiation.

We have shown that blockade of GSK-3 has acute effects on plastic processes thought to underlie learning and memory mechanisms, specifically, that GSK-3 is required for LTD and provides a mechanism by which LTP can inhibit LTD. However, whether or not these functions or dysregulation of these functions are important early or late features in the development of some, or all, of these diseases remains to be determined.

Acknowledgements

This study was supported by the MRC, Wellcome Trust and CIHR. SP was supported by a fellowship from FRM. CT was supported by fellowships from CIHR and MSFHR. GLC is a Royal Society-Wolfson Merit Award Holder. YTW is an HHMI international scholar.

Conflict of interest

The authors state no conflicts of interest.

References

Abraham WC, Bear MF (1996). Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci* **19**: 126–130.
Ali A, Hoeflich KP, Woodgett JR (2001). Glycogen synthase kinase-3: properties, functions, and regulation. *Chem Rev* **101**: 2527–2540.
Alvarez G, Munoz-Montano JR, Satrustegui J, Avila J, Bogonez E, Diaz-Nido J (2002). Regulation of tau phosphorylation and protection against beta-amyloid-induced neurodegeneration by lithium. Possible implications for Alzheimer's disease. *Bipolar Disord* **4**: 153–165.
Anderton BH (1999). Alzheimer's disease: clues from flies and worms. *Curr Biol* **9**: R106–R109.
Andreasen M, Lambert JD, Jensen MS (1989). Effects of new non-*N*-methyl-D-aspartate antagonists on synaptic transmission in the *in vitro* rat hippocampus. *J Physiol* **414**: 317–336.
Avila J, Lucas JJ, Perez M, Hernandez F (2004). Role of tau protein in both physiological and pathological conditions. *Physiol Rev* **84**: 361–384.
Bashir ZI, Alford S, Davies SN, Randall AD, Collingridge GL (1991). Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. *Nature* **349**: 156.

Bashir ZI, Jane DE, Sunter DC, Watkins JC, Collingridge GL (1993). Metabotropic glutamate receptors contribute to the induction of long-term depression in the CA1 region of the hippocampus. *Eur J Pharmacol* **239**: 265.

Bear MF, Abraham WC (1996). Long-term depression in hippocampus. *Annu Rev Neurosci* **19**: 437–462.

Beasley C, Cotter D, Khan N, Pollard C, Sheppard P, Varndell I *et al.* (2001). Glycogen synthase kinase-3beta immunoreactivity is reduced in the prefrontal cortex in schizophrenia. *Neurosci Lett* **302**: 117–120.

Beneyto M, Kristiansen LV, Oni-Orinsan A, McCullumsmith RE, Meador-Woodriff JH (2007). Abnormal glutamate receptor expression in the medial Temporal Lobe in schizophrenia and mood disorders. *Neuropsychopharmacology* **32**: 1888–1902.

Bhat RV, Budd Haeberlein SL, Avila J (2004). Glycogen synthase kinase 3: a drug target for CNS therapies. *J Neurochem* **89**: 1313–1317.

Bhat RV, Shanley J, Correll MP, Fieles WE, Keith RA, Scott CW *et al.* (2000). Regulation and localization of tyrosine216 phosphorylation of glycogen synthase kinase-3beta in cellular and animal models of neuronal degeneration. *Proc Natl Acad Sci USA* **97**: 11074–11079.

Bliss TV, Collingridge GL (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**: 31–39.

Bliss TVP, Collingridge GL, Morris RGM (2007). Synaptic plasticity in the hippocampus. In: Andersen P, Morris RGM, Amaral DG, Bliss TVP, O'Keefe J (eds). *The Hippocampus Book*, pp 343–474. Oxford University Press: USA.

Bolshakov VY, Siegelbaum SA (1994). Postsynaptic induction and presynaptic expression of hippocampal long-term depression. *Science* **264**: 1148–1152.

Bortolotto ZA, Clarke VR, Delany CM, Parry MC, Smolders I, Vignes M *et al.* (1999a). Kainate receptors are involved in synaptic plasticity. *Nature* **402**: 297.

Bortolotto ZA, Fitzjohn SM, Collingridge GL (1999b). Roles of metabotropic glutamate receptors in LTP and LTD in the hippocampus. *Curr Opin Neurobiol* **9**: 299.

Busceti CL, Biagioni F, Aronica E, Riozzi B, Storto M, Battaglia G *et al.* (2007). Induction of the Wnt inhibitor, Dickkopf-1, is associated with neurodegeneration related to temporal lobe epilepsy. *Epilepsia* **48**: 694–705.

Chen P, Gu Z, Liu W, Yan Z (2007). Glycogen synthase kinase 3 regulates *N*-methyl-D-aspartate receptor channel trafficking and function in cortical neurons. *Mol Pharmacol* **72**: 40–51.

Collingridge GL, Kehl SJ, McLennan H (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J Physiol* **334**: 33–46.

Davies SN, Collingridge GL (1989). Role of excitatory amino acid receptors in synaptic transmission in area CA1 of rat hippocampus. *Proc R Soc Lond B Biol Sci* **236**: 373.

Doble BW, Woodgett JR (2003). GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci* **116**: 1175–1186.

Du J, Gray NA, Falke CA, Chen W, Yuan P, Szabo ST *et al.* (2004). Modulation of synaptic plasticity by antimanic agents: the role of AMPA glutamate receptor subunit 1 synaptic expression. *J Neurosci* **24**: 6578–6589.

Du J, Suzuki K, Wei Y, Wang Y, Blumenthal R, Chen Z *et al.* (2007). The anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: relationship to clinical effects in mood disorders. *Neuropsychopharmacology* **32**: 793–802.

Dudek SM, Bear MF (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. *Proc Natl Acad Sci USA* **89**: 4363.

Eldar-Finkelman H (2002). Glycogen synthase kinase 3: an emerging therapeutic target. *Trends Mol Med* **8**: 126–132.

Embi N, Rylatt DB, Cohen P (1980). Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem* **107**: 519–527.

Fang X, Yu SX, Lu Y, Bast Jr RC, Woodgett JR, Mills GB (2000). Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. *Proc Natl Acad Sci USA* **97**: 11960–11965.

Fitzjohn SM, Bortolotto ZA, Palmer MJ, Doherty AJ, Ornstein PL, Schoepp DD *et al.* (1998). The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. *Neuropharmacology* **37**: 1445.

Frame S, Cohen P (2001). GSK3 takes centre stage more than 20 years after its discovery. *Biochem J* **359**: 1–16.

Frey BN, Andreazza AC, Nery FG, Martins MR, Quevedo J, Soares JC *et al.* (2007). The role of hippocampus in the pathophysiology of bipolar disorder. *Behav Pharmacol* **18**: 419–430.

Fujii S, Saito K, Miyakawa H, Ito K, Kato H (1991). Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig hippocampal slices. *Brain Res* **555**: 112–122.

Goode N, Hughes K, Woodgett JR, Parker PJ (1992). Differential regulation of glycogen synthase kinase-3 beta by protein kinase C isotypes. *J Biol Chem* **267**: 16878–16882.

Gould TD, Manji HK (2005). Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. *Neuropsychopharmacology* **30**: 1223–1237.

Graef IA, Mermelstein PG, Stankunas K, Neilson JR, Deisseroth K, Tsien RW *et al.* (1999). L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons. *Nature* **401**: 703–708.

Grimes CA, Jope RS (2001). The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol* **65**: 391–426.

Hanger DP, Hughes K, Woodgett JR, Brion JP, Anderton BH (1992). Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. *Neurosci Lett* **147**: 58–62.

Harris EW, Cotman CW (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by *N*-methyl D-aspartate antagonists. *Neurosci Lett* **70**: 132–137.

Harwood AJ (2005). Lithium and bipolar mood disorder: the inositol-depletion hypothesis revisited. *Mol Psychiatry* **10**: 117–126.

Hernandez F, Borrell J, Guaza C, Avila J, Lucas JJ (2002). Spatial learning deficit in transgenic mice that conditionally over-express GSK-3beta in the brain but do not form tau filaments. *J Neurochem* **83**: 1529–1533.

Hooper C, Markevich V, Plattner F, Killick R, Schofield E, Engel T *et al.* (2007). Glycogen synthase kinase-3 inhibition is integral to long-term potentiation. *Eur J Neurosci* **25**: 81–86.

Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgett JR (1993). Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *EMBO J* **12**: 803–808.

Jiang H, Guo W, Liang X, Rao Y (2005). Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. *Cell* **120**: 123–135.

Kirschenbaum F, Hsu SC, Cordell B, McCarthy JV (2001). Glycogen synthase kinase-3beta regulates presenilin 1 C-terminal fragment levels. *J Biol Chem* **276**: 30701–30707.

Klein PS, Melton DA (1996). A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci USA* **93**: 8455–8459.

Koh SH, Kim Y, Kim HY, Hwang S, Lee CH, Kim SH (2007). Inhibition of glycogen synthase kinase-3 suppresses the onset of symptoms and disease progression of G93A-SOD1 mouse model of ALS. *Exp Neurol* **205**: 336–346.

Kozlovsky N, Belmaker RH, Agam G (2002). GSK-3 and the neurodevelopmental hypothesis of schizophrenia. *Eur Neuropsychopharmacol* **12**: 13–25.

Lee YI, Seo M, Kim Y, Kim SY, Kang UG, Kim YS *et al.* (2005). Membrane depolarization induces the undulating phosphorylation/dephosphorylation of glycogen synthase kinase 3beta, and this dephosphorylation involves protein phosphatases 2A and 2B in SH-SY5Y human neuroblastoma cells. *J Biol Chem* **280**: 22044–22052.

Leroy K, Brion JP (1999). Developmental expression and localization of glycogen synthase kinase-3beta in rat brain. *J Chem Neuroanat* **16**: 279–293.

Man HY, Wang Q, Lu WY, Ju W, Ahmadian G, Liu L *et al.* (2003). Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. *Neuron* **38**: 611–624.

Manahan-Vaughan D, Kulla A, Frey JU (2000). Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. *J Neurosci* **20**: 8572–8576.

Morfini G, Szebenyi G, Brown H, Pant HC, Pigino G, DeBoer S *et al.* (2004). A novel CDK5-dependent pathway for regulating GSK3 activity and kinesin-driven motility in neurons. *EMBO J* **23**: 2235–2245.

Mulkey RM, Endo S, Shenolikar S, Malenka RC (1994). Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* **369**: 486–488.

Mulkey RM, Herron CE, Malenka RC (1993). An essential role for protein phosphatases in hippocampal long-term depression. *Science* **261**: 1051–1055.

Mulkey RM, Malenka RC (1992). Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* **9**: 967–975.

Peineau S, Taghibiglou C, Bradley C, Wong TP, Liu L, Lu J *et al.* (2007). LTP inhibits LTD in the hippocampus via regulation of GSK3beta. *Neuron* **53**: 703–717.

Plattner F, Angelo M, Giese KP (2006). The roles of cyclin-dependent kinase 5 and glycogen synthase kinase 3 in tau hyperphosphorylation. *J Biol Chem* **281**: 25457–25465.

Ryves WJ, Harwood AJ (2001). Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. *Biochem Biophys Res Commun* **280**: 720–725.

Selenica ML, Jensen HS, Larsen AK, Pedersen ML, Helboe L, Leist M *et al.* (2007). Efficacy of small-molecule glycogen synthase kinase-3 inhibitors in the post-natal rat model of tau hypersphosphorylation. *Br J Pharmacol* **153**: 959–979.

Szatmari E, Habas A, Yang P, Zheng JJ, Hagg T, Hetman M (2005). A positive feedback loop between glycogen synthase kinase 3beta and protein phosphatase 1 after stimulation of NR2B NMDA receptors in forebrain neurons. *J Biol Chem* **280**: 37526–37535.

Takahashi M, Tomizawa K, Kato R, Sato K, Uchida T, Fujita SC *et al.* (1994). Localization and developmental changes of tau protein kinase I/glycogen synthase kinase-3 beta in rat brain. *J Neurochem* **63**: 245–255.

Watase K, Gatchel JR, Sun Y, Emamian E, Atkinson R, Richman R *et al.* (2007). Lithium therapy improves neurological function and hippocampal dendritic arborization in a spinocerebellar ataxia type 1 mouse model. *PLoS Med* **4**: e182.

Wood NI, Morton AJ (2003). Chronic lithium chloride treatment has variable effects on motor behaviour and survival of mice transgenic for the Huntington's disease mutation. *Brain Res Bull* **61**: 375–383.

Woodgett JR (1990). Molecular cloning and expression of glycogen synthase kinase-3/factor A. *EMBO J* **9**: 2431–2438.

Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K (2005). GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell* **120**: 137–149.