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Summary

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 summarises this recent work and discusses its potential relevance to the treatment of neurological disorders.

Key words: 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; CK1, casein kinase 1; CREB, cAMP responsive element binding protein DP, depotentiation; GSK-3, glycogen synthase kinase-3; I-1, inhibitor 1; LTD, long term depression; LTP, long term potentiation; MAP1B: microtubule-associated protein 1B; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NMDAR, N-methyl-D-aspartate receptors; PDK, phosphoinositide-dependent protein kinase; PI3K, phosphatidylinositol 3-kinase, PKA, protein kinase A; PKC, protein kinase C; PP1, protein phosphatase 1; PP2A, protein phosphatese 2A; PP2B, protein phosphatase 2B; PS-1, presenilin 1; RSK, p90 ribosomal S6 kinase; S6K, p70 ribosomal S6 kinase-1.

Introduction

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 *et al.*, 2001); (Grimes *et al.*, 2001). GSK-3 dysfunction is implicated in major diseases including cancer and diabetes (Frame *et al.*, 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 PP1 and PP2A, results in the dis-inhibition of its activity (Fig. 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

(Doble *et al.*, 2003; Frame *et al.*, 2001). In addition to Akt, PKA has been shown to phosphorylate both α and β subtypes of GSK-3 (Fang *et al.*, 2000) while PKC has been shown to phosphorylate GSK-3β (Fang *et al.*, 2000; Goode *et al.*, 1992). Other regulators of GSK-3β include the mammalian target of rapamycin (mTOR) pathway and mitogen-activated protein kinase (MAPK) cascades (Frame *et al.*, 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 (Doble *et al.*, 2003; Frame *et al.*, 2001). Some of the substrates that are relevant to neuronal function are shown schematically in Figure 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 (Leroy *et al.*, 1999; Woodgett, 1990); (Takahashi *et al.*, 1994). (Fig 2) where it has been implicated in various disorders including Alzheimer's disease (Alvarez *et al.*, 2002; Anderton, 1999; Bhat *et al.*, 2004; Eldar-Finkelman, 2002; Grimes *et al.*, 2001), schizophrenia (Beasley *et al.*, 2001; Eldar-Finkelman, 2002; Kozlovsky *et al.*, 2002) and bipolar disorders (Eldar-Finkelman, 2002; Grimes *et al.*, 2001; Klein *et al.*, 1996). 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 (Avila *et al.*, 2004; Hanger *et al.*, 1992; Kirschenbaum *et al.*, 2001). 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 *et al.*, 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 *et al.*, 2001). This may account for its ability to act as a mood stabilising drug (Klein *et al.*, 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 the 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, LTP and LTD, have been extensively investigated in the pursuit of understanding the molecular and cellular basis of learning and memory (Bliss *et al*., 1993; Bear *et al.*, 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 utilised 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 receptor: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs), kainate 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 *et al.*, 1992; Mulkey *et al.*, 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 *et al.*, 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 (Dudek *et al.*, 1992; Fujii *et al.*, 1991) and another that requires the activation of mGluRs (Bashir *et al.*, 1993; Bolshakov *et al.*, 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 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 *et al.*, 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

GSK-3β is widely expressed throughout the rat CNS (Leroy *et al.*, 1999) with particularly high levels of expression in the hippocampus (Fig. 2A, B). It is expressed throughout embryonic development and into adulthood, but with a developmental peak between birth and the second week of life (Fig. 2C). In cultured hippocampal neurons it is expressed throughout the cell, including within dendritic spines (Fig. 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 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 two 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 two-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 (Fig. 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 (e.g., 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 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 depolarisation 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 the 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 (Fig. 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 (I-1), which leads to activation of PP1 (Mulkey *et al.*, 1994; Mulkey *et al.*, 1993). PP1 is also a known activator of GSK-3β via dephosphorylation of ser9 (Lee *et al.*, 2005; Morfini *et al.*, 2004; 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 (Fig. 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 account for its known "constitutive activity". As well as 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 via 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 (Fig. 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 Fig. 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 (Fig. 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 does GSK-3β have on LTP and second, what role does the LTP-induced regulation of GSK-3β activity play. With respect to the first issue, it was shown that in a transgenic animal that over-expressed GSK-3β there was a pronounced inhibition of LTP (Fig 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 over-expression 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 *et al.*, 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 co-existence 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 (Fig. 6) (Peineau *et al.*, 2007). In the first set of experiments, we utilised 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 which 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 able to completely prevent the induction of LTD (Fig. 6A). This inhibitory effect lasted for approximately one hour 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 (Fig. 6B), but a full reversal of LTP was observed if the stimuli were delivered one hour 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 that 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 (Fig. 6C, D). Using the whole-cell protocol we additionally confirmed the effects using a second PI3K inhibitor, wortmannin, and also 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 internalisation 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 internalisation 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 (Fig. 7A) and that the activity of this AMPAR-associated GSK-3β is regulated by LTP (Fig. 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

internalisation of AMPARs during the LTD process, as shown schematically in Fig. 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 (Fig. 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), while 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; Du *et al.*, 2007). Similar results have been demonstrated for the use of valporate, an antimanic drug used in the treatment of bipolar disorder that also blocks GSK-3 signalling (Du *et al.*, 2004; Du *et al.*, 2007).

Given the role of GSK-3 in tau hyperphosphorylation and it's 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, are 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 *et al.*, 2003) and spinocerebellar ataxia type 1 (lithium; Watase *et al.*, 2007).

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 dys-regulation of these functions, are important early or late features in the development of some, or all, of these diseases remains to be determined.

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Statement of conflict of interests

There are no conflicts of interest.

Figure Legends

Figure 1. Example 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 are shown. Abbreviations: PI3K, phosphatidylinositol 3-kinase; PDK, phosphoinositide-dependent protein kinase; PKA, protein Kinase A; PKC, protein kinase C, mTOR, mammalian target of rapamycin; S6K, p70 ribosomal S6 kinase-1; MAPK, mitogen-activated protein kinase; RSK, p90 ribosomal S6 kinase; CK1, casein kinase 1; PP2A, protein phosphatese 2A; PP2B, protein phosphatase 2B; I-1, inhibitor 1; PP1, protein phosphatase 1; MAP1B: microtubule-associated protein 1B; PS-1, presenilin 1; CREB, cAMP responsive element binding protein.

Figure 2. GSK-3β is widely distributed in the 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 two weeks of life. (D) Immunohistochemical analysis of the distribution of GSK-3β in cultured hippocampal neurons. Data (A-C) modified from (Leroy *et al.*, 1999) and (D) (Peineau *et al.*, 2007).

Figure 3. GSK-3 inhibitors block the induction of LTD. (A) 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 A & B depict 40 pA and 50 ms. The numbers indicate the time of the recordings shown in C & D. (C) Pooled data (mean ± SE) 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).

Figure 4. GSK-3β activity is regulated during LTD. (A) LTD is associated with an increase in GSK-3β activity (decrease in ser 9 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).

Figure 5. GSK-3β is regulated during LTP. (A) LTP is associated with a decrease in GSK-3β activity (increase in ser 9 phosphorylation). Experiments were performed in (i) dentate gyrus *in vivo* (ii) CA1 *in vivo* and (iii) CA1 *in vitro*. (B) Over-expression of GSK-3β inhibits the induction of LTP. The LTP deficit is normalised by treatment with lithium. A (i and ii) and B are from (Hooper *et al.*, 2007), and A (iii) is from (Peineau *et al.*, 2007).

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 & D) These effects are prevented by treatment with LY294002 (10 μM). In the experiments illustrated in C & 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).

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 co-immunoprecipitated 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).

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.

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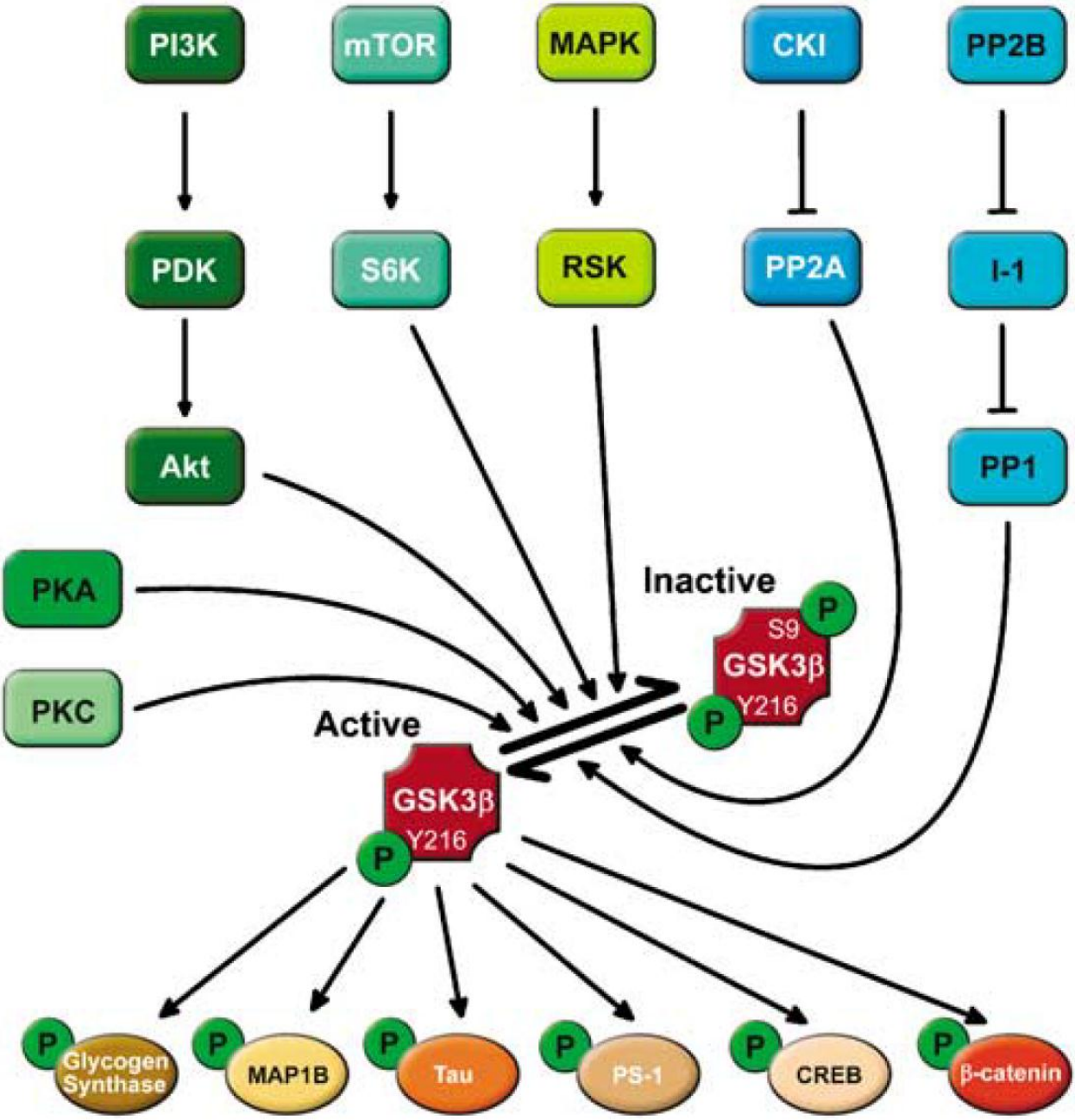
PI3K
mTOR
MAPK
CKI
PP2B
PDK
S6K
RSK
PP2A
I-1
Akt
PP1
PKA
PKC
Inactive
S9
GSK3β
Y216
P
Active
GSK3β
Y216
P
Glycogen Synthase
MAP1B
Tau
PS-1
CREB
β-catenin

Figure 1

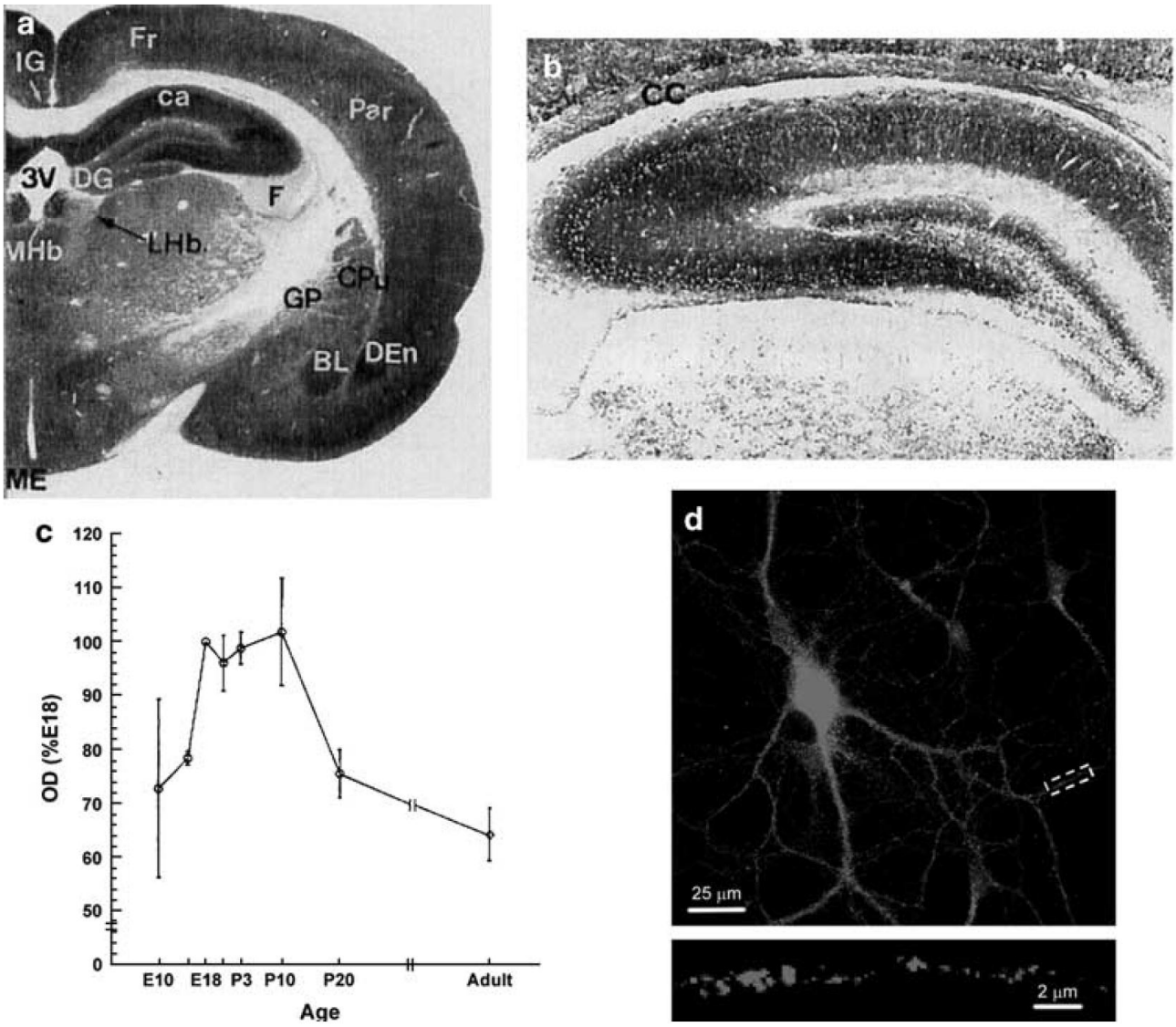
a
Fr
IG
ca
Par
3V
DG
F
MHb
LHb
GP
CPu
BL
DEn
ME
b
CC
c
OD (%E18)
120
110
100
90
80
70
60
50
0
E10
E18
P3
P10
P20
Adult
Age
d
25 µm
2 µm

Figure 2

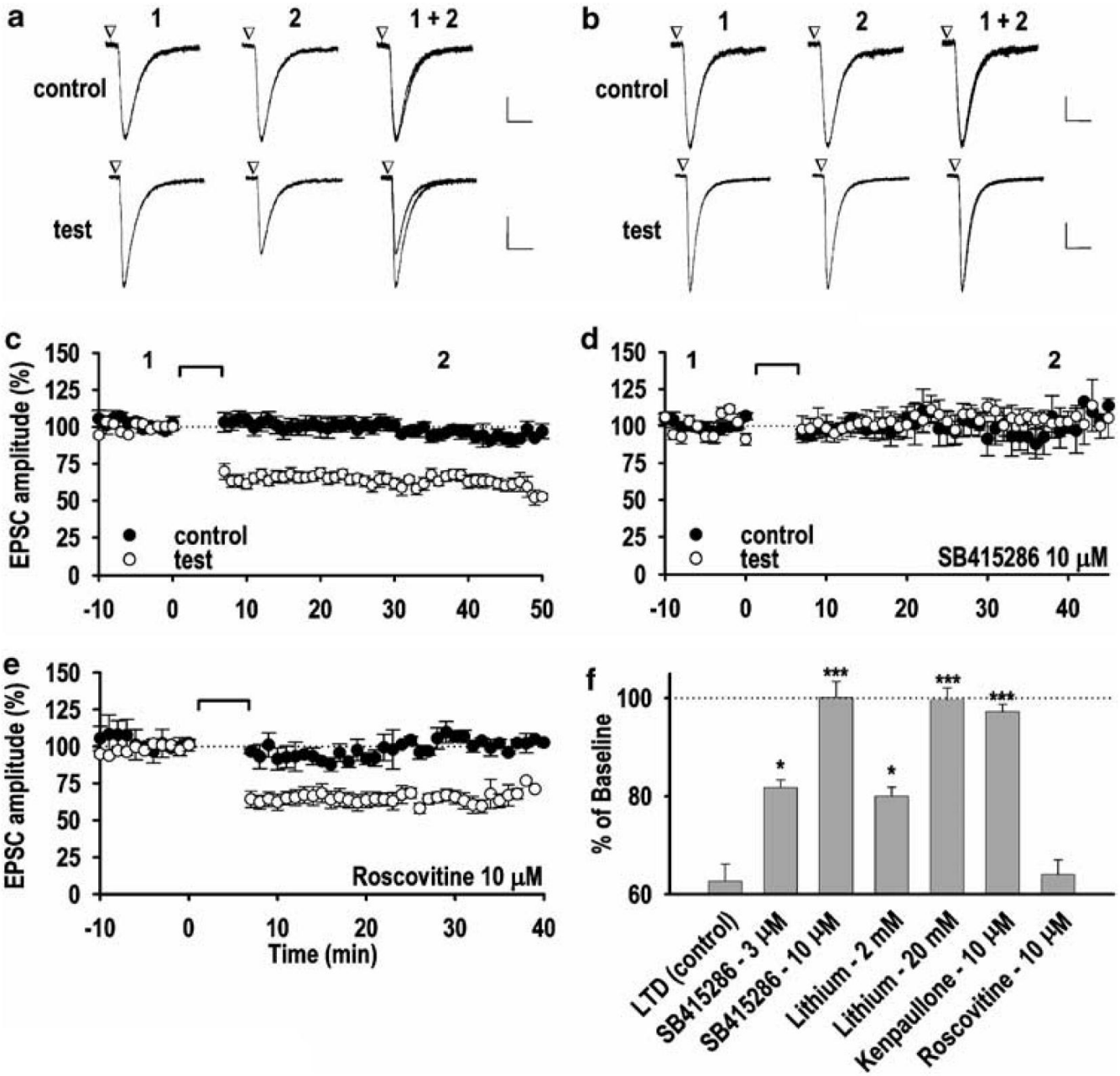
a
1
2
1 + 2
control
test
b
1
2
1 + 2
control
test
c
EPSC amplitude (%)
1
2
control
test
d
1
2
control
test
SB415286 10 µM
e
EPSC amplitude (%)
Roscovitine 10 µM
Time (min)
f
% of Baseline
LTD (control)
SB415286 - 3 µM
SB415286 - 10 µM
Lithium - 2 mM
Lithium - 20 mM
Kenpaullone - 10 µM
Roscovitine - 10 µM

Figure 3

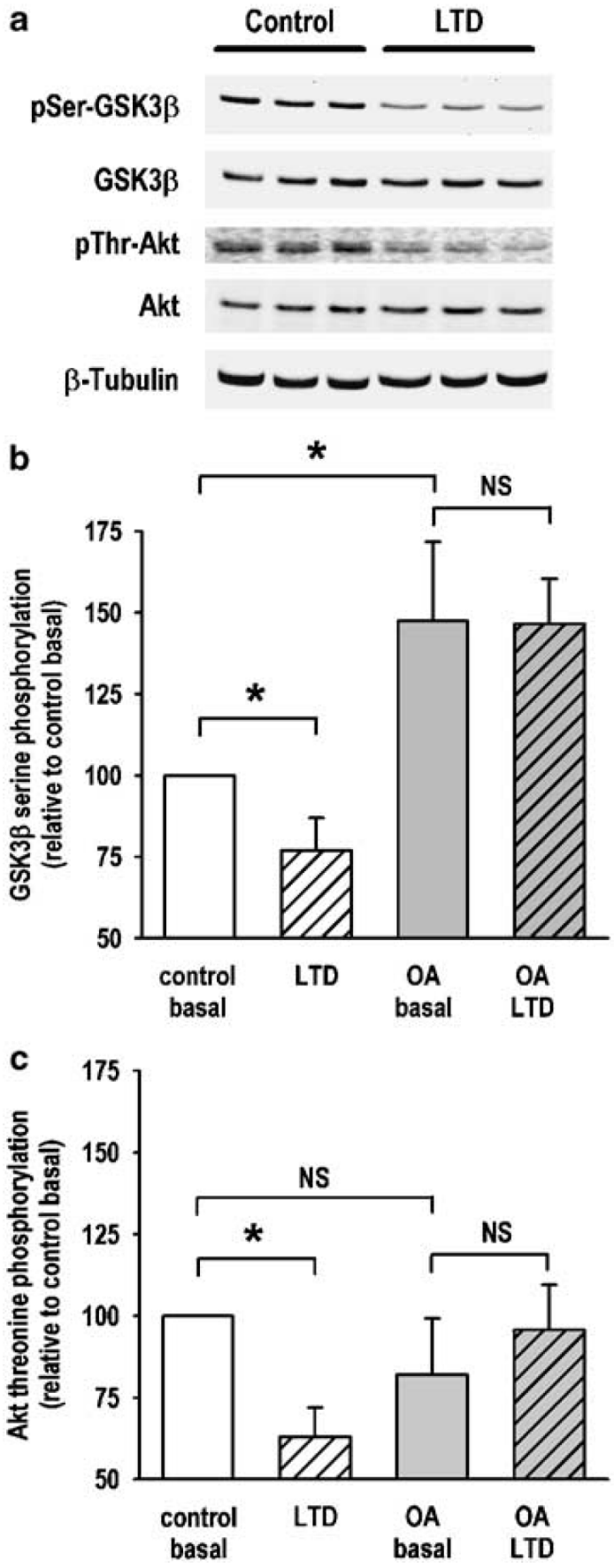
a
Control
LTD
pSer-GSK3β
GSK3β
pThr-Akt
Akt
β-Tubulin
b
*
NS
*
GSK3β serine phosphorylation (relative to control basal)
175
150
125
100
75
50
control basal
LTD
OA basal
OA LTD
c
NS
*
NS
Akt threonine phosphorylation (relative to control basal)
175
150
125
100
75
50
control basal
LTD
OA basal
OA LTD

Figure 4

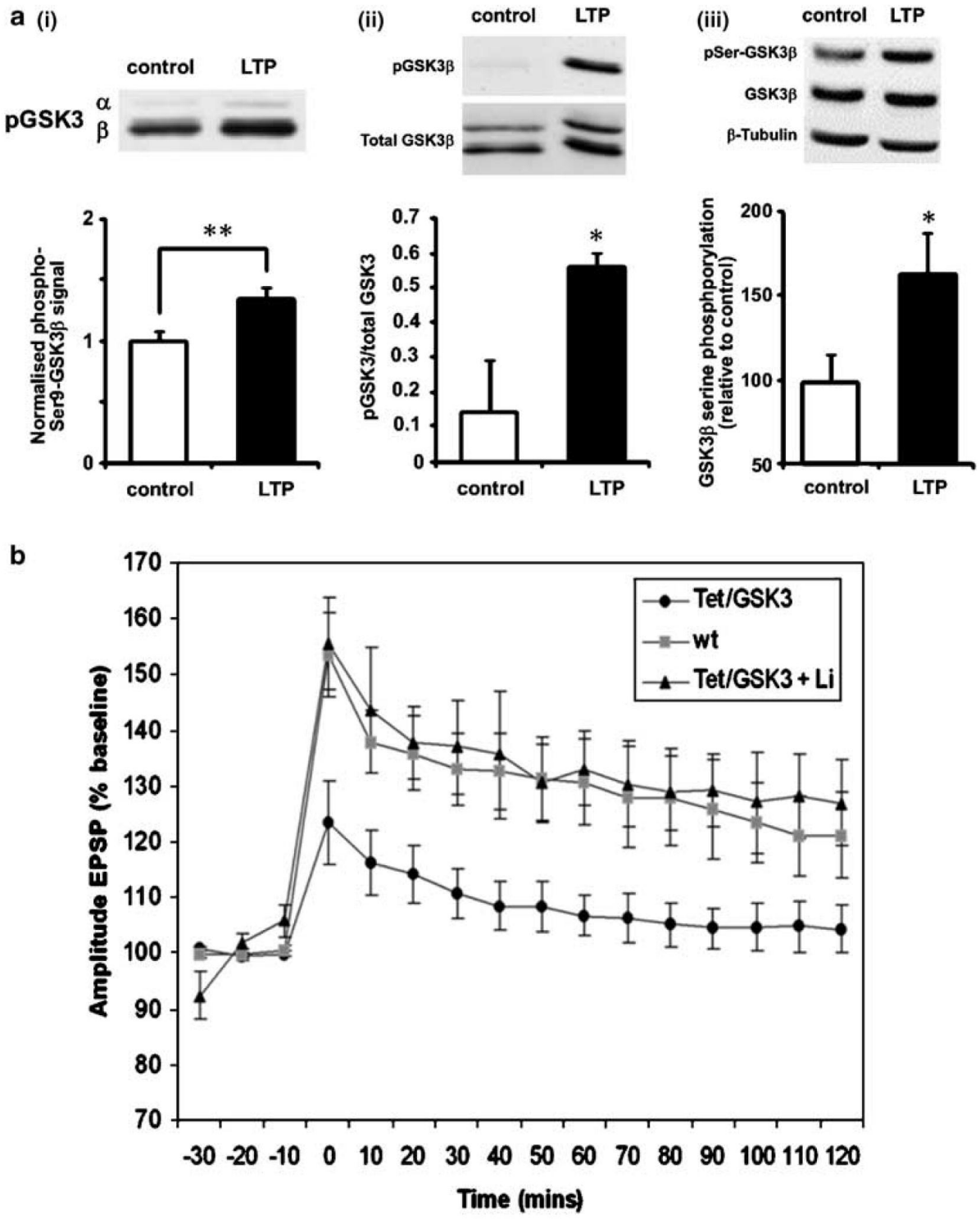
a (i)
control
LTP
pGSK3
α
β
(ii)
control
LTP
pGSK3β
Total GSK3β
(iii)
control
LTP
pSer-GSK3β
GSK3β
β-Tubulin
Normalised phospho-Ser9-GSK3β signal
**
control
LTP
pGSK3/total GSK3
*
control
LTP
GSK3β serine phosphporylation (relative to control)
*
control
LTP
b
Amplitude EPSP (% baseline)
Time (mins)
Tet/GSK3
wt
Tet/GSK3 + Li

Figure 5

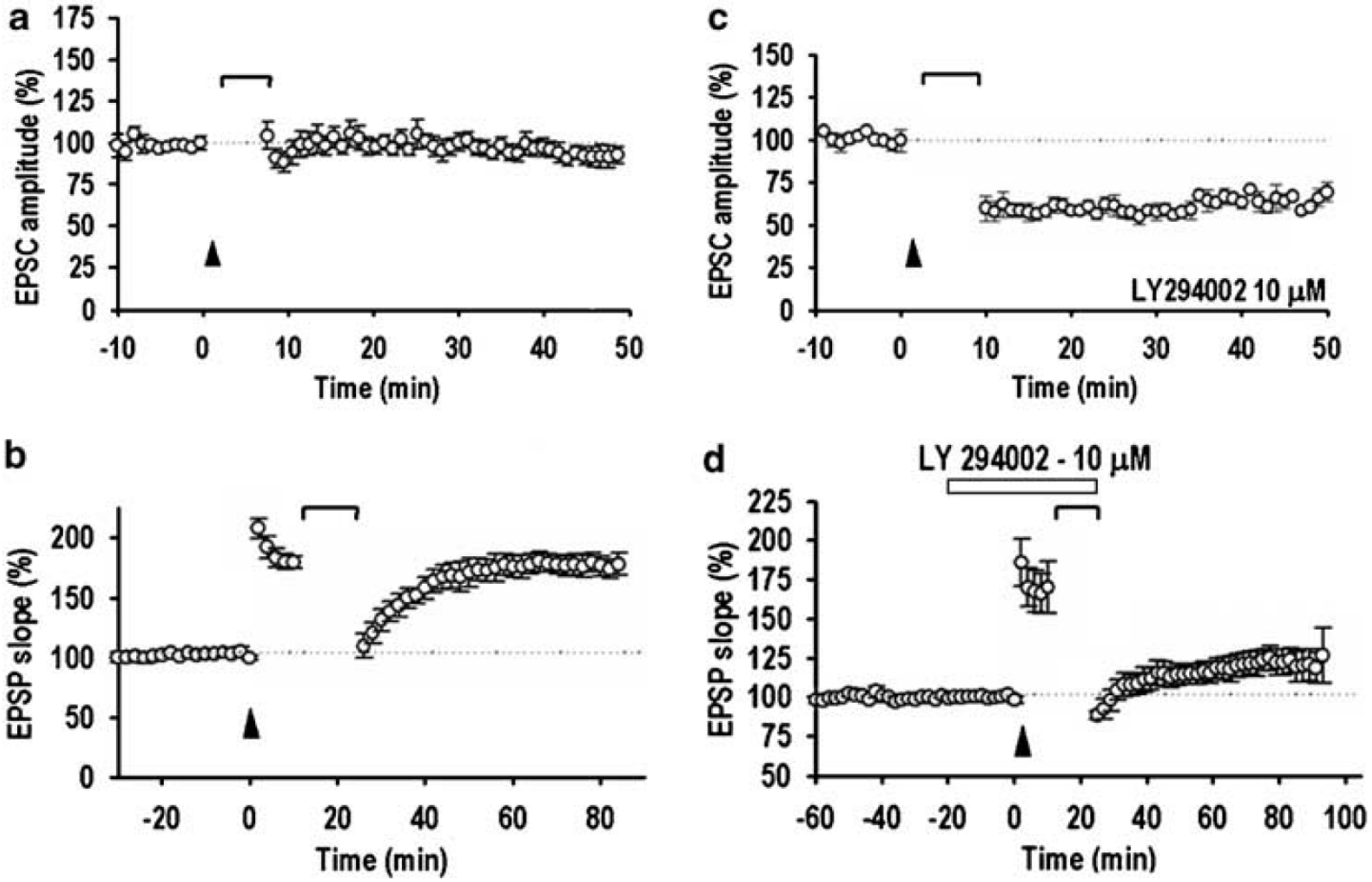
a
EPSC amplitude (%)
Time (min)
b
EPSP slope (%)
Time (min)
c
EPSC amplitude (%)
LY294002 10 µM
Time (min)
d
LY 294002 - 10 µM
EPSP slope (%)
Time (min)

Figure 6

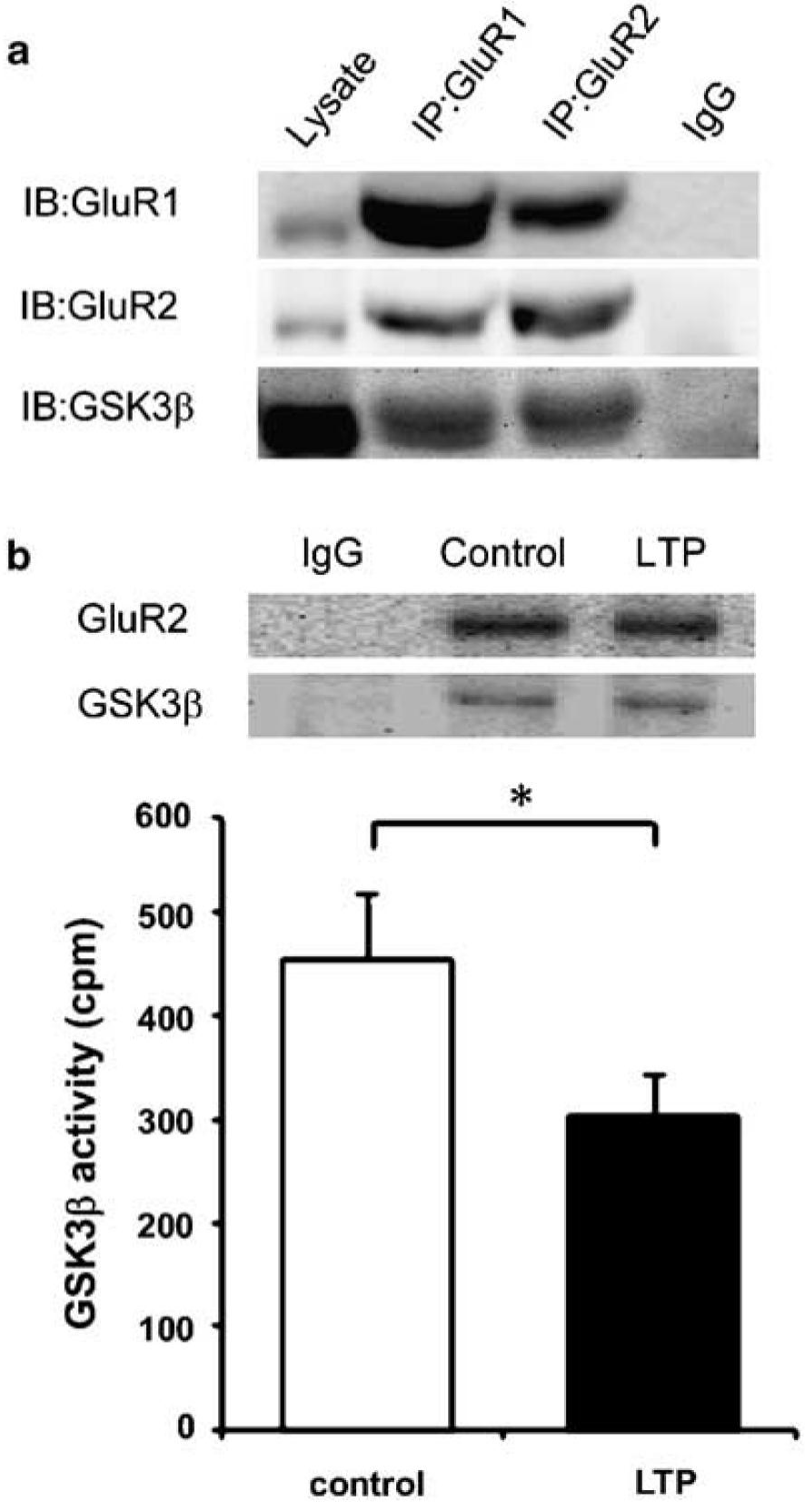
a
Lysate
IP:GluR1
IP:GluR2
IgG
IB:GluR1
IB:GluR2
IB:GSK3β
b
IgG
Control
LTP
GluR2
GSK3β
*
600
500
400
300
200
100
0
GSK3β activity (cpm)
control
LTP

Figure 7

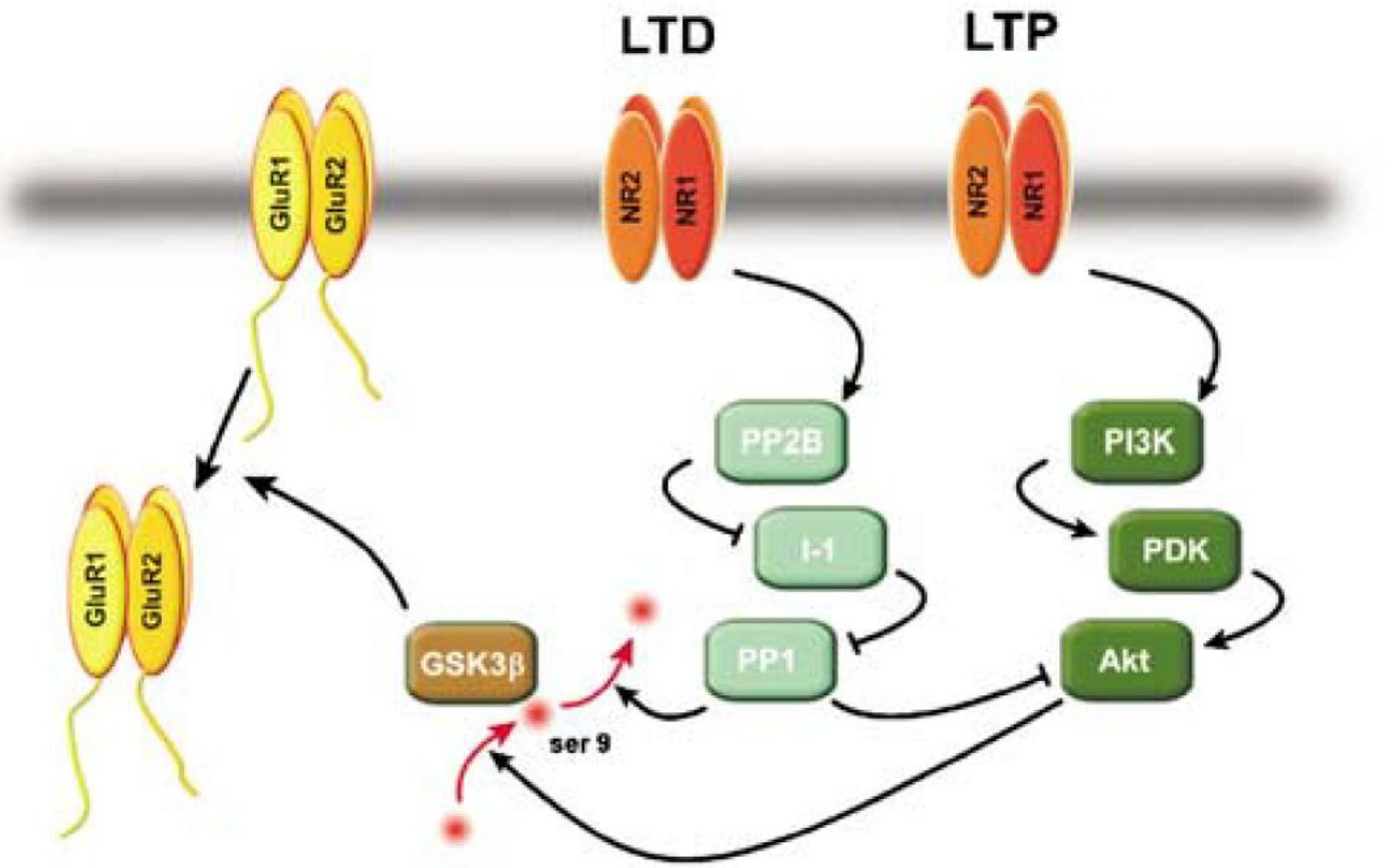
LTD
LTP
GluR1
GluR2
NR2
NR1
NR2
NR1
PP2B
PI3K
I-1
PDK
GluR1
GluR2
GSK3β
PP1
Akt
ser 9

Figure 8