



Differential regulation of STP, LTP and LTD by structurally diverse NMDA receptor subunit-specific positive allosteric modulators

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ABSTRACT

Different types of memory are thought to rely on different types of synaptic plasticity, many of which depend on the activation of the N-Methyl-D Aspartate (NMDA) subtype of glutamate receptors. Accordingly, there is considerable interest in the possibility of using positive allosteric modulators (PAMs) of NMDA receptors (NMDARs) as cognitive enhancers. Here we firstly review the evidence that NMDA receptor-dependent forms of synaptic plasticity: short-term potentiation (STP), long-term potentiation (LTP) and long-term depression (LTD) can be pharmacologically differentiated by using NMDAR ligands. These observations suggest that PAMs of NMDAR function, depending on their subtype selectivity, might differentially regulate STP, LTP and LTD. To test this hypothesis, we secondly performed experiments in rodent hippocampal slices with UBP714 (a GluN2A/2B preferring PAM), CIQ (a GluN2C/D selective PAM) and UBP709 (a pan-PAM that potentiates all GluN2 subunits). We report here, for the first time, that: (i) UBP714 potentiates sub-maximal LTP and reduces LTD; (ii) CIQ potentiates STP without affecting LTP; (iii) UBP709 enhances LTD and decreases LTP. We conclude that PAMs can differentially regulate distinct forms of NMDAR-dependent synaptic plasticity due to their subtype selectivity.

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1. Introduction

1.1. NMDA receptor-dependent plasticity and memory

Memory is essential for all animal species, including humans, - forming our personalities, defining our abilities to adapt and function in the world (Squire and Kandel, 1999). Most of our everyday memories are short-lived and, after serving their purpose, easily forgotten, whilst

selected memories can last a lifetime (Nadel and Hardt, 2011). Our memory system is not unitary, it includes different memory types (see Baddeley et al., 2002; Güell, 2017; Nadel and Hardt, 2011), which are suited for different functional purposes. Dysfunction of these cognitive processes can arise due to a large variety of brain and vascular disorders, events and injuries (Baddeley et al., 2002; Markowitsch and Staniloiu, 2012). Working and short-term memory deficits are observed in a number of brain disorders and diseases (e.g. depression, schizophrenia,

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epilepsy) where long-term memory function is largely unaffected. In contrast, long-term memory deficits are hallmarks of late Alzheimer's disease as well as other types of dementia. Memories can also be affected in many other ways, - e.g. by electroconvulsive therapy, by medicines or drugs of abuse; leading to disruption of different short and long-term memory types and producing various forms of retrograde and anterograde amnesia (Baddeley et al., 2002; Markowitsch and Staniloiu, 2012).

Encoding of memories in the brain is thought to rely on synaptic plasticity, - the ability of synapses to modulate their pre-synaptic reliability and post-synaptic potency of signal transfer, based on the history and pattern of neuronal activity (Bliss and Collingridge, 1993). Patterned electrical stimulation of excitatory glutamatergic synapses, in many areas of the mammalian brain, can induce various forms of synaptic plasticity; the induction of the majority of which relies on the activation of the NMDA subtype of glutamate receptors (NMDARs), which are central in the formation of memories (Bliss and Collingridge, 1993; Morris et al., 1986). NMDAR-dependent types of plasticity are inhibited by NMDAR antagonists, differentiating them from non-NMDA-receptor-dependent forms of plasticity (Bliss and Collingridge, 1993; Collingridge et al., 1983b; Dudek and Bear, 1992).

Of the various NMDAR-dependent types of plasticity, long-term potentiation (LTP) and long-term depression (LTD) are the most widely studied (Bliss and Lomo, 1973; Dudek and Bear, 1992). LTP denotes a stable increase in synaptic transmission, which can be maintained for hours *in vitro*, and days and even years *in vivo* (Abraham, 2003; Abraham et al., 2002). LTD is the opposite of LTP, representing a stable decrease in neurotransmission (Dudek and Bear, 1992; Dunwiddie and Lynch, 1978; Lynch et al., 1977). LTP and LTD are universally recognised as neuronal correlates of long-term memory encoding, being particularly suited to mould neuronal networks by controlling the strength of synaptic connections (Bear, 1999; Bliss and Collingridge, 1993).

In more recent years, NMDAR-dependent short-term potentiation (STP) has also attracted significant attention, due to its unique properties and features, which make it particularly suited for the encoding of the shorter-lasting memories (Volianskis et al., 2013b; Volianskis and Jensen, 2003). STP, similarly to LTP, reflects an increase in synaptic transmission, albeit of transient duration (McNaughton, 1982; Racine and Milgram, 1983; Racine et al., 1983). In contrast to LTP, which provides response stability over hours and days, STP confers the synapses with a temporal increase in strength, which depresses either back to baseline or to a stable level of LTP in response to activation of the synapses (McNaughton, 1982; Pradier et al., 2018; Racine and Milgram, 1983; Volianskis and Jensen, 2003). With STP, the strength of synapses remains stable in between the bouts of neuronal activity, and gets adjusted in response to both low and high frequency of neuronal activity (Volianskis and Jensen, 2003). In principle, this permits the creation of dynamic cell assemblies, which are thought to be important for the working memory function and also for cognition (Albright et al., 2000; Goddard, 1980).

NMDAR-dependent short-term potentiation can sometimes be confused with "short-term plasticity", which unfortunately shares the acronym (STP). The term short-term plasticity is frequently used in the literature to denote a group of NMDA receptor-independent forms of plasticity (Zucker and Regehr, 2002), such as post-tetanic potentiation (PTP). PTP is caused by pre-synaptic accumulation of $[Ca^{2+}]_i$ (Zucker and Regehr, 2002), its decay is independent of synaptic stimulation (Korshoej and Lambert, 2007; Volianskis and Jensen, 2003) and it is very short lived at the hippocampal synapses (Stevens et al., 1994; Volianskis and Jensen, 2003). The term STP referred to in this article is thus different from this short-term plasticity.

If the three types of NMDA receptor-dependent plasticity - STP, LTP and LTD - are involved in the formation of different memory types then their dysfunction is likely to be implicated in a variety of pathophysiological processes and brain disorders. It can be therefore speculated that

selective targeting of the various plasticity processes through electrical stimulation, pharmacological agents or by other means, may be beneficial for the treatment of disorders involving dysfunction of different memory types.

1.2. Modulation of NMDAR-dependent plasticity through electrical stimulation

NMDAR-dependent plasticity can be studied *in vivo* where it is traditionally induced by electrical stimulation (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; McNaughton, 1982; Morris et al., 1986) and more recently, through optogenetic means (Nabavi et al., 2014). Nonetheless, STP, LTP and LTD are most frequently studied *in vitro* (Andersen et al., 1977; Lynch et al., 1977; Malenka, 1991; Volianskis and Jensen, 2003) in rodent hippocampal slice preparations (Skrede and Westgaard, 1971) while using electrical stimulation in combination with field or whole cell recording of post-synaptic responses — electrophysiological techniques that permit high temporal resolution of the changes in the strength of synaptic responses and relative ease of drug application. During these experiments, induction of the specific types of plasticity depends both on experimental conditions and the induction paradigms used, with somewhat different results sometimes observed in extracellular and whole-cell intracellular experiments.

In extracellular experiments, when less intense high-frequency stimulation (HFS) paradigms containing only a few stimuli are applied, STP can be induced independently of LTP (McNaughton, 1982; Racine et al., 1983; Racine and Milgram, 1983; Watanabe et al., 1992). Such weak HFS is frequently defined in the literature as being "sub-threshold" for the induction of LTP (Watanabe et al., 1992). Stronger high frequency activation of NMDARs by tetanic (e.g. 100 Hz for 1 s) or theta-burst stimulation (TBS, e.g. 4 pulses at 100 Hz, repeated at 5 Hz 5–30 times) induces both STP and LTP (Larson et al., 1986; Volianskis and Jensen, 2003); whilst prolonged low frequency stimulation (LFS, e.g. 1 Hz for 15 min) induces LTD (Dudek and Bear, 1992; Lynch et al., 1977). In cases where STP and LTP are co-induced, STP is seen as a declining phase of potentiation, lasting from about 10 min and up to 1 h, that decays to a stable level of potentiated synaptic transmission, the LTP proper (Fig. 1A, black circles). The amplitude of STP depends on the frequency of HFS, whilst its duration is dependent on the number of stimuli in the induction train, as is the magnitude of LTP (Volianskis and Jensen, 2003). Whilst LTP saturates after ~2 s of TBS (Larson et al., 1986), STP can be repeatedly re-induced after saturation of LTP (Racine et al., 1983; Schulz and Fitzgibbons, 1997). The prolonged maintenance of stable LTD and LTP relies on multiple cellular mechanisms, including activation of protein phosphatases and kinases, *de novo* protein synthesis and also structural plasticity during which synaptic contacts can either be retracted or maintained (Bliss and Collingridge, 2013; Bliss et al., 2018; Collingridge et al., 2010). In contrast, STP is typically resistant to many of these treatments.

In whole cell experiments, when pairing protocols are used for the induction of potentiation, LTP is most often induced independently of STP (Chen et al., 1999; Malinow and Tsien, 1990). This is because the frequency of stimulation used for pairing is (i) too low to efficiently induce STP and (ii) too fast for its stable maintenance. It has been shown, however, that single synapses can express STP and LTP independently of each other, or indeed, a combination of both (Debanne et al., 1999).

NMDAR-dependent LTD can be induced in both intracellular and extracellular experiments (Debanne et al., 1999; Dudek and Bear, 1992; Lynch et al., 1977) while using LFS and pairing protocols (Collingridge et al., 2010). The ease of LTD induction is developmentally regulated and LTD is most readily induced in juvenile rodent hippocampal slices, at a time when STP and LTP are sometimes more difficult to induce. Notably, the subunit composition of NMDARs in the hippocampus is also developmentally regulated, with different NMDAR types being expressed during different developmental stages (Monyer et al., 1994).

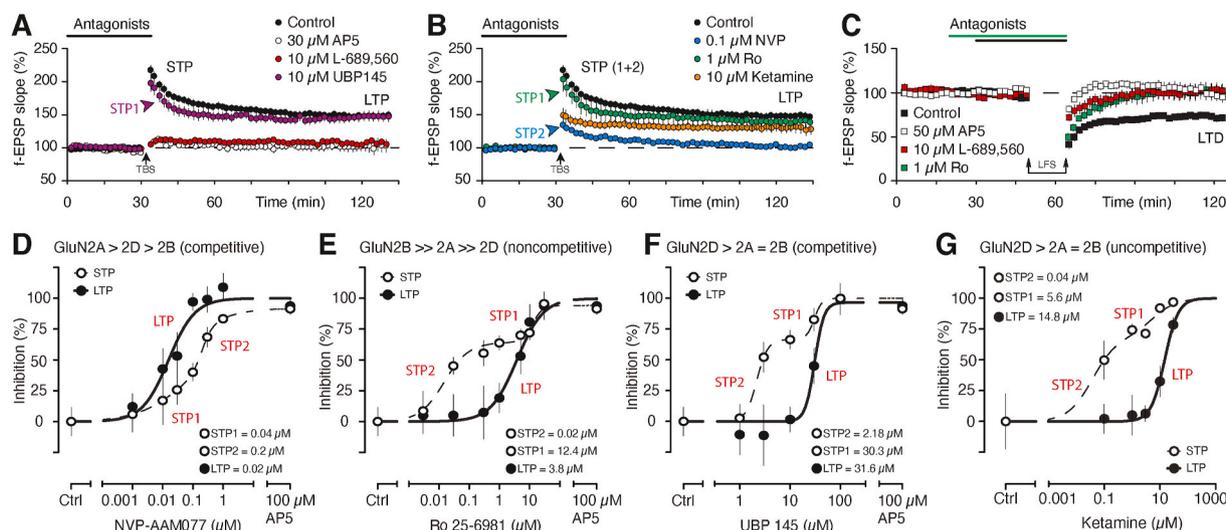


Fig. 1. NMDAR dependence of STP1, STP2, LTP and LTD.

(A) Theta-burst stimulation (TBS) induces STP that declines slowly to a stable level of LTP (black circles, control) in adult hippocampal slices. AP5 (open circles) and L-689,500 (red circles) inhibit STP and LTP (Ingram and Volianskis, 2019; Volianskis et al., 2013a). The GluN2C/2D antagonist UBP145 (purple circles) has no effect on the fast-decaying STP1 and LTP but inhibits the slow-decaying STP2 (Volianskis et al., 2013a). The bar indicates the time for the application of antagonists.

(B) The GluN2A preferring concentration of NVP (blue circles) inhibits the fast-decaying STP1 and LTP but has no effect on the slow-decaying STP2 (Volianskis et al., 2013a). Effects of the GluN2B NAM Ro (green circles) are similar to UBP145 in A: it has no effect on the fast-decaying STP1 and LTP but inhibits the slow-decaying STP2 (Volianskis et al., 2013a). Ketamine (orange circles) inhibits both STP1 and STP2 and preserves most of LTP (Ingram et al., 2018), when compared to the control STP and LTP that are induced by TBS (black circles).

(C) AP5 (open squares), L-689,560 (red squares) and Ro (green squares) inhibit LFS-induced LTD (black squares) in P14 hippocampal slices (France et al., 2017).

(D) NVP inhibits LTP more potently than STP1 and STP2 (Volianskis et al., 2013a) IC₅₀ values for STP1, STP2 and LTP are given in the abscissa. Effects of NMDAR antagonists on induction of STP and LTP were quantified in individual experiments in terms of percentage reduction (block) in the amount of STP (amplitude × τ) and in the amplitude of LTP relative to their mean levels in control experiments (i.e. without the application of antagonists). These data were pooled for each concentration of an antagonist (mean ± SEM) and used to construct concentration–response curves describing the antagonist’s effects on STP and LTP (Volianskis et al., 2013a). The potency ranking of NVP, and the other compounds, omits GluN2C, which in neurons is expressed in other brain structures than the hippocampus (Paoletti, 2011; Paoletti et al., 2013).

(E) Ro is highly potent at STP2 and inhibits STP1 and LTP at high concentrations (Volianskis et al., 2013a).

(F) UBP145 is more potent at STP2 than STP1 and LTP (Volianskis et al., 2013a).

(G) Ketamine is highly selective between STP2 and LTP and inhibits STP1 at high concentrations as demonstrated by Ingram et al. (2018), which also discusses why GluN2Ds are inhibited by ketamine, and other NMDAR channel blockers, at lower IC₅₀ values when compared to GluN2A/2B-containing NMDA receptors.

1.3. NMDA receptors, their composition and regional expression

NMDARs are ligand and voltage gated channels composed of GluN1, GluN2 and GluN3 subunits. GluN1 and GluN3 subunits bind the neurotransmitter glycine, whilst GluN2 subunits are glutamate-binding. These subunits assemble, in different combinations, into heterotetrameric NMDARs (Paoletti et al., 2013). Conventional NMDARs are composed of 2 GluN1 and 2 GluN2 subunits whilst non-conventional NMDARs incorporate GluN3 subunits, in addition to both GluN1 and GluN2 subunits (Kehoe et al., 2013). GluN1 and GluN3 subunits can also co-assemble into functional excitatory glycine receptors, which are not activated by glutamate (Chatterton et al., 2002; Grand et al., 2018). The knowledge about the role of receptors that incorporate GluN3 subunits in synaptic plasticity is still limited (Larsen et al., 2011; Pérez-Otaño et al., 2016) with research primarily focusing on the role of the glutamate binding site of the GluN2 subunit in synaptic function.

There are four different GluN2 subunits, and their co-expression with GluN1s results in functional NMDARs with different physiological properties (Monyer et al., 1992; Vicini et al., 1998; Wyllie et al., 2013). Concurrent binding of glutamate and glycine to their respective binding sites (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988), together with a depolarisation of the neuronal membrane that relieves the voltage-dependent Mg²⁺ block of the channel pore (Mayer et al., 1984; Nowak et al., 1984), is necessary for receptor activation. Receptors expressing GluN2A and GluN2B are more sensitive to the Mg²⁺ block, have higher open probability, Ca²⁺ permeability and single channel conductance compared to GluN2C and GluN2D (Monyer et al.,

1992; Stern et al., 1992; Vicini et al., 1998). GluN2D subunits have the highest agonist affinity and generate currents that can last for seconds (Monyer et al., 1992; Vicini et al., 1998). This is in stark contrast to the other receptor types, whose opening times are in the millisecond range (Monyer et al., 1992; Vicini et al., 1998).

The different GluN2 subunits have different temporo-spatial expression patterns in the CNS (Goebel and Pooch, 1999; Monyer et al., 1992, 1994, 1992; Watanabe et al., 1992, 1993a, 1993b), forming functional NMDARs whose structure and expression are developmentally regulated (reviewed in Paoletti, 2011; Paoletti et al., 2013; Stroebel et al., 2018). GluN2A subunits are minimally expressed at birth with their expression increasing with development and leading to high levels in adulthood, mostly in the hippocampus, cortex and cerebellum. Conversely, GluN2B subunits are ubiquitously expressed at birth whilst during development expression of GluN2B containing receptors becomes gradually restricted to the forebrain areas, being highly expressed in cortex and the hippocampus (Paoletti, 2011; Paoletti et al., 2013). Expression of GluN2C subunits in neurons, similarly to GluN2A subunits, increases during development, leading to a high expression in the cerebellum and the olfactory bulb but not in cortex or the hippocampus (Paoletti, 2011; Paoletti et al., 2013), where they have been reported to be expressed in astrocytes (Ravikrishnan et al., 2018). GluN2D subunits are expressed in the hippocampus and diencephalon, their expression peaks at birth and then decreases into low levels during adulthood. Different GluN2 subunits, together with the GluN1 and GluN3 subunits, form a variety of di- and tri-heteromeric combinations, with the latter being specifically prevalent in the adult brain (Stroebel et al., 2018).

The changes in GluN2 subunit expression during development may play a role in determining the ease of induction of different types of synaptic plasticity, explaining facilitated induction of STP and LTP and reduced induction of LTD during development and aging.

1.4. Targeting STP, LTP and LTD with competitive antagonists, NAMs and channel blockers

Induction of STP, LTP and LTD requires the activation of NMDARs and high concentrations of competitive antagonists ($\geq 10 \times \text{IC}_{50}$), which act at glutamate (AP5, Fig. 1A and C, open symbols) or glycine (L-689,560, Fig. 1A and C, red symbols) subunits, can inhibit the three types of plasticity (Bashir et al., 1990; Collingridge et al., 1983b; France et al., 2017; Ingram and Volianskis, 2019; Volianskis et al., 2013a, 2015). STP, LTP and LTD are also inhibited by NMDAR channel blockers such as MK-801 or ketamine (Babiec et al., 2014; Coan et al., 1987; Huang et al., 2016; Ingram et al., 2018; Izumi and Zorumski, 2014; Stringer et al., 1983; Stringer and Guyenet, 1983). It has been reported, however, that in some experiments, inhibition of the GluN1 subunit does not prevent the induction of LTD, suggesting that metabotropic action of NMDARs, elicited by glutamate binding to the GluN2 subunits, might be sufficient for induction of LTD (Malinow, 2016; Nabavi et al., 2013; Stein et al., 2021) and even LTP (Stein et al., 2021).

The identity of GluN2 subunits that are involved in the induction of both LTP and LTD has also been contested. It was observed initially that whilst 50 μM AP5 inhibited both LTP and LTD in 16- to 21-day-old Sprague Dawley rats, 10 μM of CPP was only able to inhibit LTP and not LTD (Hrabetova and Sacktor, 1997). CPP shows preference to GluN2A/2B subunits over GluN2C/2D suggesting their involvement in LTP and LTD, respectively; supported by a later study that used a GluN2C/2D preferring antagonist PPDA (Hrabetova et al., 2000). In contrast, 10 μM of a more selective GluN2C/2D antagonist UBP145 was not able to inhibit LTD in P14 slices from Wistar rats (France et al., 2017). It is worth noting, however, that the above studies differed in the age and strain of the rat (P21 Sprague Dawley vs P14 Wistar), and LTD induction paradigm (3 Hz vs 1 Hz LFS).

Similar to actions on LTD, 10 μM of UBP145 had no effect on the induction of LTP in either P14 (France et al., 2017) or adult (Volianskis et al., 2013a) rat hippocampal slices, although partial inhibition of LTP has recently been described in adult mouse hippocampal slices (Eapen et al., 2021; current issue). In both species, UBP145 inhibited the induction of a slow-decaying component of STP, termed STP2 (Volianskis et al., 2013a; France et al., 2017; Eapen et al., 2021). The fast-decaying component of STP that is not sensitive to inhibition by UBP145 is termed STP1 (Fig. 1A, purple circles). The results of UBP145 on STP were mimicked by 1 μM of Ro 25-6981 (Ro), which is a highly selective negative allosteric modulator (NAM) of GluN2B receptors. 1 μM Ro inhibited induction of STP2 and spared induction of STP1, whilst having no effect on LTP in both adult (Fig. 1B, green circles) and P14 hippocampal slices (France et al., 2017). GluN2A preferring antagonist NVP-AAM077 (NVP) was more potent against STP1 and LTP in adult than in P14 tissue. In adult rat hippocampal slices STP1 and LTP were inhibited by 0.1 μM of NVP while STP2 was unaffected (Fig. 1B, blue circles). In contrast to the adults, 0.1 μM NVP had no effect on STP and LTP in slices of P14 hippocampus whilst 1 μM of the antagonist resulted in complete inhibition of both STP and LTP, which is likely due to inhibition of non-GluN2A containing receptors by the high concentration of NVP (France et al., 2017). This suggests that the sensitivity of STP1 and LTP to GluN2A inhibition changes with development.

A preferential involvement of GluN2A subunits in LTP and GluN2B subunits in LTD has been suggested early on (Liu et al., 2004; Massey et al., 2004), but many studies could not replicate such findings (Bartlett et al., 2007; Berberich et al., 2005; Li et al., 2007; Morishita et al., 2007). It was found however that LTD inhibition could be observed reliably after a longer application of 1 μM Ro (Fig. 1C, green squares), which might reflect the slow time to achieve a steady-state concentration of

this NAM (France et al., 2017). A further complication is that the sensitivity of LTD to GluN2B inhibitors depends on the hippocampal slice orientation and/or activation of other receptors and neurotransmitter systems (Bartlett et al., 2011). The animal strain, species and age dependency of GluN2B involvement in LTD is still not established.

NMDAR channel blockers have also been shown to differentiate between STP, LTP and LTD (Ingram et al., 2018; Izumi and Zorumski, 2014). Both STP (Fig. 1B, orange circles) and LTD (Izumi and Zorumski, 2014) are inhibited by sub anaesthetic concentrations of the dissociative anaesthetic ketamine, without affecting LTP. Ketamine shows a similar concentration response profile (Fig. 1G) on STP when compared to UBP145 (Fig. 1F) and Ro (Fig. 1E). Ketamine shows very high potency at STP2 and lesser potency at STP1 and LTP, whilst inverse selectivity is observed with NVP (Fig. 1D). STP is also far more sensitive to the glycine site antagonist L-689,560 than LTP, showing that these two types of potentiation can be dissociated by targeting the GluN1 subunit (Ingram and Volianskis, 2019).

In conclusion, dissociation of STP1, STP2, LTP and LTD can be achieved by competitive, non-competitive and uncompetitive antagonists targeting different subunits of the NMDAR.

1.5. Potentiation of NMDA receptors as a strategy for targeting plasticity

Potentiation of NMDARs offers an alternative strategy for the differentiation of STP, LTP and LTD, which has been demonstrated with the NMDAR-inhibitors. In principle, this could be achieved pharmacologically in a variety of relatively direct ways, e.g. by using agonists that target glutamate and glycine binding sites on the NMDAR, depolarising treatments that relieve the Mg^{2+} block of the NMDAR or positive allosteric modulators (PAMs) that bind to other sites on NMDARs than orthosteric ligands. NMDAR function can also be facilitated in many other ways (reviewed in Collingridge et al., 2013).

Exogenous application of NMDA reliably induces LTD (Collingridge et al., 1983a; Lee et al., 1998), whilst induction of STP and LTP has proven more difficult. An initial depression of synaptic responses that is followed by a transient enhancement are observed after a brief iontophoretic application of NMDA (Collingridge et al., 1983b; Kauer et al., 1988). Such transient enhancement can be converted into a stable potentiation in the presence of high $[\text{Ca}^{2+}]_o$ and also through depolarisation (Malenka, 1991). Bath application of NMDA, similarly to iontophoresis experiments, depresses synaptic responses and results in a rebound potentiation of the field-excitatory post-synaptic potential (f-EPSP) amplitude (Asztely et al., 1991; McGuinness et al., 1991a; Volianskis et al., 2015), which is occluded in high $[\text{Ca}^{2+}]_o$ (McGuinness et al., 1991b). The decline of rebound potentiation, in contrast to STP, is not dependent on stimulation (Lee et al., 1998; Volianskis et al., 2015). STP is also different from rebound potentiation in that it potentiates both the slope and the amplitude of f-EPSPs (Asztely et al., 1991; Volianskis et al., 2015). LTP is usually not induced in response to bath application of NMDA alone, but a combination of NMDA, glycine and spermine, which is followed by AP5, produces a slow-onset LTP, which is inhibited by pre-application of AP5 (Thibault et al., 1989).

Exogenous application of glycine alone has been also shown to induce a slowly developing LTP in slices from adult rats (Shahi and Baudry, 1993; Shahi et al., 1993) whilst in neonatal slices, in which induction of LTP is unfavourable, glycine induces LTD (Shahi et al., 1993). Glycine-induced LTP was replicated in organotypic cultures (Musleh et al., 1997), dissociated hippocampal neurons (Lu et al., 2001) and also in whole-cell recordings from CA1 hippocampal neurons in slices from juvenile rats (Chen et al., 2011). Here, in the absence of $[\text{Mg}^{2+}]_o$ and under a strict control of the membrane potential, low concentrations of glycine induced LTP whilst high concentrations induced LTD (Chen et al., 2011). Glycinergic LTP and LTD are NMDAR-dependent (Chen et al., 2011; Lu et al., 2001; Shahi et al., 1993), but induction of LTD requires co-activation of NMDA and strychnine sensitive glycine receptors, inhibition of which produces a

switch from LTD to LTP (Chen et al., 2011). Other amino acids, such as serine and taurine, can also be used to induce stable chemical LTP (Chen et al., 2011). Exogenous application of glycine and structurally related amino acids were shown to promote induction of LTP with subthreshold tetanic stimulation, without affecting STP (Watanabe et al., 1992).

Induction of STP has not been demonstrated by glycine application. Notably, a fast-decaying, STP-like potentiation of miniature postsynaptic currents (mEPSCs) could be elicited in neurons that were bathed in tetrodotoxin and glycine, and briefly treated for depolarisation with high K^+ solution (Fitzjohn et al., 2001). A fast-decaying STP could also be elicited by brief depolarisations of the postsynaptic neurons during whole cell experiments in slices from juvenile Hartley guinea pigs, in the absence of any synaptic stimulation or NMDAR agonist application; - without inducing LTP or LTD (Kullmann et al., 1992).

In summary, an STP-like potentiation can be produced by depolarising treatments and not by agonist application, whilst LTP seems to be more easily induced through activation of the GluN1 site. Exogenous application of both glutamate and glycine site agonists can induce LTD-like effects.

1.6. Targeting of synaptic plasticity by using NMDA receptor PAMs

A large number of endogenous and synthetic PAMs have been described in the literature (Burnell et al., 2019; Hackos and Hanson, 2017; Monaghan et al., 2012). PAMs of NMDARs can be subunit selective and are able to increase the effects of the endogenous agonists in a fashion that is specific to activated synapses, which makes them particularly interesting for therapeutic purposes that aim to modulate synaptic transmission (Burnell et al., 2019; Costa et al., 2010; Monaghan et al., 2012; Volgraf et al., 2016).

Endogenous NMDAR-PAMs, such as histamine, spermine and neurosteroids, have been shown to potentiate LTP although their effects on NMDARs and their subunits are not always easily predictable and can depend on a variety of factors (reviewed in Monaghan et al., 2012).

Injection of histamine and spermine into anaesthetised rats potentiates LTP induced by a subthreshold tetanus *in vivo*, in the mature visual cortex (Kuo and Dringenberg, 2008), and in the dentate gyrus (Chida et al., 1992), without inducing STP. Histamine also potentiates LTP in the CA1 area of hippocampal slices (Brown et al., 1995). Both histamine (Burban et al., 2010; Williams, 1994a) and spermine (Williams, 1994b) are GluN2B preferring PAMs, with unknown effects on STP and LTD.

Application of ATP (10–70 μ M) to hippocampal slices causes a transient depression of responses, followed by a slowly developing LTP (Fujii et al., 1999; Wieraszko and Seyfried, 1989), which is $[Ca^{2+}]_o$ -dependent and is blocked by AP5 (Fujii et al., 1999, 2002). Higher concentrations of ATP (e.g. 250 μ M) produce an LTD like effect, which can be reversed by 3,4-diaminopyridine (Wieraszko and Seyfried, 1989). ATP-induced LTD is unlikely to be NMDAR-dependent as it can be induced in $[Ca^{2+}]_o$ free solutions and AP5 can transform ATP-induced LTP into an ATP-LTD (Fujii et al., 1999, 2002). ATP binds to both orthosteric and allosteric sites on NMDARs and can both potentiate and inhibit NMDARs, in a manner that depends on the glutamate concentration (Kloda et al., 2004). NMDAR-independent effects of ATP are mediated through ionotropic P2X and metabotropic P2Y receptors and also, after its hydrolysis to adenosine, through adenosine receptors (Guzman and Gerevich, 2016).

Neurosteroids, -pregnenolone sulphate (PS, Chen et al., 2010; Sabeti et al., 2007; Sliwinski et al., 2004) and 24(S)-hydrocholesterol (Paul et al., 2013)- have been shown to potentiate LTP induced by a subthreshold tetanus. Notably, some of the effects of PS on LTP are NMDAR-independent (Chen et al., 2010; Sabeti et al., 2007), whilst others depend on NMDAR function (Sliwinski et al., 2004). PS has also been shown to reduce LTD and shift the balance of transmission from depression to potentiation (Chen et al., 2010). Neither PS, which shows a preference for GluN2A/2B subunits (Cameron et al., 2012; Horak

et al., 2006; Malayev et al., 2002; Park-Chung et al., 1997), nor 24 (S)-hydrocholesterol affect STP.

A large number of synthetic NMDAR PAMs have also been described (Burnell et al., 2019; Costa et al., 2010; Hackos and Hanson, 2017; Irvine et al., 2012, 2015, 2019; Monaghan et al., 2012; Mosley et al., 2010; Sapkota et al., 2017; Volgraf et al., 2016). However, only a few of these have been tested on synaptic plasticity. SGE-201 has a similar effect on LTP as 24(S)-hydrocholesterol (Paul et al., 2013). SGE-301 is a GluN2A/GluN2B preferring PAM, which potentiates LTP and reduces STP (La et al., 2019). Potentiation of both STP and LTP has been suggested with GNE-PAMs (Hackos et al., 2016). Finally, the isoquinoline derivative CIQ, a potent and highly selective PAM of GluN2C/2D-containing NMDARs (Mullasseril et al., 2010), was shown to rescue LTP in the striatum of a mouse model of Parkinson's disease whilst blocking LTP in wild-type mice (Nouhi et al., 2018).

In conclusion, although pharmacological dissociation of STP, LTP and LTD, through selective potentiation of NMDARs, seems to be possible in principle, this remains to be demonstrated in practice. Based on our own research, and on the literature we have reviewed, we have hypothesised that potentiation of GluN2A/2B receptors would result in facilitation of LTP whilst facilitation of STP2 will be achieved through selective targeting of GluN2C/2D subunits. We hypothesised also that enhancement of all receptor subtypes with a pan-PAM can be expected to mimic agonist application and should result in a greater LTD. To test these predictions we selected three NMDAR PAMs with different pharmacological profiles: (1) the GluN2A/2B preferring coumarin derivative UBP714 (Irvine et al., 2012), (2) the GluN2C/2D preferring isoquinoline derivative CIQ (Mullasseril et al., 2010) and (3) the phenanthrene derivative, pan-PAM UBP709 (Irvine et al., 2015). We examined their effects on induction of STP, LTP and LTD in hippocampal slices from rats and mice.

2. Methods

2.1. Animals, slice preparation and experimental conditions

Experiments were performed on three research sites: at the University of Bristol (UoB) and Queen Mary University of London (QMUL) in the UK and at the Lunenfeld-Tanenbaum Research Institute (LTRI, Mount Sinai Hospital) in Canada. In the UK, synaptic plasticity experiments were performed after institutional approval and in accordance with national (UK Scientific Procedures Act, 1986) and EU guidelines for animal care in dorsal (UoB) and ventral (QMUL) hippocampal slices from adult Wistar rats (purchased from Harlan UK and Charles River UK, respectively) and in P14 Wistar rats (raised at UoB). In Canada experiments were approved by the animal care committee at The National Centre for Phenogenomics (TCP, LTRI), conforming to the Canadian Council on Animal Care guidelines, and performed in dorsal hippocampal slices from C57BL/6J mice that were bred and maintained at TCP.

Detailed experimental procedures have been published previously (Bartlett et al., 2007; France et al., 2017; Volianskis et al., 2013a; Volianskis and Jensen, 2003). Briefly, the animals were anaesthetised and decapitated after cervical dislocation, their brains were extracted and cooled down in artificial cerebrospinal fluid (ACSF). The hippocampal slices (400 μ m) were prepared by either using a Microslicer (DSK DTK-1000) for P14 rats, producing parasagittal hippocampal slices from the whole brain (France et al., 2017), or by using McIlwain tissue chopper to produce transverse hippocampal slices from either dorsal or ventral hippocampus after the hippocampal dissection from adults rats, and all ages of mice (Volianskis et al., 2010, 2013a; Volianskis and Jensen, 2003). Slices were allowed to recover for 2 h at room temperature, prior to commencing the electrophysiological recordings. Experiments in the P14 rats were conducted in an interface chamber (Bartlett et al., 2007; France et al., 2017) whilst submerged chambers were used for adult rats and all ages of mice (Volianskis et al., 2010, 2013a;

Volianskis and Jensen, 2003).

2.2. Chemicals, solutions and drug application

All salts were purchased from either Fisher Scientific or Sigma. ACSF in P14 rat experiments was composed of 124 mM NaCl, 26 mM NaHCO₃, 3 mM KCl, 1.4 mM NaH₂PO₄, 1 mM MgSO₄, 2 mM CaCl₂ and 10 mM D-glucose, saturated with 95% O₂ – 5% CO₂ at 28 ± 1 °C. In experiments in adult rats, and in mice, ACSF was composed of 124 mM NaCl, 26 mM NaHCO₃, 3.5 mM KCl, 1.25 mM NaH₂PO₄, 2 mM MgSO₄, 2 mM CaCl₂ and 10 mM D-glucose, saturated with 95% O₂ – 5% CO₂ at 37 °C.

UBP709 (Irvine et al., 2015), UBP714 (Irvine et al., 2012) and NVP-AMM077 were synthesised at the University of Bristol. CIQ was purchased from BRANDT Labs, LLC (Atlanta, US). Ro 25-6981, LY341495 and DHPG were purchased from ABCAM UK. All compounds were prepared as stock solutions and added to ACSF as indicated in the Results. UBP714 was prepared in equimolar concentrations of NaOH and stored at room temperature. UBP709 and CIQ were dissolved in DMSO, stored at room temperature and applied to slices with 1 mM 2-(hydroxypropyl)-β-cyclodextrin (purchased from Alfa Aesar) in ACSF to improve solubility (Volgraf et al., 2016), which was also used as control vehicle in interleaved experiments. The rest of the compounds were water soluble. They were prepared as stock solutions, stored frozen and added to ACSF during the experiments, as described previously (France et al., 2017; Volianskis et al., 2013a).

2.3. Electrophysiological recordings, data analysis, presentation and statistics

In all hippocampal slice experiments, f-EPSPs were recorded in the stratum radiatum of the CA1 area in response to the stimulation of the Schaffer collaterals, filtered at 3 kHz and digitised at 20 kHz (CA-1000, National Instruments) by using WinLTP (Anderson and Collingridge, 2007; Anderson et al., 2012). Baseline f-EPSPs were monitored at 0.033 Hz in P14 and at 0.067 Hz in adult slices. STP and LTP were induced by TBS (4 pulses at 100 Hz repeated at 5 Hz for 2 or 10 bursts and 5 pulses were given for 5 Hz) in rats and mice, whilst HFS (100 Hz 1 s) was used in P14 rats. LTD was induced by LFS (1 Hz, 900 pulses) or 10 Hz stimulation (900 pulses). Recordings were monitored on-line and analysed off-line to quantify changes in the slopes of the f-EPSPs.

For the analysis of STP and LTP in adult hippocampal slices (both rat and mouse) the amplitudes of STP and LTP, and the decay time constants of STP (τ), were determined as described previously (Volianskis and Jensen, 2003; Volianskis et al., 2013a) by a mono-exponential fitting routine in single experiments ($P = LTP + STP * e^{-t/\tau}$) using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA). STP is characterized by both its amplitude (percentage increase over baseline) and its duration (τ). We have therefore defined the amount of STP expressed in an individual experiment in terms of the area under its exponential decay ($STP = \text{amplitude} \times \tau$) whereas the amount of LTP was defined as amplitude (percentage increase over baseline). LTP and LTD, which were studied in P14 slices, were quantified in terms of percentage increase and decrease from baseline.

Results are presented normalised to baseline and plotted over time (mean values ± SEM). Statistical analyses between the groups were performed by using SigmaPlot and GraphPad Prism (t-tests were used if not otherwise specified, and else ANOVAs with Bonferroni post-hoc tests for multiple comparisons corrections (MCC) were used as appropriate). Statistical differences were set at $p < 0.05$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The electrophysiology of recombinant receptor currents in oocytes and HEK 293 cells have been described previously in detail in Sapkota et al. (2017).

3. Results

3.1. Effects of UBP714 and CIQ on synaptic plasticity

We have previously reported that the coumarin derivative UBP714 weakly potentiates NMDAR responses in hippocampal slices, without affecting AMPA receptor-mediated synaptic transmission (Irvine et al., 2012), however, its effects on different forms of synaptic plasticity have not yet been reported.

Application of 2 bursts of TBS (2 TBS; 8 pulses) in dorsal hippocampal slices (DHS, $n = 7$) from adult rats did not induce LTP but resulted in an STP ($25.8 \pm 6.4\%$) that declined back to baseline ($0.4 \pm 3.0\%$) with a decay time constant (τ) of 7.7 ± 2.1 min (Fig. 2A, black circles). In contrast, in the presence of 100 μ M UBP714 ($n = 9$), 2 TBS induced LTP ($23.3 \pm 6.4\%$, $t(14) = 2.948$, $p = 0.011$) but had no significant effects on the amplitude ($35.2 \pm 6.2\%$, $t(14) = 1.042$, $p = 0.32$) or the decay time constant of STP (10.6 ± 3.9 min, $t(14) = 0.6017$, $p = 0.56$), when compared to those without the application of the compound (Fig. 2A, open circles). UBP714 had no effect on the induction of STP ($52.3 \pm 5.0\%$ vs $55.6 \pm 9.1\%$; $t(16) = 0.3459$, $p = 0.74$), its decay time constant (13.6 ± 1.8 min vs 16.0 ± 5.4 min; $t(16) = 0.4993$, $p = 0.62$) or LTP ($40.2 \pm 4.8\%$ vs $32.1 \pm 8.4\%$; $t(16) = 0.9038$, $p = 0.38$) in experiments that used 10 bursts of TBS (10 TBS; 40 pulses in total), which produce saturated levels of LTP in DHS (Fig. 2A, black vs open squares, control $n = 11$ and UBP714 $n = 7$, respectively). We can therefore conclude that UBP714 potentiates LTP that is induced by subthreshold TBS without affecting STP.

Similar results were obtained in DHS from adult mice (Fig. 2B), which generally showed somewhat higher levels of STP and LTP than we observed in the DHS from rats. In the mouse slices ($n = 9$, Fig. 2B, black circles) 2 bursts of TBS induced a larger STP ($40.2 \pm 5.3\%$) that decayed slower (12.1 ± 1.6 min) to a higher LTP level of $6.0 \pm 2.6\%$ than in the corresponding rat experiments (Fig. 2B vs 2A). Nonetheless, application of UBP714 ($n = 8$, Fig. 2B, open circles) permitted enhanced induction of LTP ($19.4 \pm 3.9\%$, $t(15) = 2.919$, $p = 0.011$) without statistically affecting the amplitude of STP ($47.4 \pm 8.9\%$, $t(15) = 0.7141$, $p = 0.49$) when compared to interleaved experiments without the application of the compound. The decay time constant of STP was halved in the presence of UBP714 (6.6 ± 1.3 min, $t(15) = 2.625$, $p = 0.019$). The potentiating effects of UBP714 on LTP were not observed in 5 TBS (25 pulses) experiments in mice ($40.4 \pm 7.8\%$ vs $49.6 \pm 5.2\%$, controls and UBP714 respectively, $t(19) = 0.9608$, $p = 0.35$). The levels of STP ($63.5 \pm 9.6\%$ vs $55.0 \pm 8.0\%$, $t(19) = 0.6725$, $p = 0.51$) were also similar when compared between the controls and the UBP714 group, and there was no effect on the decay time constant of STP (12.2 ± 1.9 min vs 9.6 ± 2.0 min, $t(19) = 0.9427$, $p = 0.36$).

We next examined STP and LTP in ventral rat hippocampal slices (VHS, Fig. 2C). 10 TBS induced a lower magnitude of STP ($28.1 \pm 4.3\%$) and LTP ($15.8 \pm 2.6\%$) in VHS ($n = 14$) than DHS (Fig. 2C, black squares vs 2A and 2B) and we investigated whether UBP714 could potentiate the induction of LTP in VHS (Fig. 2C, open squares). Here, UBP714 ($n = 6$) did not affect the amplitude of STP ($29.2 \pm 4.3\%$; $p = 0.99$), but reduced its decay time constant (1.9 ± 0.4 min vs 5.9 ± 0.9 min, UBP714 vs control, $p = 0.0387$) and increased the level of LTP ($34.4 \pm 5.1\%$; $p = 0.0018$) when compared to the control without the application of the compound (Fig. 2C, black vs open squares, ANOVA with MCC was used for all analyses in VHS). In interleaved experiments, we also investigated the effects of CIQ, which selectively potentiates GluN2C/D receptors in contrast to GluN2A/2B that are potentiated by UBP714. CIQ ($n = 6$) potentiated induction of STP ($59.2 \pm 7.1\%$, $p = 0.0008$) and increased its decay time constant (10.8 ± 1.6 min, $p = 0.0095$) without affecting the induction of LTP ($22.7 \pm 2.9\%$, $p = 0.34$) when compared to the control experiments without the application of the compound (Fig. 2C, orange vs black squares, MCC).

We next investigated whether UBP714 would have any effects on LTD, and tested its induction in parasagittal P14 slices from rat (Fig. 2D)

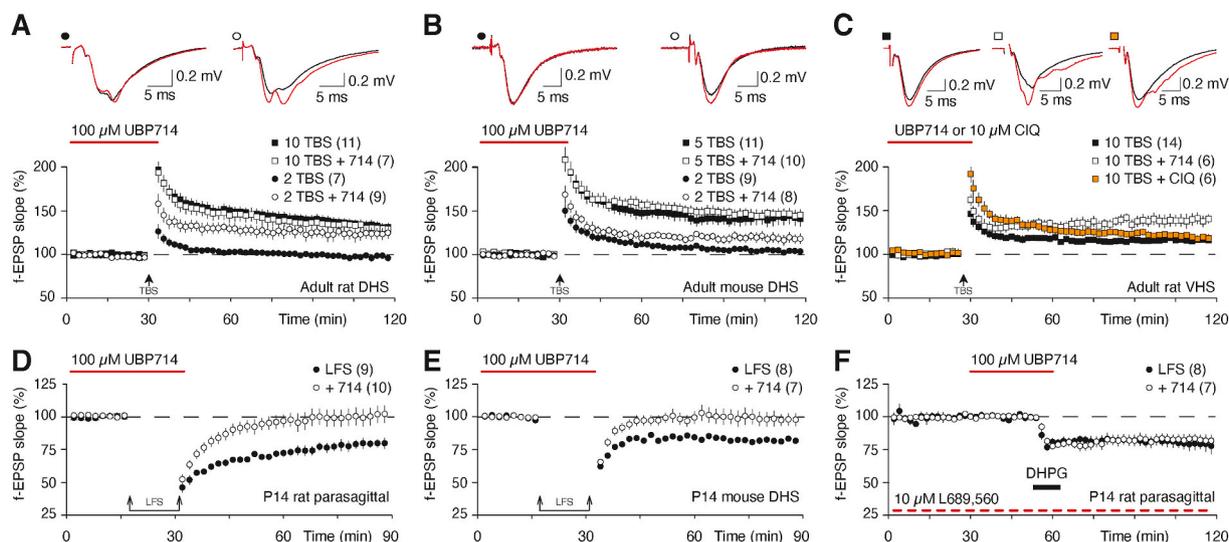


Fig. 2. Effects of UB714 and CIQ on synaptic plasticity.

(A) In adult rat dorsal hippocampal slices (DHS), a 2 burst TBS (black circles, $n = 7$) induces a small STP but not LTP, which is induced after the application of UB714 (open circles, $n = 9$). f-EPSPs from a control experiment and from an experiment with UB714 (as indicated by the symbols, last 5 min of baseline in black and last 5 min of the experiment in red). Insets show the scalebars. 10 burst TBS (black squares, $n = 11$) induces both STP and LTP and application of UB714 has no further effects on plasticity (open squares, $n = 7$).

(B) In adult mouse DHS, 2 TBS induces STP without LTP (black circles, $n = 9$), which can be induced after the application UB714 (open circles, $n = 8$). Induction of STP or LTP by 5 TBS (black squares, $n = 11$) is not affected by UB714 (open squares, $n = 10$).

(C) In adult ventral hippocampal slices (VHS) 10 TBS induces a smaller LTP (black squares, $n = 14$) than in the DHS in A. UB714 potentiates the induction of LTP by 10 TBS in VHS (open squares, $n = 6$, $F(2,23) = 9.182$; $p = 0.0012$), whereas the GluN2C/GluN2D PAM CIQ potentiates the amplitude of STP (orange squares, $n = 6$, $F(2,23) = 7.292$; $p = 0.0035$). Decay time constant of STP is reduced by UB714 and increased by CIQ ($F(2,23) = 11.43$; $p = 0.0004$). P values for the individual comparisons (after Bonferroni's MCC) are given in the Results.

(D) In parasagittal slices from P14 rats LFS induces LTD (black circles, $n = 9$), which is inhibited by UB714 (open circles, $n = 10$).

(E) In DHS from P14 mice LFS induces LTD (black circles, $n = 8$), which is inhibited by UB714 (open circles, $n = 7$).

(F) UB714 (open circles, $n = 7$) has no effect on the induction of DHPG-LTD (black circles, $n = 8$) in P14 parasagittal slices. Dashed bar indicates the application of L689,560.

and transverse DHS from P14 mice (Fig. 2E). In control experiments LFS induced robust LTD in both rats ($-22.5 \pm 3.0\%$, $n = 9$) and mice ($-17.9 \pm 1.9\%$, $n = 8$). However, in the presence of UB714 LFS failed to induce LTD in rats ($5.4 \pm 4.9\%$, $n = 10$; $t(17) = 4.724$, $p < 0.0002$) and in mice ($-1.8 \pm 5.0\%$, $n = 7$; $t(13) = 3.170$, $p = 0.0074$), when compared to their respective controls. Group I mGluR LTD (Fig. 2F), which was induced by application of $100 \mu\text{M}$ DHPG in the presence of $10 \mu\text{M}$ of GluN1 antagonist L689,560, was not affected by UB714 in P14 rat slices ($-21.1 \pm 5.2\%$ vs $-17.9 \pm 4.1\%$, $t(13) = 0.4731$, $p = 0.64$; control $n = 8$ and UB714 $n = 7$, respectively).

In summary, the GluN2A/2B preferring PAM, UB714, facilitates LTP and inhibits LTD, whereas the GluN2C/D selective PAM, CIQ, facilitates STP. This demonstrates a pharmacological dissociation between STP, LTP and LTD based on the use of PAMs.

3.2. Effects of UB709 on synaptic plasticity

The phenanthrene derivative UB709 (Fig. 3A), compound 19c in Irvine et al. (2015), in contrast to both UB714 (Irvine et al., 2012) and CIQ (Mullasseril et al., 2010), shows no NMDAR subunit preference, potentiating all GluN2 subunit function by ~ 30 – 50% , in recombinant NMDARs expressed in oocytes, at a concentration of $100 \mu\text{M}$ (Fig. 3B). We found that UB709 ($300 \mu\text{M}$) dramatically prolongs the deactivation time constant of GluN1/2A containing receptor currents ($428.6 \pm 64.4\%$, Fig. 3C, $n = 8$) in HEK 293 cells, whilst having only a modest effect on the current amplitude ($38.3 \pm 7.8\%$, Fig. 3C). We hypothesised that UB709, due to its effects on all subunits, might potentiate LTD in-line with the observations that application of exogenous GluN2 agonists (e.g. NMDA) induces LTD.

Perfusion of $300 \mu\text{M}$ UB709 to parasagittal hippocampal slices from P14 rats had no effect on baseline synaptic transmission (Fig. 3D).

However, $300 \mu\text{M}$ UB709 potentiated LTD ($-45.7 \pm 3.9\%$, $n = 9$, open circles, $p = 0.0006$, MCC) induced by 1 Hz LFS (900 stimuli), when compared to the controls ($-23.8 \pm 2.3\%$, $n = 20$, black circles, Fig. 3D and E). The effect of UB709 was concentration dependent (Fig. 3E): LTD was not significantly increased by $30 \mu\text{M}$ UB709 ($-28.2 \pm 5.8\%$, $n = 5$, $p = 0.99$, MCC) but was enhanced significantly by $100 \mu\text{M}$ ($-41.1 \pm 4.6\%$, $n = 9$, $p = 0.0016$, MCC) of the compound.

We tested UB709 on the induction of LTP in the P14 rat slices and its effects were the inverse of those on LTD (Fig. 3F and G). LTP ($63.2 \pm 6.8\%$, $n = 12$, black circles), which was induced by HFS (100 Hz, 1 s), decreased with increasing concentrations of UB709. Thus, in the presence of $300 \mu\text{M}$ UB709, LTP ($29.0 \pm 11.1\%$, $n = 6$, $p < 0.0138$, MCC) was significantly smaller than in the control, whilst in $100 \mu\text{M}$ UB709 it was not ($52.4 \pm 6.6\%$, $n = 9$, $p = 0.6$, MCC, Fig. 3G).

When 900 stimuli were delivered at a 10 Hz frequency, this protocol did not induce LTD but resulted in a brief transient depression, which returned back to baseline ($-1.2 \pm 6.0\%$, $n = 12$, Fig. 3H and J, black circles). However, in the presence of $300 \mu\text{M}$ UB709, 10 Hz stimulation resulted in a reliable induction of LTD when compared to the control experiments without the application of the compound ($-25.1 \pm 5.7\%$, $p = 0.0316$ MCC, $n = 7$, Fig. 3H and J, open circles). Induction of LTD by 10 Hz stimulation with UB709 was not inhibited by application of $50 \mu\text{M}$ AP5 (not shown) or by $1 \mu\text{M}$ of the GluN2B antagonist Ro ($-35.1 \pm 3.0\%$, $p = 0.0226$ MMC, $n = 3$, Fig. 3H and J, green circles), when compared to the control. Such LTD was also not inhibited by $100 \mu\text{M}$ of mGluR antagonist LY341495 ($-45.7 \pm 5.6\%$, $p = 0.0019$ MMC, $n = 3$, Fig. 3H and J, yellow circles), a concentration that is known to inhibit all mGluRs (Fitzjohn et al., 1998). We next tested whether coactivation of NMDARs and mGluRs was responsible for the induction of LTD by 10 Hz stimulation in the presence of UB709 (Fig. 3I and J). A co-application of $1 \mu\text{M}$ Ro and $100 \mu\text{M}$ LY341495 ($-2.4 \pm 4.5\%$, $n = 4$, $p = 0.99$ MMC,

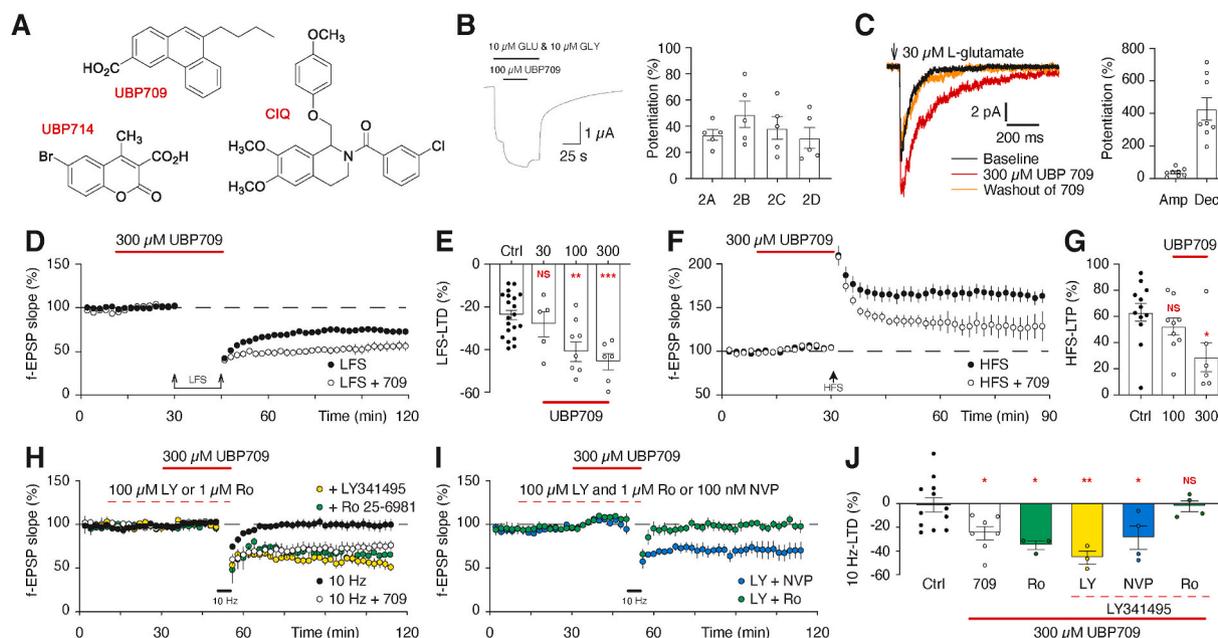


Fig. 3. UB709 is a pan-PAM that potentiates LTD and reduces LTP.

(A) The structure of UB709 when compared to UB714 and CIQ.

(B) In *Xenopus* oocytes transfected with GluN1/GluN2 subunits, UB709 potentiates recombinant GluN1/GluN2B NMDAR currents (left figure) that were evoked by 10 μ M glutamate (GLU) and 10 μ M glycine (GLY). Average potentiation of GluN1/GluN2 currents by 100 μ M UB709 (right figure): GluN2A ($33.5 \pm 4.1\%$), GluN2B ($49.2 \pm 10.1\%$), GluN2C ($38.7 \pm 8.6\%$) and GluN2D ($31.2 \pm 8.1\%$), $n = 5$ for all experiments.

(C) UB709 prolongs the decay of GluN1/2A receptor currents in HEK 293 cells (fast application of glutamate, glycine was present in the bath). The bars to the right show the mean potentiation of the amplitudes (Amp) and decay time constants (Dec) of the currents.

(D) LTD induced by LFS (black circles, $n = 20$) is potentiated by UB709 (open circles, $n = 9$). All plasticity experiments were performed in parasagittal slices from P14 rats.

(E) UB709 potentiates LTD in a concentration-dependent manner, with 300 μ M having the largest effect ($F(3,36) = 8.514$, $p = 0.0002$). P values for the individual comparisons (after Bonferroni's MCC) are given in the Results.

(F) LTP induced by HFS (black circles, $n = 12$) is inhibited by UB709 (open circles, $n = 6$).

(G) UB709 inhibits LTP in a concentration-dependent manner, with 300 μ M having the largest effect ($F(2,24) = 4.372$, $p = 0.024$), P values in the Results.

(H) 10 Hz stimulation (900 pulses) does not induce LTD (black circles, $n = 12$) under the control conditions but induces LTD after application of UB709 (open circles, $n = 7$). Such 10 Hz LTD is not inhibited by Ro 25-6981 (Ro, green circles, $n = 3$) or LY341495 (yellow circles, $n = 3$), the timing of their application is indicated by the dashed bar.

(I) Co-application of Ro and LY341495 (green circles, $n = 4$), but not NVP and LY341495 (blue circles, $n = 4$), inhibits the induction of LTD by 10 Hz stimulation after application of UB709.

(J) One way ANOVA ($F(5,27) = 5.786$, $p = 0.0009$) with MCC (in the Results) confirms that 10 Hz stimulation can induce LTD after perfusion of 709 and such LTD is only prevented by a combination of Ro and LY341495.

green circles), but not 0.1 μ M NVP and 100 μ M LY341495 ($-28.8 \pm 9.8\%$, $n = 4$, $p = 0.0444$ MMC, blue circles), inhibited the 10 Hz LTD when compared to the controls, in response to 10 Hz stimulation alone (Fig. 3J). A full LTD inhibition was also seen when 50 μ M AP5 was coapplied with 100 μ M LY341495 (not shown).

In summary, in P14 hippocampal slices, the pan-PAM UB709 potentiates the induction of LTD and reduces induction of LTP in a concentration-dependent manner. UB709 also permits induction of LTD by 10 Hz stimulation and this relies on co-activation of GluN2B containing NMDARs and mGluRs.

4. Discussion

4.1. Positive allosteric modulation as a strategy for dissociation of STP, LTP and LTD

STP, LTP and LTD have been dissociated pharmacologically by using different NMDAR antagonists (reviewed above in 1.4) and we hypothesised here that a similar pharmacological separation should be possible using PAMs of the NMDARs.

Indeed, we found that the GluN2A/2B preferring PAM UB714 enabled LTP by a subthreshold stimulus in adult DHS from both rats and mice. UB714 did not affect STP in rats but reduced its decay time

constant in the mice, which may be an indirect effect via the potentiation of LTP or STP1. We have suggested previously that triheteromeric, GluN2A/2B-containing synaptic receptors (Volianskis et al., 2013a), are specifically involved in LTP and UB714 might be particularly suited to facilitate activation of these receptors.

On the other hand, UB714 was not able to potentiate LTP induced by stronger stimulation in both rat and mouse. It also inhibited the induction of LTD in P14 hippocampal slices from both species. In this way, the effects of UB714 resemble those of PS on LTP and LTD, shifting the LTP/LTD induction towards potentiation of LTP (Chen et al., 2010).

Potentiating effects of UB714 were also seen in VHS, where it was able to facilitate induction of LTP whilst speeding up decay of STP; which once again is probably an indirect effect on STP1 and/or LTP. The GluN2C/2D PAM CIQ increased both the amplitude and the duration of STP, suggesting an effect on STP2. These results complement our previous observations that GluN2A/2B inhibition (e.g. by AP5 or NVP) reduces LTP in a concentration-dependent manner (Volianskis et al., 2013a) whilst inhibition of the slow STP2 can be achieved when using the GluN2C/2D antagonist UB145 or the GluN2B antagonist Ro (Volianskis et al., 2013a). Indeed, there are similarities between the NMDAR subtypes that are involved in STP and those involved in juvenile LTD.

Exogenous application of NMDA reliably induces LTD (reviewed in

1.5) and we hypothesised that indiscriminate potentiation of all GluN2 subunits using a pan PAM might favour LTD induction. We show here that UBP709 potentiates all GluN2s by about 30–50% and that in hippocampal slices from P14 rats UBP709 shifts the LTP/LTD induction towards LTD. To the best of our knowledge, this is the first time that a facilitation of LTD and a reduction of LTP has been demonstrated experimentally by using an NMDAR PAM.

Intriguingly, UBP709 permitted induction of LTD whilst using 10 Hz frequency of stimulation, which does not induce LTD under the control conditions. 10 Hz-induced LTD depended on co-activation of GluN2B-containing NMDARs and mGluRs, highlighting the importance of the interaction between ionotropic and metabotropic receptor signalling systems in the induction of LTD, and also their convergence. Complex pre- and post-synaptic interactions between mGluRs and NMDARs, which reciprocally facilitate receptor function, have been described previously (e.g. Fitzjohn et al., 1996; Palmer et al., 1997; Krieger et al., 2000; Luccini et al., 2007; Perroy et al., 2008; Tigaret et al., 2018; for review see Reiner and Levitz, 2018). The molecular mechanisms through which these systems participate in the induction of LTD in juvenile hippocampus remain to be addressed in future studies.

4.2. Conclusion

We have shown here that it is possible to modulate STP, LTP or LTD by using different NMDAR PAMs. These effects can be achieved by relatively weak modulation of NMDARs, which has implications for the potential therapeutic use of PAMs. Strong modulators of NMDARs, such as full agonists or potent PAMs, are likely to favour LTD and be neurotoxic. Our data further support the evidence that: (1) GluN2C/2D receptors are involved in STP, (2) GluN2A/2B receptors are involved in LTP, and (3) GluN2Bs are important for LTD, where under some circumstances they may be co-activated with mGluRs.

Compounds, drugs and amino acids

24(S)-hydrocholesterol ((3S,8S,9S,10R,13R,14S,17R)-17-[(2R)-5-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol); AP5 (D(-)-2-Amino-5-phosphonopentanoic acid); ATP (Adenosine 5'-triphosphate disodium salt); CIQ ((3-Chlorophenyl) [3,4-dihydro-6,7-dimethoxy-1-[(4-methoxyphenoxy)methyl]-2(1H)-isoquinolinyl]methanone); CPP ((RS)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic); DHPG ((S)-3,5-Dihydroxyphenylglycine); Glycine (Aminoethanoic acid); Histamine (2-(4-Imidazolyl)ethylamine dihydrochloride); Ketamine (2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride); L-689,560 (*trans*-2-Carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4-tetrahydroquinoline); LY341495 ((2S)-2-Amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl)propanoic acid); MK-801 ((5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate); NMDA (N-Methyl-D-aspartic acid); NVP (NVP-AAM077); PPDA ((2S*,3R*)-1-(Phenanthren-2-carbonyl)piperazine-2,3-dicarboxylic acid); PS (pregnenolone sulphate, ((3 β)-3-(Sulfooxy)-pregn-5-en-20-one sodium salt); Ro 25-6981 (α R, β S)- α -(4-Hydroxyphenyl)- β -methyl-4-(phenylmethyl)-1-piperidinepropanol maleate); SGE-201 ((3S,8S,9S,10R,13R,14S,17R)-17-((R)-5-hydroxy-5-methylhexan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol); SGE-301 ((3S,8S,9S,10R,13R,14S,17R)-17-((R)-5-hydroxy-5-methylhexan-2-yl)-3,10,13-trimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol); Spermine (N,N'-Bis(3-aminopropyl)-1,4-butanediamine tetrahydrochloride); Taurine (2-Aminoethylsulfonic acid); Tetrodotoxin (Octahydro-12-(hydroxymethyl)-2-imino-5,9,7,10a-dimethano-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol); UBP145 ((2R*,3S*)-1-(9-bromophenanthrene-3-carbonyl)piperazine-2,3-dicarboxylic acid); UBP709 (9-*n*-butylphenanthrene-3-carboxylic

acid); UBP714 (6-bromo-4-methylcoumarin-3-carboxylic acid).

Author contribution

GF, RV, RI, NB, AV contributed and analysed the data. MWI, GF, ESB, RR, KS, BMC, DAC were involved in synthesis and characterisation of compounds. SMD, ATMT, DTM, JG, ZAB, DEJ, GLC, AV were involved in design and supervision of the studies. RV and AV wrote the manuscript and all authors edited and approved the final version.

Declaration of competing interest

The authors have no competing interests to declare.

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