Differentiation of short-term potentiation (STP), long-term potentiation (LTP) and long-term depression (LTD) by structurally diverse NMDA receptor subunit-specific positive allosteric modulators


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 and reduces LTD; and (iii) UBP709 reduces LTD. 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, dementia, etc.).
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 Stanišiūtė, 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, 1992; 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-tetanization potentiation (PTP). PTP is caused by pre-synaptic accumulation of [Ca^{2+}] (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 LTD 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 LTD 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 LTD (Volianskis and Jensen, 2003). Whilst LTD 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 LTD 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, LTD 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 LTD and (ii) too fast for its stable maintenance. It has been shown, however, that single synapses can express STP and LTD 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 LTD 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).
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 regulated of the channel pore (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-Otano 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 GluN1 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 Dingleline, 1988), together with a depolarisation of the neuronal membrane that relieves the voltage-dependent Mg$^{2+}$ 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 Mg$^{2+}$ block, have higher open probability, Ca$^{2+}$ 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 temporospatial 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 Paolelli, 2011; Paolelli et al., 2013; Stroebel et al., 2018). GluN2A subunits are minimally expressed at birth with the 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 (Paolelli, 2011; Paolelli et al., 2013). Expression of GluN2C subunits in neurones, 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 (Paolelli, 2011; Paolelli 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).

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. APS (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., 2016), when compared to the control STP and LTP that are induced by TBS (black circles).

(C) APS (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 × t) 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 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 GluN2D subunits are inhibited by ketamine, and other NMDAR channel blockers, at lower IC50 values when compared to GluN2A/2B-containing NMDA receptors.
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 (≥ 10 × IC50), which act at glutamate (AP5, Fig. 1A and C, open symbols) or glycine (L-glutamate; Shahi and Babiec et al., 2014). Here, in the absence of 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 metabolotropic action of NMDARs, elicited by glutamate binding to the GluN2 subunits, might be sufficient for induction of LTD (Malinow et al., 2016; Nabavi et al., 2013; Stein et al., 2021) and even LTD (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 (Harabetova 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 (Harabetova 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 LTD in either P14 (France et al., 2017) or adult (Volianskis et al., 2013a) rat hippocampal slices, although partial inhibition of LTD 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 LTD, termed STP2 (Volianskis et al., 2013a; France et al., 2017; Eapen et al., 2021). The fast-decaying component of LTD that is not sensitive to inhibition by UBP145 is termed STP1 (Fig. 1A, purple circles). The results of UBP145 on LTD were mimicked by 1 μM of Ro 26-6981 (Ro), which is a highly selective non-competitive allosteric modulator (NAM) of GluN2B receptors. 1 μM Ro inhibited induction of STP2 and spared induction of STP1, whilst having no effect on LTD 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 LTD in adult than in P14 tissue. In adult rat hippocampal slices STP1 and LTD 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 LTD in slices of P14 hippocampus whilst 1 μM of the antagonist resulted in complete inhibition of both LTD and STP, 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 LTD to GluN2A inhibition changes with development.

A preferential involvement of GluN2A subunits in LTD 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 induction 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 anesthetic concentrations of the dissociative anesthetic 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 LTD, whilst inverse selectivity is observed with NVP (Fig. 1D). LTD 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, LTD 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 Mg2+ 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 LTD 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 [Ca2+]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 [Ca2+]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). LTD 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). LTD 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 LTD in slices from adult rats (Shahi and Baudry, 1993; Shahi et al., 1993) whilst in neonatal slices, in which induction of LTD is unfavourable, glycine induces LTD (Shahi et al., 1993). Glycine-induced LTD was replicated in organotypic cultures (Muselet 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 Mg2+ 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 LTD 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., 2016; 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 (Burnan 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 μM) to hippocampal slices causes a transient depression of responses, followed by a slowly developing LTP (Fuji et al., 1999; Wierszak and Seyfried, 1989), which is [Ca²⁺]₀-dependent and is blocked by AP5 (Fuji et al., 1999, 2002). Higher concentrations of ATP (e.g. 250 μM) produce an LTD like effect, which can be reversed by 3,4-diaminopyridine (Wierszak and Seyfried, 1989). ATP-induced LTD is unlikely to be NMDAR-dependent as it can be induced in [Ca²⁺]₀-free solutions and AP5 can transform ATP-induced LTP into an ATP-LTD (Fuji 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 (Klöda 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., 2016; Hackos and Hanson, 2017; Irvine et al., 2012, 2015, 2019; Monaghan et al., 2012; Mosley et al., 2010; Saptota 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 LTD 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 would 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 (L TRI, 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 μm) 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;
All salts were purchased from either Fisher Scientific or Sigma. ACSF in P14 rat experiments was composed of 124 mM NaCl, 26 mM NaHCO$_3$, 3 mM KCl, 1.4 mM NaH$_2$PO$_4$, 1 mM MgSO$_4$, 2 mM CaCl$_2$ and 10 mM d-glucose, saturated with 95% O$_2$ – 5% CO$_2$ at 28 ± 1 °C. In experiments in adult rats, and in mice, ACSF was composed of 124 mM NaCl, 26 mM NaHCO$_3$, 3.5 mM KCl, 1.25 mM NaH$_2$PO$_4$, 2 mM MgSO$_4$, 2 mM CaCl$_2$ and 10 mM d-glucose, saturated with 95% O$_2$ – 5% CO$_2$ 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. The rest of the compounds were water soluble. They were prepared as stock solutions, stored frozen and stored at room temperature. UBP709 and CIQ were dissolved in DMSO, stored at room temperature and applied to slices with 1 mM DMSO, stored at room temperature and applied to slices with 1 mM DMSO, and stored at room temperature. UBP709 and CIQ were dissolved in DMSO, stored at room temperature and applied to slices with 1 mM DMSO, and stored at room temperature. UBP709 and CIQ were dissolved in DMSO, stored at room temperature and applied to slices with 1 mM DMSO, and stored at room temperature. UBP709 and CIQ were dissolved in DMSO, stored at room temperature and applied to slices with 1 mM DMSO, and stored at room temperature. UBP709 and CIQ were dissolved in DMSO, stored at room temperature and applied to slices with 1 mM DMSO, and stored at room temperature.
and transverse DHS from P14 mice (Fig. 2E). In control experiments LFS induced robust LTD in both rats (−22.5 ± 3.0%, n = 9) and mice (−17.9 ± 1.9%, n = 8). However, in the presence of UBP714 LTD failed to induce LTD in rats (5.4 ± 4.9%, n = 10; t(17) = 4.724, p < 0.0002) and in mice (−1.8 ± 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 μM DHPG in the presence of 10 μM of GluN1 antagonist L689,560, was not affected by UBP714 in P14 rat slices (−21.1 ± 5.2% vs. −17.9 ± 4.1%; t(13) = 0.4731, p = 0.64; control n = 8 and UBP714 n = 7, respectively).

In summary, the GluN2A/2B preferring PAM, UBP714, facilitates LTD and inhibits LTD, whereas the GluN2C/D selective PIQ, CIQ, facilitates STP. This demonstrates a pharmacological dissociation between STP, LTD and LTD based on the use of PAMs.

3.2. Effects of UBP709 on synaptic plasticity

The phenanthrene derivative UBP709 (Fig. 3A), compound 19c in Irvine et al. (2015), in contrast to both UBP714 (Irvine et al., 2012) and CIQ (Mullasserril 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 μM (Fig. 3B). We found that UBP709 (300 μM) dramatically prolongs the deactivation time constant of GluN1/2A containing receptor currents (428.6 ± 64.4%, Fig. 3C, n = 8) in HEK 293 cells, whilst having only a modest effect on the current amplitude (38.3 ± 7.8%, Fig. 3D). We hypothesised that UBP709, 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 μM UBP709 to parasagittal hippocampal slices from P14 rats had no effect on baseline synaptic transmission (Fig. 3D). However, 300 μM UBP709 potentiated LTD (−45.7 ± 3.9%, n = 9, open circles, p = 0.0006, MCC) induced by 1 Hz LFS (900 stimuli), when compared to the controls (−23.8 ± 2.3%, n = 20, black circles, Fig. 3D and E). The effect of UBP709 was concentration dependent (Fig. 3E): LTD was not significantly increased by 30 μM UBP709 (−28.2 ± 5.8, n = 5, p = 0.99, MCC) but was enhanced significantly by 100 μM (−41.1 ± 4.6, n = 9, p = 0.0016, MCC) of the compound.

We tested UBP709 on the induction of LTD in the P14 rat slices and its effects were the inverse of those on LTD (Fig. 3F and G). LTD (63.2 ± 6.8%, n = 12, black circles), which was induced by HFS (100 Hz, 1 s), decreased with increasing concentrations of UBP709. Thus, in the presence of 300 μM UBP709, LTD (29.0 ± 11.1%, n = 6, p < 0.0138, MCC) was significantly smaller than in the control, whilst in 100 μM UBP709 it was not (52.4 ± 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 ± 6.0%, n = 12, Fig. 3H and J, black circles). However, in the presence of 300 μM UBP709, 10 Hz stimulation resulted in a reliable induction of LTD when compared to the control experiments without the application of the compound (−25.1 ± 5.7%, p = 0.0316 MCC, n = 7, Fig. 3H and J, open circles). Induction of LTD by 10 Hz stimulation with UBP709 was not inhibited by application of 50 μM AP5 (not shown) or by 1 μM of the GluN2B antagonist Ro (−35.1 ± 3.0%, p = 0.0226 MCC, n = 3, Fig. 3H and J, green circles), when compared to the control. Such LTD was also not inhibited by 100 μM of mGluR antagonist LY341495 (−45.7 ± 5.6%, p = 0.0019 MCC, 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 UBP709 (Fig. 3I and J). A co-application of 1 μM Ro and 100 μM LY341495 (−2.4 ± 4.5%, n = 4, p = 0.99 MCC,
Indeed, there are similarities between the GluN2A/2B preferring PAM UBP714 and CIQ. Indeed, we found that the GluN2A/2B preferring PAM UBP714 enabled LTD by a subthreshold stimulus in adult DHS from both rats and mice. UBP714 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, GluN2C/2D-containing synaptic receptors (Volianskis et al., 2013a), which once again is probably an indirect effect on STP1 and/or LTP. The effects of UBP714 resemble those of PS on LTP and LTD, shifting the potentiation of LTD towards potentiation of LTP (Chen et al., 2010). POTentiating effects of UBP714 were also seen in VHS, where it was able to facilitate activation of these receptors.

In the other hand, UBP714 was not able to potentiate LTD induced by stronger stimulation in both rat and mouse. It also inhibited the induction of LTD after application of UBP709 (open circles, n = 4). 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.

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 hypothesized here that a similar pharmacological separation should be possible using PAMs of the NMDARs.

Indeed, we found that the GluN2A/2B preferring PAM UBP714 enabled LTD by a subthreshold stimulus in adult DHS from both rats and mice. UBP714 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 UBP714 might be particular suited to facilitate activation of these receptors.

On the other hand, UBP714 was not able to potentiate LTD 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 UBP714 resemble those of PS on LTP and LTD, shifting the LTD/LTD induction towards potentiation of LTP (Chen et al., 2010).

POTentiating effects of UBP714 were also seen in VHS, where it was able to facilitate induction of LTD 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 LTD, suggesting an effect on STP2. These results complement our previous observations that GluN2A/2B inhibition (e.g. by AP5 or NVP) reduces LTD in a concentration-dependent manner (Volianskis et al., 2013a) whilst inhibition of the slow STP2 can be achieved when using the GluN2C/2D antagonist UBP145 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; Luccioni 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 LTD, 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 LTD, (3) ‘The Neuroscience Catalyst (GLC.), (3) ‘The Neuroscience Catalyst Foundation Grant #154276 (GLC.), (4) NIH R01MH062052 (MDT and JDE), (5) MRC G0601812, MR/K023098/1 (GLC) and (6) BBSRC BB/L001977/1 (JDE), (7) GLC is supported by the Krembil Family Chair in Alzheimer’s Research.

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