Group I mGluR Induced LTD of NMDAR-synaptic Transmission at the Schaffer Collateral but not Temperoammonic Input to CA1

Stephen Fitzjohn1 Zafar Bashir1 and Paul Farrow2* 

1University of Bristol, Physiology and Pharmacology; 2Ipsen Bioinnovation, Global Drug Discovery, Neurology, UK

Abstract: NMDA receptors are composed of multiple subunits and are crucial in the induction of synaptic plasticity and learning and memory. In this study, application of the group I mGlu receptor agonist, DHPG, caused LTD of NMDA-EPSCs (DHPG-LTD$_{NMDA}$) of the Schaffer collateral, but not of NMDA-EPSCs of the temperoammonic pathway onto CA1 neurons of the hippocampus. DHPG-LTD$_{NMDA}$ did not alter the sensitivity of NMDA-EPSC to the GluN2B-antagonist, Ro25-6981, indicating that the postsynaptic NMDA receptor subunit composition remained unchanged following DHPG-LTD$_{NMDA}$. Furthermore, blockade of GluN2B receptors did not affect the induction of DHPG-LTD$_{NMDA}$. These results demonstrate a difference in the plasticity of NMDA receptors between two synapses onto the same CA1 neuron, but indicate that the subunit composition of NMDA receptors does not account for this difference.

Keywords: NMDA, CA1, DHPG, hippocampus, mGluR, plasticity.

INTRODUCTION

Principal neurons of the CA1 region receive input from the entorhinal cortex (EC) via two pathways [1]: (i) The direct temporoammonic (TA) pathway, which consists of afferents from layer III of EC and forms synapses with CA1 neurons in the stratum lacunosum moleculare; and (ii) the indirect pathway, which originates from layer II of EC and forms part of the trisynaptic pathway that terminates in Schaffer collateral (SC) synapses on CA1 neurons in the stratum radiatum.

NMDARs are heteromeric assemblies [2] of two essential GluN1 subunits and two GluN2 or GluN3 subunits. There are four GluN2 subunits (A–D) of which the GluN2A and GluN2B predominate in the forebrain. NMDARs are generally thought to be minimally active during basal synaptic transmission, but they have a key role to play during the induction of synaptic plasticity [3]. Although classically regarded as relatively stationary at the synapse, NMDA receptors undergo lateral diffusion [4] and various forms of plasticity [5-8]. Furthermore, the subunit composition of NMDA receptors can vary with development [9, 10] and following different forms of synaptic activity [11, 12].

Recently we have shown that LTD of NMDA receptor-mediated synaptic transmission can be induced by theta frequency stimulation at SC but not TA synapses and involves activation of group I mGluRs (termed mGluR-LTD; [13]). The reason for the lack of activity-dependent mGluR-LTD at TA-CA1 synapses is not clear but could be due to the afferent stimulation patterns in TA-CA1 not being ideal to activate group I mGluRs. Therefore to overcome this issue we have now used pharmacological activation of group I mGluRs to investigate whether LTD can be induced at TA-CA1 hippocampal synapses and furthermore to probe the role of GluN2B subunits in DHPG-LTD$_{NMDA}$.

Here we demonstrate that pharmacological activation of group I mGluRs induces synaptic plasticity of NMDA receptors at the SC pathway [14] but fails to induce LTD in the TA-CA1 pathway. In addition, the NR2B-selective antagonist Ro25-6981 was used to show that the subunit composition of NMDARs remains unchanged following group I mGluR-induced LTD.

MATERIALS AND METHODS

P14 male Wistar rats were killed by cervical dislocation in accordance with United Kingdom Animal (Scientific Procedures) legislation. Brains were removed and placed in ice-cold aCSF consisting of the following (in mM): NaCl 124, KCl 3, NaHCO$_3$ 26, NaH$_2$PO$_4$ 1.25, CaCl$_2$ 2, MgSO$_4$ 1, D-glucose 10 (bubbled with 95% O$_2$/5% CO$_2$). Parasagittal slices (400 µm) were cut and the hippocampus isolated. Each “n” value is from a slice from a different animal. Standard techniques were used to record excitatory postsynaptic potentials (EPSC) in response to stimulation (100 µs, 3–10 V) of the SC or TA pathways. In those experiments in which both SC-CA1 and TA-CA1 inputs were studied, alternate stimulation of both pathways was conducted in each slice. Electrodes (4–7 M) were filled with (in mM): CsMeSO$_4$, 130, NaCl 8, Mg-ATP 4, Na-GTP 0.3, EGTA 0.5, HEPES 10, QX-314 5, pH adjusted to 7.2–7.3 using CsOH and osmolarity to 275–290 mOsm with sucrose. CA1 pyramidal cells were voltage-clamped at -40 mV. Picrotoxin (50 µM) and NBQX (5 µM) were applied to isolate EPSC$_{NMDA}$. Responses were recorded and analyzed using WinLTP [15]. (RS)-DHPG (100 µM; Tocris Bioscience) was used to activate group I mGluRs and Ro25-6981 ([16]; 5 µM; Tocris...
Bioscience) as an antagonist of GluNB receptors. Experiments were carried out at room temperature (19-23 °C). Changes in synaptic strength were expressed relative to the normalized baseline (mean ± SEM) and significance tested using Student’s t-tests 30 min after LTD induction or 50 min following application of Ro25-6981. The Group II mGluR agonist DCG-IV was applied at the end of experiments to ensure selective stimulation of TA and SC inputs [17].

RESULTS

DHPG Causes LTD of NMDA Receptor-Mediated EPSCs in the SC but not the TA Pathway

After a baseline period of 10 minutes, the group I mGluR agonist (RS)-DHPG (100 µM; 10 minutes) was applied. An initial depression of NMDA receptor-mediated EPSCs (EPSC\textsubscript{NMDA}) was observed in both pathways (at the end of DHPG application responses were: SC 53.1 ± 3.3 %; TA 33.5 ± 4.4 % below baseline; P < 0.05 compared to baseline; n = 18; Fig. 1). Thirty minutes following washout of DHPG, LTD of EPSC\textsubscript{NMDA} (LTD\textsubscript{NMDA}) was observed at the SC pathway (34.9 ± 2.7 % depression; p=0.0012; n=18), which was consistent with published findings [14, 18, 19]. However, EPSC\textsubscript{NMDA} evoked by TA pathway stimulation recovered to baseline levels following DHPG washout (15.2 ± 4.2% below baseline; p = 0.764; n = 18).

THE POPULATION OF NMDA RECEPTOR SUBUNITS IS SIMILAR BETWEEN PATHWAYS

The difference in LTD\textsubscript{NMDA} between SC-CA1 and TA-CA1 might be due to differences in NMDAR subunits at the different synapses. Different NMDA receptor subunits are associated with different EPSC characteristics and the EPSC decay time constant (τ) can therefore be used as an indication of receptor subunit population at the synapse [20]. We have previously shown that the decay constant of EPSC\textsubscript{NMDA} in TA and SC synapses is similar [13], indicating a similar population of NMDA receptor subunits at the two synapses. In order to clarify this further, Ro25-6981 (Ro; a potent and selective GluN2B receptor antagonist) was applied (1 µM) and a depression of EPSC\textsubscript{NMDA} was observed in both pathways (Fig. 2). After a period of 1 hour, EPSC\textsubscript{NMDA} at the SC and the TA pathways had reduced by similar amounts (SC: 55.8 ± 7.0%; TA: 43.3 ± 5.2%; p = 0.18, n = 6), confirming our previous work suggesting that a similar population of NMDA receptor subunits are expressed at these two synaptic populations [13].

DHPG-LTD\textsubscript{NMDA} OF THE SC PATHWAY IS UNAFFECTED BY Ro25-6981

The above result suggests that it is unlikely that differences in NMDAR subunit composition within the TA-CA1 and SC-CA1 synapses explain the difference in LTD\textsubscript{NMDA} between the two inputs. However, the role of NMDA receptor subunits in the induction of LTD was not clear. Therefore, to examine directly whether the induction of LTD in the SC pathway was dependent on GluN2B activation, DHPG was applied in the presence of Ro (after 1 hour Ro application). DHPG again caused a depression of the SC pathway (30.5 ± 9.6%; Fig. 3) that was not significantly different from DHPG-LTD\textsubscript{NMDA} induced under control conditions (p = 0.617). This suggests that...
Fig. (2). **Ro25-6981 Depresses EPSC\textsubscript{NMDA} in both Pathways.** Bath application of the GluN2B antagonist Ro25-6981 (5 µM; 50 min) causes a similar depression ($p = 0.18, n = 6$) of EPSC\textsubscript{NMDA} in the SC-CA1 (red circles; 55.8 ± 7.0%) and the TA-CA1 (blue circles; 43.3 ± 5.2%) pathways. Data is normalised to the mean peak amplitude measured during the baseline period. Representative traces are shown in the top right corner (pink/light blue: baseline SC/TA, respectively; red/dark blue: following Ro25-6981 application in the SC/TA, respectively). Traces are averages of the 10 min of data from baseline and from the last 10 min of the experiment. Scale bar: 250 ms, 50 pA.

![Graph showing the depression of EPSC\textsubscript{NMDA} in both pathways with Ro25-6981 application.](image)

Fig. (3). **DHPG-LTD\textsubscript{NMDA} Unchanged in the Presence of Ro25-6981.** A Following bath application of the GluN2B antagonist Ro25-6981 (5 µM; 50 min), DHPG (100 µM; 10 min) caused a depression (30.5 ± 9.6%, $n = 6$) of NMDAR-mediated EPSCs in the SC-CA1 that was not significantly different ($p = 0.617$) from the magnitude of DHPG-LTD\textsubscript{NMDA} in the absence of Ro25-6981 (34.9 ± 2.7%). B Graph comparing the magnitude of DHPG-LTD\textsubscript{NMDA} before (pink circles) and after (red circles) application of Ro25-6981. Data is normalised to the mean peak amplitude measured during the baseline (pre-DHPG) period. Representative traces are shown in the top right corner (light pink: baseline; dark pink: following Ro25-6981 application; red: following Ro25-6981 and DHPG). Traces are averages of the 10 min of data from baseline, last 10 min of Ro25-6981 and from the last 10 min of the experiment. Scale bar: 250 ms, 50 pA.

![Graph showing the comparison of DHPG-LTD\textsubscript{NMDA} with and without Ro25-6981.](image)
DHPG-LTD\textsubscript{NMDA} in the SC pathway is independent of GluN2B subunit activation.

**Ro25-6981 Causes a Similar Depression of Both Pathways Following DHPG Application**

To examine whether there was a change in NMDAR subunit composition following LTD at the SC synapse, we compared the effect of Ro before and following DHPG-LTD; a change in efficacy of GluN2B-selective antagonists is often taken as an indication of a change in subunit composition of NMDARs (*i.e.* an increase or decrease in the complement of GluN2B receptors; *e.g.* [11]).

Thus, thirty minutes following DHPG application (100 µM; 10 minutes) when LTD was stably expressed, Ro25-6981 (1 µM; 1 hour) was bath applied. Application of DHPG caused LTD\textsubscript{NMDA} (34.0 ± 4.3 % depression). Following Ro25-6981 application, a further depression of EPSC\textsubscript{NMDA} was observed (63.5 ± 4.2% of pre-DHPG level; Fig. 4) that was not significantly different to the depression caused by Ro25-6981 under control conditions (p = 0.589), indicating that a change in GluN2B subunit composition does not occur as a result of DHPG-LTD\textsubscript{NMDA}.

**DISCUSSION**

Recently we have demonstrated a form of LTD\textsubscript{NMDA} that can be induced by synaptic stimulation at SC but not TA synapses in the hippocampus, using a short theta frequency stimulation (TFS; [13]). This form of LTD involves activation of group I mGluRs and produces metaplasticity such that the induction of subsequent LTP at the SC input is inhibited. In the current study we have utilised pharmacological activation of group I mGluRs using the agonist DHPG to elucidate whether the lack of mGluR-LTD of EPSC\textsubscript{NMDA} at the TA input to CA1 in P14 animals is due to insufficient activation of mGluRs by TFS. We now show that even with agonist activation of group I mGluRs, LTD of EPSC\textsubscript{NMDA} is not seen at the TA input. Instead, despite an initial transient depression, responses recover to baseline levels upon washout of the agonist. A previous study, using 4 - 6 week old animals, has shown that LTD can be induced.
by DHPG at TA-CA1 input (although of smaller magnitude than at SC synapses; [19]). This raises the possibility that either developmental changes may impact on the ability to induce mGluR-LTD of EPSC_{NMDA} at TA-CA1 synapses or that there is a difference in the ability to induce LTD of EPSC_{NMDA} versus LTD of EPSC_{AMPA} at TA-CA1 synapses. However, it is not possible at this stage to be able to make conclusive statements as to the impact of development on mGluR-LTD of EPSC_{NMDA} or differences between LTD of EPSC_{NMDA} versus LTD of EPSC_{AMPA} at TA-CA1 synapses.

Plasticity of EPSC_{NMDA} may involve a subunit switch in NMDAR subunits [11, 21]. As such, the ability to induce such plasticity may be dependent on the subunit composition of NMDARs in the basal state. Use of GluN2B subunit-selective antagonists, such as ifenprodil and Ro25-6981, readily allows comparison of subunit composition [11, 21]. We found that Ro25-6981 produced the same the magnitude of depression in SC and TA inputs. Similar to our previous work where we found no difference in the decay time constant of LTD_{NMDA} at the two inputs to CA1 [13], this suggests that the inability to induce LTD_{NMDA} at TA-CA1 synapses is not due to differences in GluN2B NMDA subunit composition.

The sensitivity of EPSC_{NMDA} to Ro25-6981 in the SC input was not altered following induction of LTD_{NMDA} by DHPG. Thus, a change in relative GluN2A/GluN2B subunit expression, which is seen in some forms of plasticity [11], does not underlie LTD. The precise mechanism of this form of LTD has not yet been studied. mGluR-LTD of EPSC_{AMPA} has been extensively investigated although there is no consensus on the precise mechanism of LTD and multiple signalling cascades have been implicated [22]. LTD_{NMDA} induced by TFS is expected to be postsynaptically expressed, as this form of LTD is specific for EPSC_{NMDA} vs EPSC_{AMPA}; postsynaptic expression of pharmacologically-induced LTD_{NMDA} has also been demonstrated [14, 18].

The function of LTD_{NMDA} is not currently known. However, it affects subsequent induction of long-term potentiation (LTP; [13]) and is therefore a form of metaplasity that will regulate the acquisition of hippocampal-dependent spatial information [23]. Future work will aim to elucidate the precise signalling and expression mechanisms underlying this LTD as well as its role in learning and memory.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] van Strien, N.M.; Cappaert, N.L.; Witter, M.P. The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. Nat. Rev. Neurosci., 2009, 10, 272-282. http://dx.doi.org/10.1038/nrn2614

[2] Cull-Candy, S.; Brickley, S.; Farrant, M. NMDA receptor subunits: diversity, development and disease. Curr. Opin. Neurobiol., 2001, 11, 327-335. http://dx.doi.org/10.1016/S0959-4388(00)00215-4

[3] Collingridge, G.L.; Kehl, S.J.; McLeman, H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commisural pathway of the rat hippocampus. J. Physiol., 1983, 334, 33-46. http://dx.doi.org/10.1113/jphysiol.1983.sp014478

[4] Groc, L.; Heine, M.; Cognet, L.; Brickley, K.; Stephenson, F.A.; Lounis, B.; Choquet, D. Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. Nat. Neurosci., 2004, 7, 696-696. http://dx.doi.org/10.1038/nn1270

[5] Bashir, Z.I.; Alford, S.; Davies, S.N.; Randall, A.D.; Collingridge, G.L. Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature, 1991, 349, 156-158. http://dx.doi.org/10.1038/349156a0

[6] Selig, D.K.; Lee, H.K.; Bear, M.F.; Malenka, R.C. Reexamination of the effects of MCPG on hippocampal LTP, LTD, and depotentiation. J. Neurophysiol., 1995, 74, 1075-1082. http://dx.doi.org/10.1152/1068-1440

[7] Montgomery, J.M.; Selcher, J.C.; Hanson, J.E.; Madison, D.V. Dynamin-dependent NMDA endocytosis during LTD and its dependence on synaptic state. BMC Neurosci., 2005, 6, 48. http://dx.doi.org/10.1186/1471-2202-6-48

[8] Harney, S.C.; Rowan, M.; Anwyl, R. Long-term depression of NMDA receptor-mediated synaptic transmission is dependent on activation of metabotropic glutamate receptors and is altered to long-term potentiation by low intracellular calcium buffering. J. Neurosci., 2006, 26, 1128-1132.

[9] Sheng, M.; Cummings, J.; Roldan, L.A.; Jan, Y.N.; Jan, L.Y. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature, 1994, 368, 144-147.

[10] Tovar, K.R.; Westbrook, G.L. Mobile NMDA receptors at hippocampal synapses. Neuron, 2002, 34, 255-264. http://dx.doi.org/10.1016/S0896-6273(02)00586-X

[11] Bellone, C.; Nicoll, R.A. Rapid bidirectional switching of synaptic NMDA receptors. Neuron, 2007, 55, 779-785. http://dx.doi.org/10.1016/j.neuron.2007.07.035

[12] Yashiro K; Philpot, B.D. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology, 2008, 55, 1081-1094. http://dx.doi.org/10.1016/j.neuropharm.2008.07.046

[13] Bihouri, M.; Farrow, P.A.; Motee, A.; Yan, X.; Battaglia, G.; Di Menna, L.; Riozzi, B.; Nicoletti, F.; Fitzjohn, S.M.; Bashir, Z.I. mGlu receptor-induced LTD of NMDA receptor transmission selectively at Schaffer collateral-CA1 synapses mediates metaplasticity. J. Neurosci., 2014, 34, 12223-12229. http://dx.doi.org/10.1523/JNEUROSCI.0753-14.2014

[14] Snyder, E.M.; Philpot, B.D.; Huber, K.M.; Dong, X.; Fallon, J.R.; Ikonomovic, M.F. Internalization of ionotropic glutamate receptors in response to mGluR activation. Nat. Neurosci., 2004, 7, 1079-1085. http://dx.doi.org/10.1038/nn746

[15] Anderson, W.W.; Collingridge, G.L. Capabilities of the WinLTP data acquisition program extending beyond basic LTP experimental functions. J. Neurosci. Methods, 2007, 162, 246-56. http://dx.doi.org/10.1016/j.jneumeth.2006.12.018

[16] Fischer, G.; Metul, V.; Trube, G.; Malherbe, P.; Kew, J.N.; Mohacsi, E.; Heitz, M.P.; Kemp, J.A. Ro 25-6981, a highly potent and selective blocker of N-methyl-D-aspartate receptors containing the NR2B subunit. Characterization in vitro. J. Pharmacol. Exp. Ther., 1997, 283, 1285-1292.

[17] Kew, J.N.; Ducarre, J.M.; Pflimlin, M.C.; Metul, V.; Kemp, J.A. Activity-dependent presynaptic autoinhibition by group II metabotropic glutamate receptors at the perforant path inputs to the dentate gyrus and CA1. Neuropharmacology, 2001, 40, 20-27. http://dx.doi.org/10.1016/S0028-3908(00)00118-0

[18] Ireland, D.R.; Abraham, W.C. Mechanisms of Group 1 mGluR-Dependent Long-Term Depression of NMDA Receptor-Mediated Transmission at Schaffer Collateral-CA1 Synapses. J. Neurophysiol., 2009, 101, 1375-1385. http://dx.doi.org/10.1152/jn.00643.2008

[19] Xu, J.Y.; Chen, R.; Zhang, J.; Chen, C. Endocannabinoids differentially modulate synaptic plasticity in rat hippocampal CA1 pyramidal neurons. PLoS One, 2010, 5, e10306. http://dx.doi.org/10.1371/journal.pone.0010306

[20] Cull-Candy, S.G.; Leszekiewicz, D.N. Role of distinct NMDA receptor subtypes at central synapses. Sci. STKE, 2004, 2004(255), re16. http://dx.doi.org/10.1126/stke.2552004re16
Matta, J.A.; Ashby, M.C.; Sanz-Clemente, A.; Roche, K.W.; Isaac, J.T. mGluR5 and NMDA receptors drive the experience- and activity-dependent NMDA receptor NR2B to NR2A subunit switch. *Neuron*, 2011, 70, 339-351. http://dx.doi.org/10.1016/j.neuron.2011.02.045

Gladding, C.M.; Fitzjohn, S.M.; Molnar, E. Metabotropic glutamate receptor-mediated long-term depression: molecular mechanisms. *Pharmacol. Rev.*, 2009, 61, 395-412. http://dx.doi.org/10.1124/pr.109.001735

Abraham, W.C. Metaplasticity: tuning synapses and networks for plasticity. *Nat. Rev. Neurosci.*, 2008, 9, 387. http://dx.doi.org/10.1038/nrn2356