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An analysis of the stimulus requirements for setting the molecular switch reveals a lower threshold for metaplasticity than synaptic plasticity

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ABSTRACT

The requirements for the synaptic activation of metabotropic glutamate (mGlu) receptors and for the induction of metaplasticity in the hippocampus are not known. In the present study, we have investigated the synaptic activation of $mGlu_5$ receptors and the setting of the molecular switch, a form of metaplasticity, at CA1 synapses in the mouse hippocampus. We find that as few as eight stimuli (delivered at 100 Hz) are sufficient to set the molecular switch, since a subsequent tetanus delivered to the same input is able to induce long-term potentiation (LTP) in the presence of the mGlu receptor antagonist MCPG ((S)-α-methyl-4-carboxyphenylglycine). In addition, we find that the molecular switch can be activated over a wide frequency range. When 10 shocks were delivered the threshold frequency was 4 Hz. The ability of 10 shocks (delivered at 100 Hz) to set the molecular switch was lost in the $mGlu_5$ knockout. These data show that $mGlu_5$ receptors can be activated synaptically and metaplasticity can be induced by relatively few stimuli. Indeed, metaplasticity was induced by stimuli that were subthreshold for the induction of LTP *per se*. Thus, metaplasticity has a lower threshold than the synaptic plasticity that it regulates.

1. Introduction

Most excitatory synaptic transmission in the vertebrate nervous system utilises L-glutamate as the neurotransmitter. The actions of L-glutamate are mediated, primarily, via the activation of ionotropic glutamate receptors, named after the selective agonists, AMPA, NMDA and kainate. In addition, L-glutamate activates the G-protein coupled family of metabotropic glutamate (mGlu) receptors of which eight subtypes exist ($mGlu_1$–$mGlu_8$) (Pin and Duvoisin, 1995). Activation of mGlu receptors is primarily thought to be modulatory in nature, though these receptors can contribute to a slow component of synaptic transmission and trigger the induction of synaptic plasticity (Anwyl, 1999; Massey and Bashir, 2007). In general, it is believed that whilst ionotropic glutamate receptors can be readily activated by single stimuli or short trains of stimuli, the activation of mGlu receptors requires more prolonged periods of high frequency transmission. For example, at Schaffer collateral–commissural synapses in the CA1 region of the hippocampus mGlu receptor-mediated long-term depression (LTD) requires long trains of paired-pulse stimuli (Moult et al., 2008). Surprisingly, given the importance of mGlu receptors as drug targets, little is known about the optimum requirements for the synaptic activation of mGlu receptors in the hippocampus.

NMDA receptor-dependent long-term potentiation (LTP) of AMPA receptor-mediated synaptic transmission in the CA1 region of the hippocampus is the most widely studied experimental system for exploring the molecular basis of memory (Bliss and Collingridge, 1993). The ability of synapses to undergo changes in synaptic efficacy is also subject to modification, via a process termed metaplasticity – the plasticity of synaptic plasticity (Abraham and Bear, 1996). There is growing evidence that mGlu receptors are important in various forms of metaplasticity. For example, activation of mGlu receptors facilitates the subsequent induction of NMDA receptor-dependent LTP (Cohen and Abraham, 1996) and inhibits the induction of mGlu receptor-dependent LTD (Rush et al., 2002; Wu et al., 2004).

We have described a different form of mGlu receptor-mediated metaplasticity at these synapses. Previously, we found that the synaptic activation of mGlu receptors renders the subsequent induction of LTP in the same input insensitive to the mGlu receptor antagonist (*S*)-α-methyl-4-carboxyphenylglycine (MCPG) (Bortolotto et al., 1994). To explain these results, we proposed the existence

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of an mGlu receptor-mediated molecular switch that has to be set, either during or prior to the tetanus, for LTP to be induced. Once set, the switch stays on for hours where it negates the need for the synaptic activation of mGlu receptors during the induction of additional LTP. Initially, we proposed a model that involved the activation of a single class of MCPG-sensitive mGlu receptor in both switch setting and the induction of LTP (Watkins and Collingridge, 1994). However, when we tested the effects of the potent broad spectrum ($mGlu_1$–$mGlu_8$) mGlu receptor antagonist LY341495 on both the setting of the molecular switch and the induction of LTP the results were not compatible with this simple explanation (Fitzjohn et al., 1998). We found that LY341495 blocked the setting of the molecular switch but not the induction of LTP *per se*. This suggests that there are two MCPG-sensitive receptors involved in the process; one that is required for the induction of LTP, and which has a novel pharmacology, and one that is required for setting the molecular switch, and which could be one of the known mGlu receptor subtypes. Subsequently, we were able to identify the receptor responsible for setting the molecular switch as $mGlu_5$. Thus, the setting of the molecular switch was (i) mimicked by the $mGlu_5$ agonist, (RS)-2-chloro-5-hydroxyphenylglycine (CHPG), (ii) blocked by the $mGlu_5$ antagonist 2-methyl-6-(phenylethynyl)-pyridine hydrochloride (MPEP) and (iii) the molecular switch could not be set in the $mGlu_5^{-/-}$ mouse (Bortolotto et al., 2005).

In the present investigation, we used the setting of the molecular switch as a means to monitor the synaptic activation of $mGlu_5$ receptors and the induction of metaplasticity. We were particularly interested to know what the minimum number of stimuli and minimum frequency are to set the molecular switch.

2. Methods

Experiments were performed on transverse hippocampal slices obtained from 3 to 8 week-old mice, using the $mGlu_5$ receptor knockout strain and genotyping as described previously (Jia et al., 1998). Mice were anaesthetised with halothane and decapitated. Slices (400 μm thick) were prepared using a mechanical tissue slicer and maintained in a recording chamber at a temperature of 30 ± 1 °C. They were perfused continuously at 2 ml/min with a solution comprised of the following: 124 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 1.25 mM Na_2HPO_4, and 10 mM D-glucose. The solution was equilibrated with 95% O_2/5% CO_2. Two fully independent inputs were stimulated alternately, with 15 s separating the stimulation of each input. The stimulus intensity was set so as to obtain field EPSP slopes of approximately 50% of that at which a population spike was just detectable. All slices were naive (i.e., had not received any prior experimental manipulation) such that, whenever tested, the induction of LTP was invariably blocked reversibly by 200 μM MCPG. Synaptic responses were displayed, and field EPSP slopes were measured and plotted on-line using software written in house (Anderson and Collingridge, 2001); available from www/ltp-program.com. Each experiment was conducted on a separate slice, each having been obtained from a separate mouse on a different day. The *n* values refer to the number of times the experiment was performed. Data are presented as mean ± s.e.m. LTP was defined as a statistically significant ($P < 0.05$) stable increase in field EPSP slope, measured at least 60 min following a single tetanus (100 Hz, 1 s, test intensity). Potentiation is expressed as percentage baseline (i.e., 100% equals no change).

3. Results

3.1. The number of stimuli required to set the molecular switch

In the first set of experiments, we kept the frequency of stimulation fixed at 100 Hz but varied the number of stimuli delivered. In each case, we delivered a single train with the stimulus intensity kept the same as the test intensity. Previously, we used a single train of 100 shocks to set the molecular switch (Bortolotto et al., 1994). Here, we first tested the effects of delivering 10 stimuli (Fig. 1A and 2D). To our surprise this stimulus, which induced a very small LTP (of 18 ± 6%), was able to fully set the molecular switch, such that MCPG was unable to block the induction of subsequent LTP in the same input. Thus, a stable LTP, followed for 2 h, of 91 ± 8% was induced by the standard tetanus

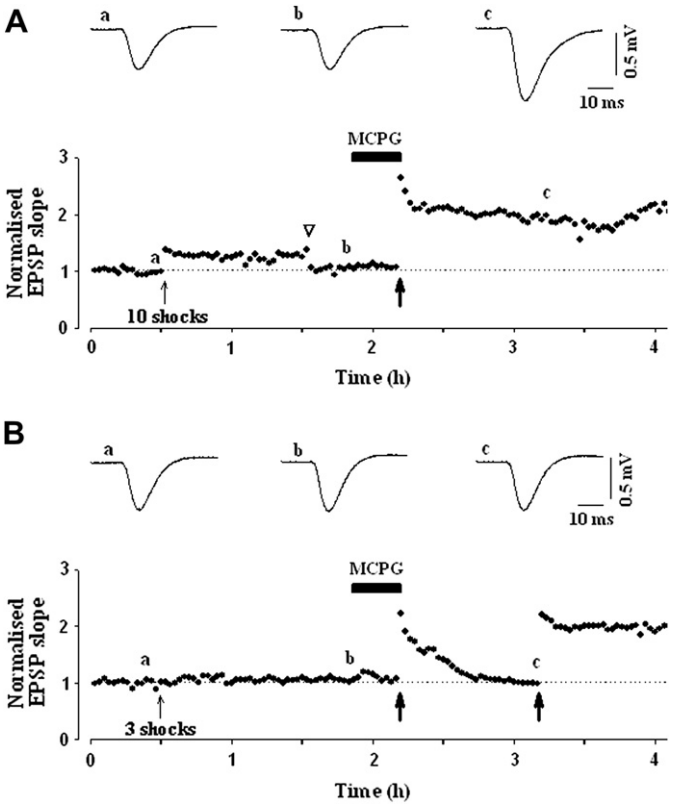

Fig. 1. A few stimuli are sufficient to set the molecular switch. (A) A single representative experiment that shows field EPSP slope plotted vs time. A brief conditioning train (10 shocks at 100 Hz) was delivered at the time indicated (thin arrow) and this induced a small LTP, which persisted for at least 1 h. The stimulus intensity was then reduced (triangle) and a new baseline obtained. MCPG (200 μM) was then applied for 20 min before tetanus (100 shocks at 100 Hz) was delivered (thick arrow). This resulted in a substantial LTP which persisted for the remainder of the experiment. (B) A similar experiment but where the conditioning train comprised just 3 shocks (at 100 Hz). This did not induce LTP or set the molecular switch, since MCPG blocked the induction of LTP, but not short-term potentiation. To ensure that the lack of LTP was due to block of induction by MCPG a second tetanus was delivered after washout of LTP. In these, and subsequent, figures each point is the mean of four successive responses. The traces are averages of four responses obtained at the times indicated by letters.

(100 Hz, 1 s, test intensity; $n = 3$). We next tested the effects of delivering just three stimuli and found that this was unable to set the molecular switch, since MCPG invariably blocked the subsequent induction of LTP (Fig. 1B and 2A; $n = 3$). Lastly, we investigated the effects of two intermediate numbers of stimuli (5 and 8 shocks). We found that 5 shocks was approximately the threshold for setting the molecular switch, since there was a trend for a small LTP (10 ± 6%) when the tetanus was delivered in the presence of MCPG and there was also a trend for the LTP (67 ± 11%) following washout of MCPG to be smaller ($n = 4$; Fig. 2B). The effect of 8 shocks was to clearly set the molecular switch ($n = 4$; Fig. 2C), albeit possibly not to the same extent as 10 shocks since the LTP induced in the presence of MCPG was smaller (61 ± 5%; Fig. 2C). Thus, $mGlu_5$ receptors are activated sufficiently to induce metaplasticity by very few synaptic stimuli. The level of LTP induced by the conditioning train and by the 100 shocks delivered in the presence of MCPG is compared in Fig. 2E and F, respectively.

3.2. The frequency-dependence for setting the molecular switch

Given that 10 stimuli were sufficient to maximally set the molecular switch we delivered 10 shocks at a variety of stimulation