Directional gating of synaptic plasticity by GPCRs and their distinct downstream signalling pathways

Bo Gong and Yu Tian Wang*

Department of Medicine, Brain Research Center, University of British Columbia, Vancouver, British Columbia, Canada

*Correspondence to: ytwang@interchange.ubc.ca

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Synaptic plasticity, the activity-dependent modification of synaptic strength, plays a fundamental role in learning and memory as well as in developmental maturation of neuronal circuitry. However, how synaptic plasticity is induced and regulated remains poorly understood. In this issue of *The EMBO Journal*, Yang and colleagues present sets of exciting data, suggesting that G-protein-coupled receptors (GPCRs) selectively execute distinct signalling pathways to differentially regulate induction

thresholds of hippocampal long-term potentiation (LTP) and long-term depression (LTD), thereby governing the direction of synaptic plasticity. These results shed significant light on our current understanding of how bidirectional synaptic plasticity is regulated.

Synaptic plasticity has been demonstrated at synapses in various brain regions; the most well-characterized forms are LTP and LTD at hippocampal CA1 glutamatergic synapses (Collingridge *et al.*, 2004). In experimental models, LTP

Figure 1 GPCRs regulate the direction of synaptic plasticity via activating distinct signalling pathways. Synaptic NMDA receptors, both GluN2A- and GluN2B-containing, play key roles in the induction of various forms of synaptic plasticity at the hippocampal CA1 glutamatergic synapse. Under the basal level of GluN2A and GluN2B ratio, stimulation with a train of pulses at frequencies from 1 to 100 Hz produces a frequency and plasticity (LTD–LTP) curve, with maximum LTD and LTP being, respectively, induced at 1 and 100 Hz. Activation of PAC1R with its agonist PACAP38 activates Src and thereby results in tyrosine phosphorylation and consequent functional upregulation of GluN2A-containing NMDARs, resulting in an increase in the ratio of functional GluN2A and GluN2B. The increased ratio in turn causes a left shift of frequency and plasticity curve, favouring LTP induction. In contrast, activation of D1R by the receptor agonist SKF81297 triggers Fyn-specific tyrosine phosphorylation and functional upregulation of GluN2B, causing a reduction of GluN2A and GluN2B ratio. This decreased ratio results in a right shift of the curve, favouring LTD induction. The ability of GPCRs to differentially activate distinct downstream signalling pathways involved in synaptic plasticity suggests the potential roles of GPCRs in governing the direction of synaptic plasticity.

and LTD can be, respectively, induced by high-frequency stimulation (HFS) and low-frequency stimulation (LFS) via activation of the *N*-methyl-D-aspartic acid (NMDA) subtype ionotropic glutamate receptor (NMDAR). However, how HFS and LFS activate NMDARs and thereby lead to synaptic plasticity remains poorly understood and highly controversial. It is even more unclear how the bidirectional synaptic plasticity is produced and regulated in response to physiological or pathological changes.

Functional NMDARs consist primarily of two GluN1 subunits and two GluN2 subunits, with GluN2A and GluN2B subunits being the most common NMDAR subunits found in the cortical and hippocampal regions of the adult brain (Cull-Candy *et al*, 2001). GluN2A and GluN2B subunits may confer distinct gating and pharmacological properties to NMDARs and couple them to distinct intracellular signalling machineries (Cull-Candy *et al*, 2001). Moreover, the ratio of these two subpopulations of NMDARs at the glutamatergic synapse is dynamically regulated in an activity-dependent manner (Bellone and Nicoll, 2007; Cho *et al*, 2009; Xu *et al*, 2009). Although controversial, GluN2A- and GluN2B-containing NMDARs have been suggested to have differential roles in regulating the direction of synaptic plasticity (Collingridge *et al*, 2004; Morishita *et al*, 2007). Among the factors shown to regulate NMDAR function, Src family tyrosine kinases may be the best characterized, with both Src and Fyn able to upregulate NMDAR function, and thus LTP induction (Salter and Kalia, 2004). However, if these kinases modulate NMDAR function in a NMDAR subunit-specific manner remains unknown. To explore this concept, Yang *et al* (2012) investigated the potential subunit-specific regulation of NMDARs by Src and Fyn using whole-cell patch clamp recording of NMDAR-mediated currents from acutely dissociated CA1 hippocampal neurons or from rat hippocampal slices. They found that intracellular perfusion of recombinant Src or Fyn increased the NMDAR-mediated currents. By applying subunit-preferential antagonists of GluN2A- or GluN2B-containing NMDARs, or by using neurons obtained from GluN2A knockout mice, they discovered that Src and Fyn differentially enhanced currents gated through GluN2A- and GluN2B-containing NMDARs, respectively.

Can physiological or pathological factors differentially activate Src or Fyn, thereby exerting subunit-specific regulation of NMDAR function? To answer this question, Yang *et al* focused their investigation on the role of GPCRs, specifically pituitary adenylate cyclase activating peptide receptor (PAC1R) and dopamine D1 receptor (D1R), both of which have recently been shown to potentiate NMDARs through Src family kinases (Macdonald *et al*, 2005; Hu *et al*, 2010). Indeed, they found that activation of PAC1R specifically increased GluN2A-NMDAR-mediated currents without affecting currents gated through GluN2B-NMDARs, and this potentiation was prevented by the Src-specific inhibitory peptide Src(40–58) (Salter and Kalia, 2004). To rule out the contribution of Fyn, the authors developed a novel-specific Fyn inhibitory peptide Fyn(39–57), and demonstrated that it had little effect on PAC1R potentiation. In contrast, activation of D1R potentiated GluN2B- (but not GluN2A-) NMDAR-mediated currents, and this potentiation was specifically eliminated by Fyn(39–57), but not by Src(40–58). The authors further demonstrated that stimulation of PAC1Rs resulted in a selective activation of Src kinase and consequent

tyrosine phosphorylation of the GluN2A subunit, whereas activation of D1Rs led to a specific increase in Fyn-mediated tyrosine phosphorylation of the GluN2B subunit. To provide convincing evidence that these subunit-differential modulations are indeed the result of tyrosine phosphorylation of the respective NMDAR subunits, the authors then performed electrophysiological experiments using neurons from two knockin mouse lines GluN2A(Y1325F) and GluN2B(Y1472F), in which the tyrosine phosphorylation residues in native GluN2A and GluN2B subunits were, respectively, replaced with non-phosphorylatable phenylalanine residues. As expected, the authors found that PAC1R-mediated potentiation of NMDA currents was lost in neurons from GluN2A(Y1325F) mice (but maintained in neurons from GluN2B(Y1472F) mice), while D1R-mediated enhancement of NMDA currents was only observed in neurons from GluN2A(Y1325F) mice. Together, as illustrated in Figure 1, the authors have made a very convincing case that PAC1R and D1R, respectively, enhance function of GluN2A- and GluN2B-containing NMDARs by differentially activating Src- and Fyn-mediated phosphorylation of respective NMDAR subunits.

Given the coupling of NMDARs to the induction of synaptic plasticity, it is then reasonable to ask if activation of the two GPCRs can selectively affect the induction of LTP or LTD at CA1 synapses. Yang and colleagues investigated the effects of pharmacological activation of PAC1R and D1R on the induction of LTP and LTD by recording the field excitatory postsynaptic potentials from hippocampal slices. Consistent with differential roles of NMDAR subunits in governing directions of synaptic plasticity, the authors observed that activation of PAC1Rs reduces the induction threshold of LTP, while stimulation of D1Rs favours LTD induction (Figure 1). Facilitation of LTP by PAC1R and LTD by D1R were, respectively, prevented in the brain slices obtained from GluN2A(Y1325F) and GluN2B(Y1472F) knockin mice, supporting the differential involvements of Src-mediated GluN2A phosphorylation and Fyn-mediated GluN2B phosphorylation.

Taken together, the authors' results have demonstrated that activation of PAC1R and D1R can control the direction of synaptic plasticity at the hippocampal CA1 synapse by differentially regulating NMDAR_{EPSCs} in a subunit-specific fashion (Figure 1). Specifically, PAC1R enhances the function of GluN2A-containing NMDARs by increasing Src phosphorylation of GluN2A subunit at Y1325, whereas D1R upregulates GluN2B-containing NMDARs through increased Fyn phosphorylation of GluN2B at Y1472. Moreover, by regulating the ratio of functional GluN2A- and GluN2B-containing NMDARs, PAC1R and D1R in turn modulate the direction of synaptic plasticity, favouring the production of LTP and LTD, respectively.

While consistent with the recently proposed hypothesis that GluN2A and GluN2B may have preferential roles in the induction of hippocampal CA1 LTP and LTD (Collingridge *et al*, 2004; but see also Morishita *et al*, 2007), the current study further emphasizes the importance of GluN2A/GluN2B ratios in regulating LTP and LTD thresholds: increased ratio favours LTP, while reduced ratio promotes LTD. However, this seems to contradict some recent studies where the reduction and increase in the GluN2A/GluN2B ratio appeared to, respectively, favour LTP (Cho *et al*, 2009; Xu *et al*, 2009) and LTD (Xu *et al*, 2009). Therefore, the direction of plasticity change is likely modulated not only by the GluN2A/GluN2B

ratio, but also by additional factors such as experimental conditions, developmental stages, and brain regions.

Under many experimental conditions, LTP and LTD are usually induced by HFS and LFS stimulating protocols, respectively, but it remains essentially unknown how LTP and LTD are physiologically or pathologically generated in animals. To this end, the identification of different GPCRs as the endogenous upstream regulators of NMDA receptor subpopulations, and hence regulators of synaptic plasticity, is the major novelty of Yang and colleagues' work. Future studies are needed to investigate if and how PAC1R and/or D1R are critically involved in the production of LTP or LTD in animals under physiological or pathological conditions. Given the fact that Src family kinases may be required for LTP induced by HFS in hippocampal slices (Salter and Kalia, 2004), an equally intriguing question would be whether these GPCRs are actually required for LTP/LTD induced by HFS/LFS experimental paradigms. In line with this conjecture, it would be interesting to determine if ligands for various GPCRs co-exist in the glutamatergic presynaptic terminals and, if so, can be differentially co-released with glutamate in a frequency-dependent manner, thereby contributing to either HFS-induced LTP or LFS-induced LTD.

The findings by Yang and colleagues establish an exciting mechanistic model by which GPCRs can govern the direction of synaptic plasticity by determining the contributions of GluN2A- and GluN2B-NMDARs through differential tyrosine phosphorylation of respective NMDA receptor subtypes. Additional studies further validating this model under physiological and pathological conditions will greatly improve our understanding of the molecular mechanisms underlying synaptic plasticity and cognitive brain functions. In addition, NMDARs, depending on their subunit composition and/or subcellular localization, may also have complex roles in mediating neuronal survival and death (Lai *et al*, 2011). Considering that neurotoxicity produced by over-activation of NMDARs is widely accepted to be a common mechanism for neuronal loss in a number of acute brain injuries and chronic neurodegenerative diseases, Yang and colleagues' finding of the differential regulation of NMDAR subunits by different GPCRs could have wider implications beyond synaptic plasticity.

Conflict of interest

The authors declare that they have no conflict of interest.

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