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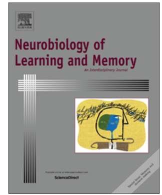

Evidence for glycinergic GluN1/GluN3 NMDA receptors in hippocampal metaplasticity

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Hebbian, or associative, forms of synaptic plasticity are considered the molecular basis of learning and memory. However, associative synaptic modifications, including long-term potentiation (LTP) and depression (LTD), can form positive feedback loops which must be constrained for neural networks to remain stable. One proposed constraint mechanism is metaplasticity, a process whereby synaptic changes shift the threshold for subsequent plasticity. Metaplasticity has been functionally observed but the molecular basis is not well understood. Here, we report that stimulation which induces LTP recruits GluN2B-lacking GluN1/GluN3 NMDA receptors (NMDARs) to excitatory synapses of hippocampal pyramidal neurons. These unconventional receptors may compete against conventional GluN1/GluN2 NMDARs to favor synaptic depotentiation in response to subsequent "LTP-inducing" stimulation. These results implicate glycinergic GluN1/GluN3 NMDAR as molecular brakes on excessive synaptic strengthening, suggesting a role for these receptors in the brain that has previously been elusive.

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1. Introduction

Learning and memory occurs through modification of synapses via Hebbian, or associative, synaptic plasticity. Strong, synchronous stimulation induces long-term potentiation (LTP) of excitatory synapses, while weak, asynchronous stimulation induces long-term depression (LTD) (Dan & Poo, 2004; Magee & Johnston, 1997). However, the positive reinforcement of associative plasticity can lead to runaway synaptic potentiation or depression. To safeguard against this outcome, a process termed metaplasticity is proposed to constrain synaptic change (Abraham, 2008). The influential Bienenstock–Cooper–Munro (BCM) model of metaplasticity posits that changes in synaptic strength alter the threshold for subsequent plasticity, thus diminishing the rate of return on synaptic modification (Bienenstock, Cooper, & Munro, 1982). This "sliding threshold" model predicts that stimuli that previously caused LTP may fall below the newly increased threshold, thereby causing LTD instead. The BCM model is supported by evidence from transgenic mice overexpressing constitutively active CaMKIIα: these mice exhibit excessive LTP under basal conditions and undergo LTD at electrical stimulation frequencies that normally produce LTP (Mayford, Wang, Kandel, & O'Dell, 1995). However, the molecular mechanisms underlying metaplasticity are poorly understood.

NMDA receptors (NMDARs) play a critical role in numerous types of plasticity, with differential calcium influx through NMDARs determining synaptic change in response to stimulation. High levels of NMDAR-gated calcium entry activate kinases, particularly CaMKIIα, that are required for long term potentiation (LTP) (Lisman, Schulman, & Cline, 2002; Malenka et al., 1989; Malinow, Schulman, & Tsien, 1989), whereas low-level calcium transients preferentially activate phosphatases that are required for long term depression (LTD) (Malenka & Bear, 2004). NMDARs are heterotetramers of two obligatory GluN1 subunits with combinations of GluN2 and/or GluN3 subunits that modulate channel properties, and NMDAR subunit composition plays a crucial role in the metaplastic adjustment of LTP/LTD induction threshold (Lee, Yasuda, & Ehlers, 2010; Philpot, Cho, & Bear, 2007; Xu et al., 2009; Yashiro & Philpot, 2008; Zorumski & Izumi, 2012).

The GluN3 subfamily (GluN3A and GluN3B) are the most recently identified NMDAR subunits (Low & Wee, 2010) and both are widely expressed in brain (Wee, Zhang, Khanna, & Low,

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2008; Wong et al., 2002). GluN3A is primarily expressed early, while GluN3B levels increase over development (Fukaya, Hayashi, & Watanabe, 2005; Matsuda, Kamiya, Matsuda, & Yuzaki, 2002). Little is known about the role of GluN3 receptors. GluN3 may be a dominant negative subunit, constraining NMDAR-mediated calcium influx. Overexpression of GluN3 with GluN1/GluN2 to form triheteromeric receptors reduces calcium permeability and current responses in various heterologous or neuronal systems (Chatterton et al., 2002; Ciabarra et al., 1995; Das et al., 1998; Matsuda, Fletcher, Kamiya, & Yuzaki, 2003; Matsuda et al., 2002; Nishi, Hinds, Lu, Kawata, & Hayashi, 2001; Sucher et al., 1995). Conversely, GluN3A knockout mice have increased NMDA currents (Das et al., 1998; Tong et al., 2008), also suggesting that GluN3 subunits dampen NMDAR signaling. GluN3 subunits, like GluN1, bind the NMDAR co-agonist glycine (or D-serine), but not glutamate. Thus, GluN1/GluN3 receptors lacking the glutamate-binding GluN2 subunit would form a new type of excitatory receptor gated by glycine alone. Within the CNS, such receptors have only been observed in optic nerve (Piña-Crespo et al., 2010), and their function in brain plasticity is unknown.

2. Materials and methods

Georgetown University's Institutional Animal Care and Use Committee (IACUC) reviewed and approved all procedures and animal use in this study (protocol 14-038). All animal use was conducted in accordance with the guidelines of Georgetown University's IACUC. Mice and rats were euthanized by carbon dioxide inhalation and subsequent decapitation.

2.1. Primary hippocampal cultures

Hippocampal cultures (~150 cells mm^{-2}) were prepared from E19 rat embryo brains as described (Evers et al., 2010; Lee et al., 2011; Sala et al., 2001). Briefly, dissociated neurons were plated on coverslips coated with poly-D-lysine (30 μg/ml) and laminin (2 μg/ml). Cultures were grown in Neurobasal medium (Invitrogen) supplemented with B27 (Invitrogen), 0.5 mM glutamine and 12.5 μM glutamate, or SM1 (Stem Cell Technologies), 0.5 mM glutamine and 25 μM glutamate. Neurons were analyzed at >21 DIV for mature stages.

2.2. Transfection and RNA interference

Neurons were transfected using Lipofectamine 2000 (Invitrogen) at DIV21 for 2–3 days prior to treatment with chemical LTP or vehicle control. Constructs were obtained from the following sources: pEGFP (Invitrogen), CaMKII (gift from J. Lisman, Brandeis University), HA-GluN3B (gift from M. Yuzaki, Keio University School of Medicine; as described in Matsuda et al. (2003)). GluN3B shRNA (clone 4-1, GGTACAAGTCTTCAGGCTT corresponding to nucleotides 2378–2396 of NM_133308.2; primer pair FWD: GATC CCCGGTACAAGTCTTCAGGCTTTTCAAGAGAAAGCCTGAAGACTTGTA CCTTT-TTA and REV: AGCTTAAAAAGGTACAAGTCTTCAGGCTTTCTC TTG-AAAAGCCTGAAGACTTGTACCGGG) or scrambled shRNA (GGT AAAAGTCTTAAGGCTT, primer pair FWD: GATCCCCGGTAAA AGT C-TTAAGGCTTTTCAAGAGAAAGCCTTAAGACTTTTACCTTTTTA and REV: AGCTTA-AAAAGGTAAAAGTCTTAAGGCTTTCTCTTGAAAAGCCT TAAGACTTTTACCGGG) was inserted into pSuper vector. All primers were purchased from Integrated DNA Technologies.

2.3. Chemical LTP and pharmacology

Neurons were stimulated for 5 min with 100 μM glycine (Sigma) to activate NMDARs, with 1 μM strychnine (Sigma) included to block glycine receptors. Reagents were obtained from the following sources: APV (Sigma), MK-801 (Ascent), ALX-5407 (Tocris), KN-62 (Cayman), 5,7-DCK (Tocris), okadaic acid (Tocris).

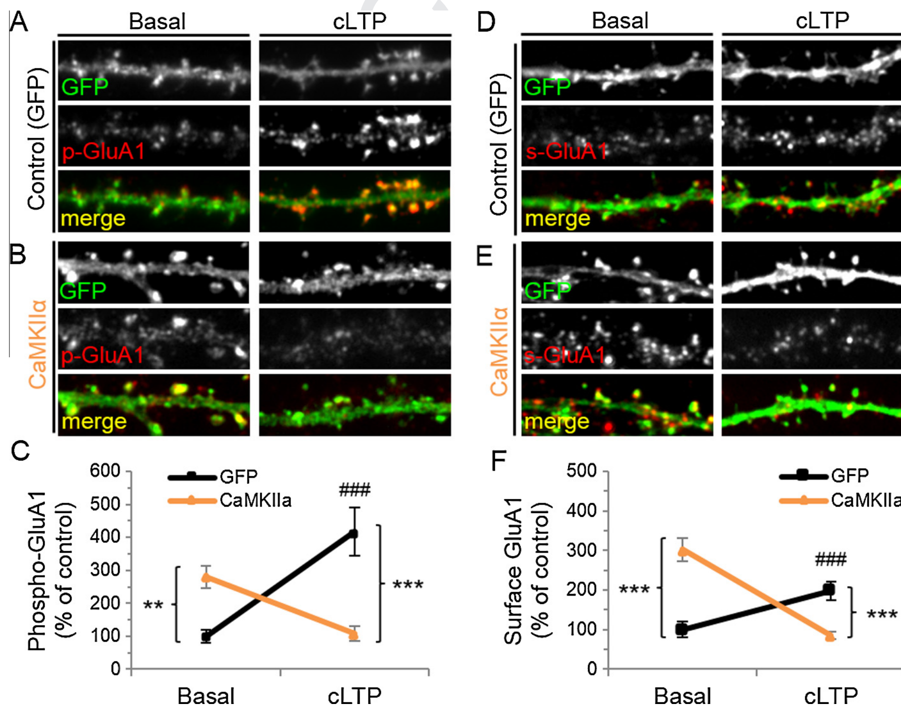

Fig. 1. CaMKIIα overexpression induces metaplasticity. Mature cultured hippocampal neurons were transfected with GFP (A, D) or GFP-CaMKIIα (B, E) and treated with chemical LTP (cLTP) or vehicle (basal). Neurons were stained for GFP and either phospho-serine 831 of GluA1 (p-GluA1) (A–C), or live labeled for surface GluA1 (sGluA1) (D–F). (C, F) Quantification of GluA1 subunits phosphorylated at serine 831 (C) or expressed at the cell surface (F). Data for each group are normalized to basal control GFP cells and are represented as mean ± SEM (*N* = 10–23/neurons per condition). $^{###}p < 0.0001$ vs. basal, interaction effect (two-way ANOVA, treatment vs. genotype); $^{**}p < 0.01$, $^{***}p < 0.0001$ vs. control (Bonferroni *post hoc* test, see Table S1). Scale, 5 μm.

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