Neuroscience 272 (2014) 271–285

POTENTIATION OF CONVERGENT SYNAPTIC INPUTS ONTO PYRAMIDAL NEURONS IN SOMATOSENSORY CORTEX: DEPENDENCE ON BRAIN WAVE FREQUENCIES AND NMDA RECEPTOR SUBUNIT COMPOSITION

J. PILLI AND S. S. KUMAR*

Department of Biomedical Sciences, College of Medicine & Program in Neuroscience, Florida State University, 1115 W. Call Street, Tallahassee, FL 32306-4300, United States

Abstract—*N*-methyl-D-aspartate receptors (NMDARs) at layer (L)1/primary whisker motor cortex synaptic inputs are distinct from thalamic/striatal (Str) synaptic inputs onto L5 pyramidal neurons in the rat somatosensory cortex. However, the consequences of differential expression of putative GluN3A-containing triheteromeric NMDARs at L1 inputs and GluN2A-containing diheteromeric NMDARs at Str inputs on plasticity of the underlying synapses at the respective inputs remain unknown. Here we demonstrate that L1, but not Str, synapses are potentiated following delta burst stimulation (dBS). This potentiation is blocked by D-serine and/or intracellular 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) suggesting that it is subunit-specific and dependent on elevations in intracellular Ca^{2+}. Interestingly, ifenprodil, the GluN2B-preferring antagonist, suppresses baseline L1 responses but does not prevent induction of dBS-evoked potentiation. Unlike L1, Str synapses are maximally potentiated following theta burst stimulation (tBS) and this potentiation is blocked with BAPTA and/or the GluN2A-preferring antagonist NVP-AAM077. We show further that while dBS is both necessary and sufficient to potentiate L1 synapses, tBS is most effective in potentiating Str synapses. Our data suggest distinct potentiating paradigms for the two convergent inputs onto pyramidal neurons in the somatosensory cortex and co-dependence of synaptic potentiation on brain wave-tuned frequencies of burst stimulation and subunit composition of underlying NMDARs. A model for predicting the likelihood of enhancing synaptic efficacy is proposed based on Ca^{2+} influx through these receptors and integration of EPSPs at these inputs. Together, these findings raise the possibility of input-specific enhancements of synaptic efficacy in neurons as a function of the animal's behavioral state and/or arousal *in vivo*.

Key words: triheteromeric NMDA receptors, synapse-specific potentiation, GluN3A subunit, somatosensory cortex, electrophysiology.

INTRODUCTION

N-methyl-D-aspartate receptors (NMDARs) are critically implicated in synaptic plasticity – the basis for learning and memory (Bliss and Lomo, 1973; Collingridge et al., 1983). However, the rich diversity of subunit compositions manifest developmentally, neuronally, and as we have shown, even synaptically at the single-cell level (Kumar and Huguenard, 2003), suggests that not all NMDARs are alike. While subunit composition is known to influence receptor function, the role specific subunits play in enabling NMDARs to mediate/modulate plasticity at discrete synaptic inputs onto cortical neurons is still poorly understood. The difficulty of isolating non-overlapping excitatory inputs onto neurons in cortical slices and/or potentiating intracortical synapses combined with the limited availability of subunit-specific compounds, have all hindered a better understanding of NMDAR stoichiometry and function. To this end, we recently showed that, just as in the frontal cortex, NMDARs underlying convergent synaptic inputs onto L5 pyramidal neurons in the somatosensory cortex show pathway-specific differences in subunit composition – stimulation in the striatum (Str) activated subcortical and/or thalamocortical afferents that operated via conventional GluN2A-containing diheteromeric NMDARs, while intracortical stimulation in L1 activated posterior medial nucleus and/or primary whisker motor cortex afferents that operated via putative GluN3A-containing triheteromeric NMDARs (Pilli and Kumar, 2012). In assaying synaptic integration at these inputs, we unwittingly discovered a stimulating paradigm – delta burst stimulation (dBS, not to be mistaken with deep brain stimulation) that specifically potentiated L1, but not Str synapses, prompting the present study of synapse-specific differences in potentiating mechanisms at these convergent inputs.

NMDARs are heterotetrameric proteins whose subunits are derived from three gene families, *GRIN1*

*Corresponding author. Address: Department of Biomedical Sciences, College of Medicine, Suite 3300-B, Florida State University, 1115 West Call Street, Tallahassee, FL 32306-4300, United States. Tel: +1-850-645-8336; fax: +1-850-644-5781.
E-mail address: sanjay.kumar@med.fsu.edu (S. S. Kumar).

Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; dBS, delta burst stimulation; EPSP, excitatory postsynaptic potential; HEPES, hydroxyethyl piperazineethanesulfonic acid; NMDARs, *N*-methyl-D-aspartate receptors; PB, phosphate buffer; PPF, paired-pulse facilitation; PPR, paired-pulse ratio; tBS, theta burst stimulation.

http://dx.doi.org/10.1016/j.neuroscience.2014.04.062

(codes for GluN1) – *GRIN3* (GluN3). All NMDARs contain one or more of the obligatory GluN1 subunits, which when assembled with either GluN2 (GluN2A-GluN2D) or GluN3 (GluN3A-GluN3B) subunits of the same type, give rise to diheteromeric NMDARs. While GluN2-containing diheteromeric (conventional) NMDARs are glutamate-activated and Ca^{2+}-permeable, GluN3-containing diheteromeric NMDARs have been shown in expression systems to be activated by glycine and have reduced Ca^{2+}-permeability (Das et al., 1998; Chatterton et al., 2002). Triheteromeric NMDARs, on the other hand, contain three different types of subunits, one of which is invariably GluN1.

To explore mechanisms underlying differences in plasticity of L1 versus Str synapses, we assayed the effects of the best known subunit-preferring compounds available on the induction and expression of burst stimulation-evoked potentiation at the respective inputs. These compounds, together with electrophysiological measurements of the voltage-dependent and kinetic properties of EPSCs, enabled us to distinguish between di- and triheteromeric NMDARs at these synapses and infer their subunit composition (Pilli and Kumar, 2012). We observed that NMDARs at L1 inputs had a threshold-like activation at hyperpolarized holding potentials (around −40 mV) with EPSCs showing strong outward rectification of their current–voltage relationships (IVs). In contrast, NMDAR-mediated EPSCs at Str inputs onto the same neurons had IVs that were more typical, displaying prominent regions of negative slope and reversing polarity close to the expected reversal potential of 0 mV. Furthermore, ion exchange experiments suggested that NMDARs at L1 synapses had higher Ca^{2+} permeability compared with those at Str inputs, likely due to enhanced selectivity for Ca^{2+} over Na^+ ($0 < P_{Na} \ll 1$) as substantiated by the hyperpolarized non-zero reversal potentials of their EPSCs. Pharmacology suggested a triheteromeric NMDAR at L1 inputs with features common to glutamate-activated GluN1/GluN2-containing and glycine-activated GluN1/GluN3-containing diheteromeric NMDARs.

Although we acknowledge the limitations of the pharmacological approach relating NMDAR function and receptor subunit composition (Neyton and Paoletti, 2006) and the need to corroborate subunit stoichiometry by either knocking out the GluN3A subunit and/or expressing/isolating these receptors in a heterologous expression system, one of the underlying themes of the present work is to assay differences in the effects these compounds have on NMDAR-mediated plasticity at convergent synaptic inputs onto single neurons. To address whether potentiation of L1 and Str synapses is dependent on elevations in intracellular Ca^{2+}, we assayed the intracellular effects of the fast Ca^{2+} chelator 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA). Intrigued by the observation that a low-burst frequency (in the lower delta band, 0.1–4 Hz) potentiated L1, but not Str inputs, we shortened the interval of burst-stimulation to determine if a higher burst frequency was more efficacious in potentiating Str synaptic inputs. The interval of burst-stimulation was varied to physiologically relevant theta (4–7 Hz), alpha-(8–12 Hz) and gamma-(25–100 Hz) frequency bands, used routinely in studying hippocampal LTP, to assay induction/expression of synaptic potentiation at the respective inputs. We determined which of the commonly occurring brain waves provided the "characteristic frequency" for burst stimulation-evoked potentiation of L1 and Str inputs and whether application of these tetanizing paradigms was necessary and sufficient to potentiate the respective synapses. To better understand frequency dependence of burst stimulation-evoked potentiation, we assayed integration of EPSPs during bursts to propose a simple, yet testable model for the likelihood of potentiating these synapses as a function of Ca^{2+}-threshold, "burst efficiency" and Ca^{2+} influx per burst. Pathway-specific differences in NMDAR subunit composition may endow individual pyramidal neurons with the ability to respond differentially based on the animal's behavioral state and/or arousal *in vivo*.

EXPERIMENTAL PROCEDURES

In vitro slice preparation and electrophysiology

Sprague–Dawley rats (male) ranging in age between postnatal days 14 and 24 were used in this study. All experiments were carried out in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Florida State University Institutional Animal Care and Use Committee. Rats were deeply anesthetized with urethane (1.5 g/kg ip) before being decapitated. Thalamocortical slices (Agmon and Connors, 1991) (450 μm thick) were cut from the excised brains in a chilled (4 °C) low-Ca^{2+}, low-Na^+ "cutting solution" containing the following (in mM): 230 sucrose, 10 D-glucose, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, and 0.5 $CaCl_2$ (equilibrated with 95% O_2/5% CO_2). Slices were allowed to equilibrate in oxygenated artificial cerebrospinal fluid (aCSF) (in mM: 126 NaCl, 26 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, and 10 D-glucose, pH 7.4), first at 32 °C for 1 h and subsequently at room temperature before being transferred to the recording chamber. Current-clamp recordings were obtained at 32 ± 1 °C from layer 5B pyramidal neurons (visualized through a 63×/0.90, water immersion objective under IR-DIC optics) on-column with the barrel field (L4). Recording electrodes (1.2–2 μm tip diameters; 3–6 MΩ) contained the following (in mM): 105 potassium gluconate, 30 KCl, 10 HEPES, 10 phosphocreatine, 4 MgATP and 0.3 GTP (adjusted to pH 7.3 with KOH). For chelating intracellular Ca^{2+}, 5 mM BAPTA (Sigma, St. Louis, MO, USA) was included with the internal solution. Slices were maintained in oxygenated (95% O_2/5% CO_2) aCSF, and drugs and chemicals were applied via bath perfusion. Stimulating electrodes placed in L1 (S_1: bipolar CE-2C75, 25-μm tip diameters; FHC, good for intracortical stimulation) and in Str (S_2: concentric bipolar CB-ARC75, 25/125 μm inner/outer tip diameters; FHC, good for evoking single-fiber responses from within fiber bundles) delivered constant current pulses 50–100 μs in duration and 100–500 μA in amplitude at low frequencies (0.1–0.3 Hz) to activate