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Ca^{2+}-PERMEABLE AMPA RECEPTORS MEDIATE INDUCTION OF TEST PULSE DEPRESSION OF NAIVE SYNAPSES IN RAT VISUAL CORTICAL SLICES AT EARLY POSTNATAL STAGE

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Abstract—Synaptic depression in the hippocampus at early postnatal stage can be induced by test pulse stimulation (<1 Hz). However, the receptor mechanism for induction of this synaptic depression is unclear. In the present study, we used whole-cell patch clamp recording *in vitro* to investigate how excitatory and inhibitory synapses onto layer II/III pyramidal neurons of the primary visual cortex adapt to test pulse activation from a previously non-activated (naive) state. We found that excitatory postsynaptic currents (EPSCs) of pyramidal neurons were rapidly depressed by 0.1 Hz stimulation in acutely prepared slices from rats at 11–12 postnatal days, while this phenomena disappeared in slices from young adolescent rats (23–24 postnatal days). By contrast, inhibitory postsynaptic currents (IPSCs) were relatively stable following 0.1 Hz stimulation of rat slices at the same early postnatal stage. Moreover, the test pulse depression of EPSCs was associated with a decrease in $1/\text{coefficient of variation (CV)}^2$ and no change in the paired-pulse ratio. These data imply silencing of synapses and no significant change either in postsynaptic receptor density or presynaptic terminal release probability. This synaptic depression was unaffected by the competitive NMDA receptor antagonist D-APV. Ca^{2+}-permeable AMPA receptor selective antagonists, Naspm or IEM-1460, prevented the induction of the test pulse depression. These data suggest that EPSCs, but not IPSCs, were rapidly depressed by test pulse stimulation in rats at early postnatal stage via a Ca^{2+}-permeable AMPA receptor-dependent mechanism.

Key words: synaptic plasticity, test pulse stimulation, whole-cell patch clamp recording, brain slice.

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Abbreviations: ACSF, artificial cerebrospinal fluid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CV, coefficient of variation; D-APV, D-2-amino-5-phosphonovaleric acid; EPSCs, excitatory postsynaptic currents; HFS, high-frequency stimulation; IEM-1460, 1-trimethylammonio-5-(1-adamantane-methylammoniopentane) dibromide; IPSCs, inhibitory postsynaptic currents; LTD, long-term depression; LTP, long-term potentiation; Naspm, 1-naphthyl acetyl spermine; NMDA, *N*-methyl-D-aspartic acid; P, postnatal day; PPR, paired-pulse ratio; QX-314, lidocaine N-ethyl bromide.

Long-term potentiation (LTP) and long-term depression (LTD) form the basis of one theory of learning and memory formation (Bliss and Collingridge, 1993; Martin et al., 2000). In the protocol of inducing LTP, higher frequency stimulation (≥2 Hz in visual cortex or 100 Hz in hippocampus) is commonly used as tetanus stimuli. By contrast, a lower frequency stimulation (≥1 Hz) is always used as tetanus to induce LTD. A much lower frequency stimulation (<1 Hz) is always used as the test pulse stimulation to obtain baseline before tetanus and to probe whether LTP or LTD formation after tetanus; such low frequency stimulation should not induce either LTP or LTD in general experiments of synaptic plasticity. However, this low frequency test pulse stimulation was shown to induce synaptic depression at naive hippocampal CA3-CA1 synapses in the rat at early postnatal stage (Xiao et al., 2004), and more recently was found at the perforant path-dentate granule cell synapses and the synapses onto interneurons in mature hippocampal CA1 stratum radiatum (Abrahamsson et al., 2005; Riebe et al., 2009). This kind of synaptic efficacy depression can be examined using either field potential or perforated patch-clamp recordings (Abrahamsson et al., 2007; Strandberg et al., 2009). Indeed, several previous studies suggested that activation at intervals of tens of seconds resulted in depression of synaptic efficacy (Castellucci and Kandel, 1974; Teyler and Alger, 1976). Furthermore, such synaptic depression was linked to behavioral habituation (Christoffersen, 1997), and has been suggested as a prerequisite for developmental LTP in the hippocampal CA1 area (Abrahamsson et al., 2008).

This test pulse depression phenomenon was reported to require a rise in postsynaptic Ca^{2+}, but was independent of activation of *N*-methyl-D-aspartic acid (NMDA) receptors, metabotropic glutamate receptors or voltage-gated calcium channels (Xiao et al., 2004); these data have been subsequently confirmed in several other studies (Abrahamsson et al., 2005, 2007; Riebe et al., 2009; Strandberg et al., 2009). However, the receptor mechanism for induction of the test pulse depression remains unclear.

In the present study, we examined whether this test pulse depression could be induced at the primary visual cortex of rats at early postnatal stage. The primary visual cortex is a well-established brain region used for the study of experience-dependent plasticity, as visual experience is an important factor for changes in synaptic plasticity in brain visual systems.

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EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on either postnatal day (P) 11–12 or P 23–24 Sprague–Dawley rats obtained from the animal experiment center of Xi'an Jiaotong University, China. The animals were housed in a standard environment on a 12/12 h light/dark cycle with light on at 7:00. The use and care of animals in this study followed the guidelines of the Xi'an Jiaotong University Animal Research Advisory Committee, and all efforts were made to minimize animal suffering and reduce experimental animal numbers.

Preparation of brain slices

Rats were initially anesthetized with ether and then immersed in ice-cold water, with the nose exposed, for 3 min to reduce brain temperature. Immediately after decapitation, the brain was removed and placed in ice-cold (1–4 °C) artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2 and 5% CO_2 (pH 7.4). The ACSF consisted of 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose (osmolality, 305–310 mOsm/kg). A block of tissue containing the visual cortex was cut into 400 μm-thick slices with a vibratome (Campden Instruments, London, UK). Slices were transferred to an incubating chamber containing oxygenated ACSF and incubated for at least 1.5 h at room temperature (20 °C) prior to electrophysiological recording.

Whole-cell patch clamp recording

Slices were individually transferred to a submerged recording chamber and perfused with oxygenated ACSF at 2.5–3.0 ml/min. The temperature of the recording chamber was continuously maintained at 31±0.5 °C and controlled by a custom-made temperature controller. The slices were placed on an upright infrared video microscope with differential interference contrast (DIC) optics (Olympus BX51WI, Tokyo, Japan) mounted on a Gibraltar X-Y table. Slices were observed through a ×40 water immersion objective using an infrared-sensitive camera (DAGE-MTI, IR-1000, USA). Layer II/III pyramidal neurons of the primary visual cortex were visually selected under the upright microscope (Mason and Larkman, 1990). Patch-clamp recordings were performed in the whole cell configuration. Unpolished and uncoated patch pipettes (1.5 mm OD, 1.1 mm ID) with a resistance of 3–6 MΩ were pulled using a horizontal puller (model P–97, Sutter Instruments, Novato, CA, USA) and used as recording electrodes. For recording of excitatory postsynaptic currents (EPSCs), the pipette solution contained 130 mM cesium methanesulfonate, 4 mM $MgCl_2$, 5 mM QX-314, 0.6 mM EGTA, 20 mM HEPES, 4 mM Na_2ATP, and 0.4 mM Na_3GTP (pH 7.3–7.4, adjusted with CsOH, 280–290 mOsm/kg). For recording of inhibitory postsynaptic currents (IPSCs), the pipette solution contained 110 mM CsCl, 5 mM $MgCl_2$, 5 mM QX-314, 0.6 mM EGTA, 40 mM HEPES, 2 mM Na_2ATP, and 0.3 mM Na_3GTP (pH 7.3–7.4 adjusted with CsOH, 290–295 mOsm/kg). Tight seals (>1 GΩ) were formed on the bodies of the pyramidal neurons. Series resistance was monitored continuously using a 5 ms, 10 mV hyperpolarizing pulse and compensation was not employed. Statistical comparisons were performed only when series resistance was <20 MΩ and did not change by more than 20%. Liquid junction potentials were measured and calculated to be approximately 8 mV (EPSCs recording) or 4 mV (IPSCs recording), and they were not corrected.

Evoked synaptic activity recording

For recording EPSC and IPSC, the holding potentials were both set to −70 mV. A monopolar glass stimulating electrode (1–2 MΩ when filled with ASCF) was placed 50–100 μm lateral to the recording pipette. All stimulus pulses were 0.1 ms duration and the frequency was changed according to the experimental protocol. The evoked EPSCs or IPSCs were recorded using an Axopatch 200B amplifier and digitized using a digidata 1322A board operated by pCLAMP 9.2 software (Molecular Devices, Sunnyvale, CA, USA). Currents were filtered at 2 kHz and digitized at 10 kHz.

To record EPSCs, picrotoxin (100 μM) was always present in the ACSF to block $GABA_A$ receptor-mediated activity, whereas to record IPSCs, CNQX (10 μM) and D-APV (50 μM) were applied in the ACSF to block ionotropic glutamate receptor-mediated activity. The evoked IPSCs disappeared following application of the ionotropic GABA receptors channels antagonist picrotoxin in the ACSF (data not shown).

To examine whether excitatory synaptic transmission is altered by the test-frequency stimulation, naive synapses (without previous stimulation) were used. As such, it was not possible to adjust the position of the stimulus electrode or the stimulus intensity to obtain EPSCs of a certain magnitude. Instead, the pre-set intensity stimulation (usually 350 μA) was used as the initial stimulus in all the cases to induce the initial EPSC, and the amplitudes of the initial EPSC in different experiments varied greatly from 15.0 to 151.0 pA. To examine whether the test pulse EPSC depression was affected by high frequency stimulation (HFS; typically used in inducing LTP), three trains of 100 pulses at 100 Hz with a 30 s train interval was applied immediately after obtaining the initial EPSC. Due to the considerable EPSC-to-EPSC variability, the EPSCs were binned (bin size=6) and normalized with respect to the average of the first six EPSCs ("initial EPSC"). For comparison of all the responses in each experiment, the peak amplitude of the EPSCs from all the other binned EPSCs were normalized to that of the first (or "initial") binned EPSC. To quantify the depression or potentiation of EPSCs caused by stimulation, the normalized value of three binned EPSCs evoked by stimulation of the 103–120th EPSCs ("late EPSC") were compared with the initial EPSCs across experiments. The IPSCs were binned in the same manner as that of EPSCs.

Synaptic response measurement

The peak amplitude of EPSC or IPSC was measured as the difference between the baseline level immediately preceding the stimulation artifact and the mean amplitude during a 1 ms time window around the negative peak. Pairs of EPSCs were evoked by paired-pulse stimulation with an interval of 50 ms at 0.1 Hz. A paired-pulse ratio (PPR) was calculated by dividing the average second and first binned EPSCs in order to correct for spurious facilitation that can be caused by random amplitude fluctuation (Kim and Alger, 2001). EPSC variability (coefficient of variation (CV)) was estimated as standard deviation/mean. The $1/CV^2$ values were calculated from 20 to 40 consecutive EPSCs.

Drugs

Picrotoxin, 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX), D-2-amino-5-phosphonovalerate (D-APV), lidocaine N-ethyl bromide (QX-314), and 1-naphthyl acetyl spermine (Naspm) were purchased from Sigma-Aldrich (St Louis, MO, USA). 1-trimethylammonio-5-(1-adamantane-methylammoniopentane) dibromide (IEM-1460) was purchased from Tocris BioScience (Evansville, MO, USA). All drugs except for QX-314 were applied at known concentrations by changing the perfusion line.

Statistical analysis

Numerical and graphed data were expressed as mean±standard error of the mean (SEM) and normalized relative to the initial value. Statistical significance for paired and independent samples was evaluated using Student's *t*-test. The significance level was established at $P<0.05$. In all cases, *n* refers to the number of slices tested.