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Ni^{2+}-sensitive T-type Ca^{2+} channel currents are regulated in parallel with synaptic and visual response plasticity in visual cortex

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ABSTRACT

Visual cortical neurons undergo depression and potentiation of their visual responses to stimulation of the deprived and non-deprived eyes, respectively, after monocular deprivation. This modification occurs predominantly during an early postnatal period in normal development, and this critical period is postponed until adulthood in animals reared in darkness from birth. We have proposed that Ni^{2+}-sensitive T-type Ca^{2+} channel-dependent long-term potentiation (T-LTP) mediates the potentiation of non-deprived eye responses. In this study, to investigate the development of Ni^{2+}-sensitive T-type Ca^{2+} channels, presumed $Ca_V3.2$ channels, we performed whole-cell recordings from layer 2/3 pyramidal neurons in rat visual cortical slices. T-type Ca^{2+} channel currents were activated by voltage steps from −100 mV to −40 mV under a pharmacological blockade of Na^+ and K^+ channels. We estimated presumed $Ca_V3.2$ currents from the currents obtained after subtraction of the currents in the presence of Ni^{2+} (50 μM) from those in control solution. The estimated currents were very small before eye opening, peaked during the critical period and then returned to a small value by adulthood. Dark rearing prevented the developmental decline in these currents until adulthood. These results suggest that the regulation of $Ca_V3.2$ currents underlies the developmental changes in T-LTP and ocular dominance plasticity.

1. Introduction

Visual cortical neurons respond selectively to features of visual stimulation (Hubel, 1982). The response selectivity is refined and maintained by visual experience during a postnatal period called the critical period (Wiesel, 1982). It has been hypothesized that activity-dependent long-term modification of synaptic transmission is an initial process attaining this experience-dependent refinement of cortical functions (Bear et al., 1987; Katz and Shatz, 1996; Singer, 1995; Zhang and Poo, 2001). Visual cortical neurons undergo depression and potentiation of their visual responses to stimulation of the deprived and non-deprived eyes, respectively, after monocular deprivation during the critical period (Frenkel and Bear, 2004). Therefore, ocular dominance plasticity is a model suitable to test that hypothesis. Recent studies have suggested that long-term potentiation (LTP) and depression (LTD) of excitatory synaptic transmission contribute to ocular dominance plasticity. One line of evidence supports the view that N-methyl-D-aspartate (NMDA) receptor-dependent LTD mediates the depression of deprived eye responses following monocular deprivation (Heynen et al., 2003; Liu et al., 2008; Yoon et al., 2009). On the other hand, our pharmacological study has suggested that LTP induced by low-frequency stimulation mediates the potentiation of non-deprived eye responses (Yoshimura et al., 2008).

The induction of this LTP requires the activation of T-type voltage-gated Ca^{2+} channels in postsynaptic cells (Komatsu and Iwakiri, 1992; Yoshimura et al., 2008). In layer 2/3 neurons, T-type Ca^{2+} channel-dependent LTP (T-LTP) occurs mostly during the critical period in both cats and rats in normal development (Komatsu et al., 1988; Yoshimura et al., 2008), while it is induced even in adulthood when rats are kept in darkness from birth (Yoshimura et al., 2008), just like the ocular dominance shift produced by monocular deprivation (Hubel and Wiesel, 1970; Cynader and Mitchell, 1980; Mower et al., 1981). LTP induction was prevented by

Abbreviations: LTP, long-term potentiation; LTD, long-term depression; NMDA, N-methyl-D-aspartate; T-LTP, T-type Ca^{2+} channel-dependent LTP; PD, postnatal day; ACSF, artificial cerebrospinal fluid; TEA, tetraethylammonium; TTX, tetrodotoxin; DL-APV, DL-2-amino-5-phosphnovaleric acid; BMI, bicuculine methiodide; DNQX, 6,7-dinitroquinoxaline-2,3-dione.

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organic T-type Ca^{2+} channel blockers as well as by a low concentration of Ni^{2+} (Yoshimura et al., 2008). It is known that there are three subtypes of T-type Ca^{2+} channels, $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ (Perez-Reyes et al., 1998; Cribbs et al., 1998; Lee et al., 1999a; McRory et al., 2001). Among these subtypes, only $Ca_V3.2$ exhibits high sensitivity to Ni^{2+} (Lee et al., 1999b), suggesting that this subtype is required for the induction of T-LTP. In the present study, we examined the postnatal development of Ni^{2+}-sensitive T-type Ca^{2+} channel, presumed $Ca_V3.2$, currents in layer 2/3 pyramidal neurons. The results suggest that the regulation of $Ca_V3.2$ currents underlies the developmental changes in T-LTP and ocular dominance plasticity.

2. Materials and methods

All of the experiments were carried out under a protocol approved by the Experimental Animal Care Committee, Research Institute of Environmental Medicine, Nagoya University and National Institute for Physiological Sciences.

2.1. Slice preparations

Experiments were conducted using pigmented (Long–Evans) rats at postnatal day (PD) 8–12, 20–30 and 60–90. Some of the rats were reared in a completely dark room from birth until adulthood (PD 60–90). As described previously (Komatsu, 1994; Yoshimura et al., 2003), coronal slices of primary visual cortex (300 μm thick) were prepared from rats under deep anesthesia with isoflurane. Then the slices were kept at 33 °C for 1 h in an interface-type recovery chamber perfused with an artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 3 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose. Thereafter, the slices were maintained in a submerged-type chamber at room temperature.

2.2. Analysis of Ca^{2+} channel currents

Ca^{2+} channel currents were recorded from small pyramidal cells in the upper part of layer 2/3 under visualization of cells with an infrared differential interference contrast microscope equipped with a 40×, 0.8NA water immersion lens (BX51WI, Olympus, Tokyo, Japan). Whole-cell voltage-clamp recording was conducted using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA), which was controlled and monitored with a personal computer running pClamp 8 (Axon Instruments). A P-97 puller (Sutter Instruments, Novato, CA, USA) was used to make patch pipettes (borosilicate glass BF150-110-10; Sutter Instruments). The pipettes had a resistance of 4–5 MΩ when filled with an internal solution containing (in mM): 140 CsCl, 10 HEPES, 2 $MgCl_2$, 0.2 EGTA, 10 Cs-BAPTA, 3 MgATP, 0.5 Na_2GTP and 10 tetraethylammonium (TEA)-Cl (pH 7.3 adjusted with CsOH). The external recording solution used to isolate Ca^{2+} channel currents contained (in mM): 100 choline-Cl, 3 CsCl, 1.3 $MgCl_2$, 2.4 $CaCl_2$, 26 choline-HCO_3, 20 TEA-Cl, 1 lidocaine, 0.001 tetrodotoxin (TTX), 0.1 DL-2-amino-5-phosphnovaleric acid (DL-APV), 0.04 6,7-dinitroquinoxaline-2,3-dione (DNQX), 0.02 bicuculine methiodide (BMI) and 10 glucose. Cell capacitance values were taken directly from readings of the amplifier after electronic subtraction of the capacitive transients. Series resistance was electronically compensated by 60–80%. The liquid junction potential was <5 mV and was not compensated. All recordings were performed at room temperature (24–26 °C). Only those cells exhibiting adequate voltage control (judged by monoexponential decay of capacitive currents) were included in the analysis. A standard P/4 protocol was used for leakage subtraction. Currents were low-pass filtered at a cutoff frequency of 2 kHz and were acquired at 20 kHz. Data were analyzed with Clampfit8 (Axon Instruments) and Prizm 4 (GraphPad Software, San Diego, CA, USA).

2.3. Chemical compounds

The drugs employed in this study were obtained from the following sources: DL-APV and DNQX from Tocris (Bristol, UK); BMI, lidocaine and nimodipine from Sigma (St. Louis, MO, USA); TTX and TEA-Cl from Wako (Osaka, Japan); BAPTA tetracesium salt from Molecular Probes (Eugene, OR, USA); ω-agatoxin IVA and ω-conotoxin GVIA from Peptide Institute (Osaka, Japan); ML 218 from Alamone Labs (Jerusalem, Israel).

2.4. Statistical analysis

Data were presented as mean ± SEM. Statistical analyses were performed using Mann–Whitney or Wilcoxon test when two groups were compared, and one-way repeated-measures ANOVA test followed by Tukey's test or Kruskal–Wallis test followed by Dunn's test when more than two groups were compared. *P* values of less than 0.05 were considered significant.

3. Results

We conducted whole-cell voltage-clamp recordings in visual cortical slices obtained from rats at PD 8–12 soon before eye opening, PD 20–30 during the critical period and PD 60–90 in adulthood. In addition, similar recordings were performed using slices obtained from rats reared in darkness from birth until adulthood (PD 60–90). To ensure an adequate voltage-clamp, we selected small pyramidal cells located in the upper part of layer 2/3 for the analysis.

3.1. Separation of Ni^{2+}-sensitive T-type Ca^{2+} channel currents

We first characterized Ca^{2+} channel currents recorded from layer 2/3 pyramidal cells at PD 20–30, when T-LTP is easily induced (Yoshimura et al., 2008). To record Ca^{2+} currents isolated from K^+ and Na^+ currents, we used a Cs^+-based internal solution containing the K^+ channel blocker TEA (10 mM) and a modified ACSF containing TEA (20 mM) and the Na^+ channel blockers TTX (1 μM) and lidocaine (1 mM). To further reduce the currents carried by monovalent cations, Na^+ and K^+ were replaced with choline and Cs^+, respectively, in the ACSF. Ca^{2+} channels were activated by depolarizing voltage steps from the holding potential of −100 mV to various test membrane potentials between −90 and +20 mV (Fig. 1A). Coapplication of Ni^{2+}-insensitive high-voltage activated (L-, N- and P/Q-type) Ca^{2+} channel blockers at high doses (10 μM nimodipine, 3 μM ω-conotxin GVIA and 1 μM ω-agatoxin IVA) suppressed the currents evoked by depolarizing steps to −30 mV or more, but not those evoked by depolarization to −40 mV or less (Fig. 1A). The latter currents may be mediated by low-voltage activated (T-type) Ca^{2+} channels and/or Ni^{2+}-sensitive high-voltage activated (R-type) Ca^{2+} channels (Fox et al., 1987; Narahashi et al., 1987; Zhang et al., 1993; Lee et al., 1999a; Foehring et al., 2000; McRory et al., 2001).

If the currents activated by voltage steps to the lower membrane potentials were indeed mediated by T-type and/or R-type Ca^{2+} channels, they would be inactivated even around the resting membrane potentials (Fox et al., 1987; Zhang et al., 1993; Lee et al., 1999a; Foehring et al., 2000; McRory et al., 2001). To characterize the inactivation of these currents, hyperpolarizing prepulses from the holding potentials of −40 mV to various membrane potentials between −110 and −50 mV (10 mV step) were applied for 1 s before the membrane potential returned to −40 mV (Fig. 1B). Ca^{2+} currents evoked by the offset of hyperpolarizing prepulse potentials between −80 and −50 mV were significantly smaller (Tukey's test, $P < 0.0005$, $n = 10$ cells), compared with the currents when the