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Research report

CRMP1 and CRMP4 are required for proper orientation of dendrites of cerebral pyramidal neurons in the developing mouse brain

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ABSTRACT

Neural circuit formation is a critical process in brain development. Axon guidance molecules, their receptors, and intracellular mediators are important to establish neural circuits. Collapsin response mediator proteins (CRMPs) are known intercellular mediators of a number of repulsive guidance molecules. Studies of mutant mice suggest roles of CRMPs in dendrite development. However, molecular mechanisms of CRMP-mediated dendritic development remain to elucidate. In this study, we show abnormal orientation of basal dendrites (extension to deeper side) of layer V pyramidal neurons in the cerebral cortex of CRMP4−/− mice. Moreover, we observed severe abnormality in orientation of the basal dendrites of these neurons in double knockout of CRMP1 and 4, suggesting redundant functions of these two genes. Redundant gene functions were also observed in proximal bifurcation phenotype in apical dendrites of hippocampal CA1 pyramidal neurons. These results indicate that CRMP1 and CRMP4 regulate proper orientation of the basal dendrites of layer V neurons in the cerebral cortex.

1. Introduction

Development of proper neural circuits is necessary for normal brain function. During axonal development, semaphorin3A (Sema3A), a repulsive cue, plays critical roles by inducing growth cone collapse (Nakamura et al., 2000) through several phosphorylation events including kinase activation of Cdk5 and GSK3β.

Collapsin response mediator proteins (CRMPs) have five isoforms (CRMP1–5) (Wang et al., 1996), which regulate the intercellular signaling by Sema3A, neurotrophins and myelin-associated inhibitors (MAIs) (Goshima et al., 1995; Nakamura et al., 2000; Tamagnone and Comoglio et al., 2000; Alabed et al., 2010). CRMP1–4 share high homology and form heterotetramers (Wang et al., 1997). Furthermore, CRMP1, CRMP2, and CRMP4 bind to actin and microtubules (Fukata et al., 2002; Alabed et al., 2010; Higurashi et al., 2012) and are phosphorylated by Cdk5 and GSK3β in Sema3A-induced growth cone collapse signaling pathway (Uchida et al., 2005).

Previous studies also indicate the involvement of external guidance cues, such as Sema3A in dendritic development. Sema3A−/− mice exhibit abnormal apical dendrite bifurcation of hippocampal CA1 pyramidal neurons (proximal bifurcation) (Nakamura et al., 2009; Schlomann et al., 2009). CRMP4−/− mice show similar phenotype (Niisato et al., 2012)., Further, CRMP1−/−; CRMP2ki/ki mice exhibit abnormal development of basal dendrites of layer V neurons in the cerebral cortex (Yamashita et al., 2012). Molecular mechanisms of CRMP-mediated dendritic development in the cerebral cortex, however, remain unclear.

In this study, we investigated the involvement of CRMP4 and CRMP1 in dendritic development in layer V pyramidal neurons in the cerebral cortex using CRMP4−/− mice (Niisato et al., 2012) and CRMP1−/− mice (Yamashita et al., 2013). We observed that CRMP4−/− single mutant mice exhibited abnormal orientation of

Abbreviations: ANOVA, analysis of variance; CA1, cornu ammonis 1; Cdk, cyclin dependent kinase; CNS, central nervous system; CRMP, collapsin response mediator protein; CRMP2ki/ki, CRMP2 knock-in; EDTA, ethylenediaminetetraacetic acid; GFP, green fluorescent protein; GSK, glycogen synthase kinase; MAIs, myelin-associated inhibitors; O/N, overnight; PFA, paraformaldehyde; RT, room temperature; Sema3A, semaphorin3A; WT, wild-type

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basal dendrites (dendritic extension to deeper side) in the cortex layer V neurons. We next generated double knockout of CRMP1 and CRMP4 (CRMP1/4−/−) mice and found high incidence of the same abnormal orientation of basal dendrites as CRMP4−/− mice. Our results suggest that CRMP4 and CRMP1 regulate proper development of basal dendrite morphology of layer V pyramidal neurons of cerebral cortex.

2. Results

2.1. Abnormal orientation of basal dendrites in layer V pyramidal neurons in the cerebral cortex of CRMP4−/− mice

We have previously reported that loss of CRMP4 causes abnormal bifurcation of apical dendrites in CA1 pyramidal neurons (Niisato et al., 2012). In addition, CRMP1−/− and CRMP2ki/ki double mutant mice show abnormal development of basal dendrites of cerebral cortex layer V neurons (Yamashita et al., 2012). Thus, in this study we investigated the role of CRMP4 in development of basal dendrites of cerebral cortex layer V neurons. To visualize neuronal morphology, we crossed CRMP4 mutant mice with GFP-M mice in which a few of layer V neurons were GFP-positive (Feng et al., 2000). We analyzed basal dendrites in layer V neurons of the somatosensory cortex of CRMP4+/+ and CRMP4−/− mice at 3 weeks of age (Fig. 1A). We divided surroundings of pyramidal neurons in four areas (45–135°, 135–225°. 225–315°, and 315–45°) and counted the numbers of basal dendrites in each area (Fig. 1B). We observed that pyramidal neurons in the cerebral cortex layer V in CRMP4−/− mice exhibited higher numbers of basal dendrites in the 225–315° area, while were reduced in the 45°135° area compared to those in CRMP4+/+ mice (Fig. 1C). Increased numbers of dendrites in the 225–315° area may represent two different phenotypes; increased branching and/or altered orientation of primary dendrites. Thus, this result suggested that branching and/or orientation of basal dendrites of the pyramidal neurons in layer V in the cerebral cortex was altered in CRMP4−/− mice.

2.2. Intense abnormal orientation of basal dendrites of cortical pyramidal neurons in CRMP1/4−/− mice

As previously reported (Laeremans et al., 2013; Wang and Strittmatter, 1996), CRMP1 and CRMP4 are expressed in layer V

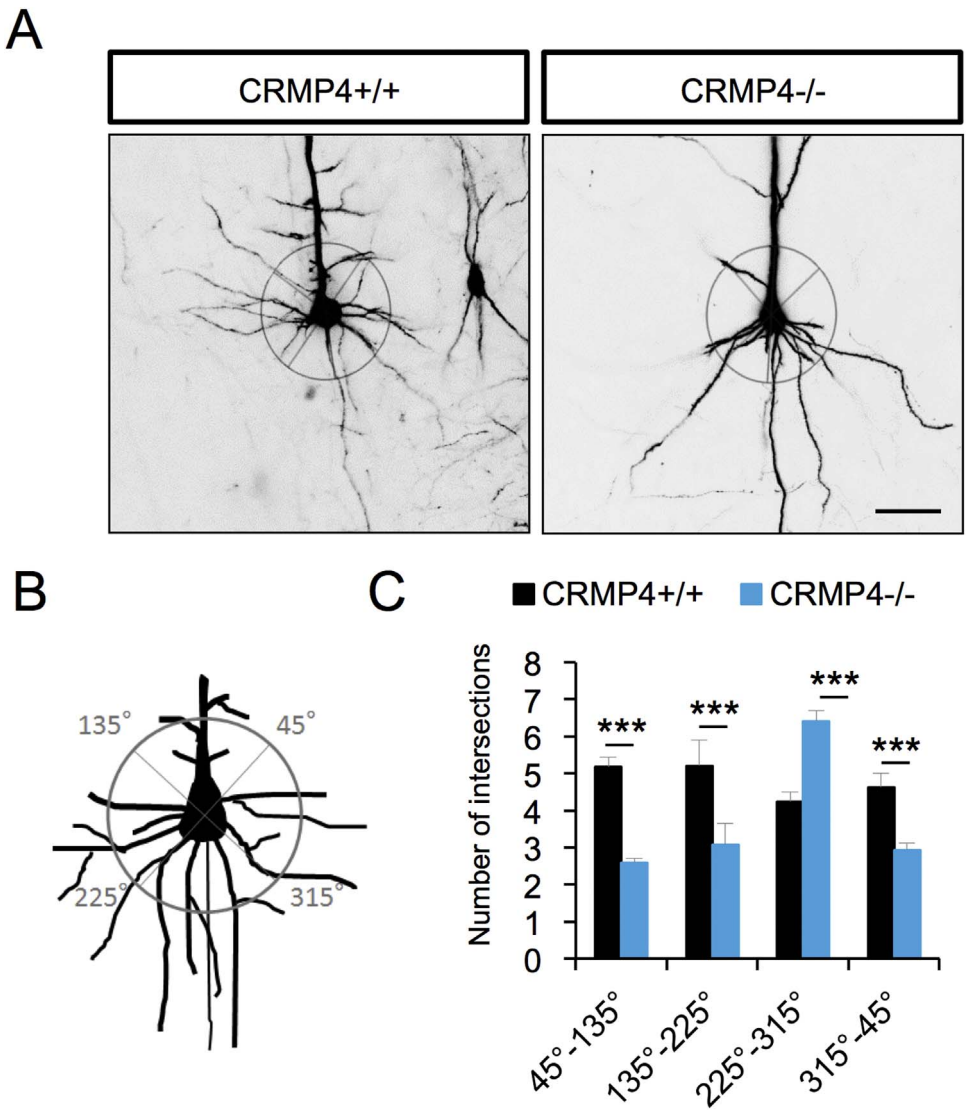

Fig. 1. CRMP4 deletion induced abnormal distribution of basal dendrites of cerebral cortex layer V neurons. A, Representative images of GFP-labeled basal dendrites of cerebral cortex layer V neurons of CRMP4+/+ and CRMP4−/− mice at 3 weeks of age. Scale bar, 50 μm. **B,** Quantitative analysis for distribution of basal dendrites. Basal dendrites were divided into four areas with 50 μm radial circle from the center of neuronal soma. **C**, The graph represents the average numbers of basal dendrites in each area. Data are shown as mean ± S.E.M for n =12 from 3 mice of each genotype. ***, $p < 0.001$. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple-comparison test.