Role of Cdk5rap2 in neocortical inhibition and excitation balance

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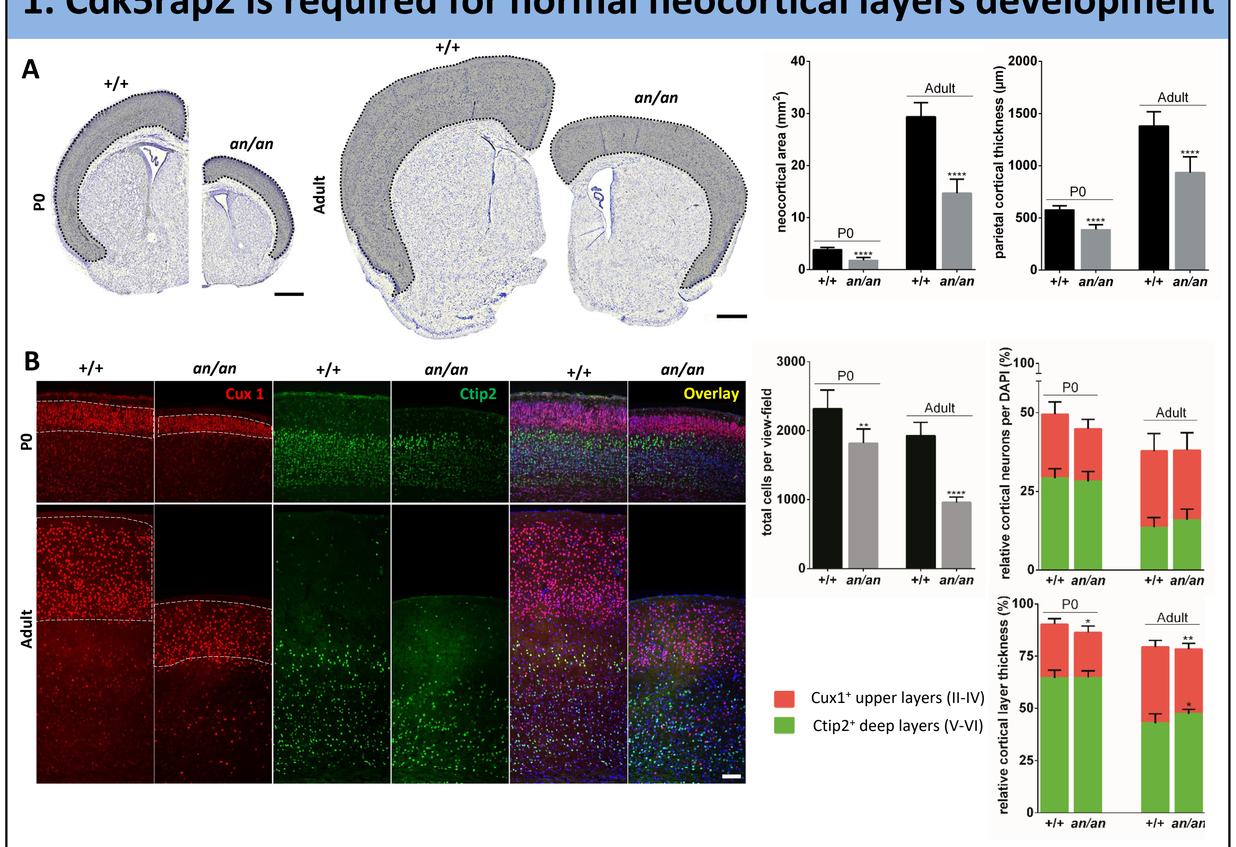
INTRODUCTION

Autosomal recessive primary microcephaly type 3 (MCPH3) is characterized by congenital microcephaly and intellectual disability. Further features include hyperactivity and seizures. The disease is caused by biallelic mutations in the Cyclin-dependent kinase 5 regulatory subunit-associated protein 2 gene *CDK5RAP2*. In the mouse, *Cdk5rap2* mutations similarly result in reduced brain size and a strikingly thin neocortex already at early stages of neurogenesis that persists through adulthood. The microcephaly phenotype in MCPH arises from a neural stem cell proliferation defect. Here, we report a novel role for Cdk5rap2 in the regulation of dendritic development and synaptogenesis of neocortical layer 2/3 pyramidal neurons using a combined morphological and electrophysiological approach.

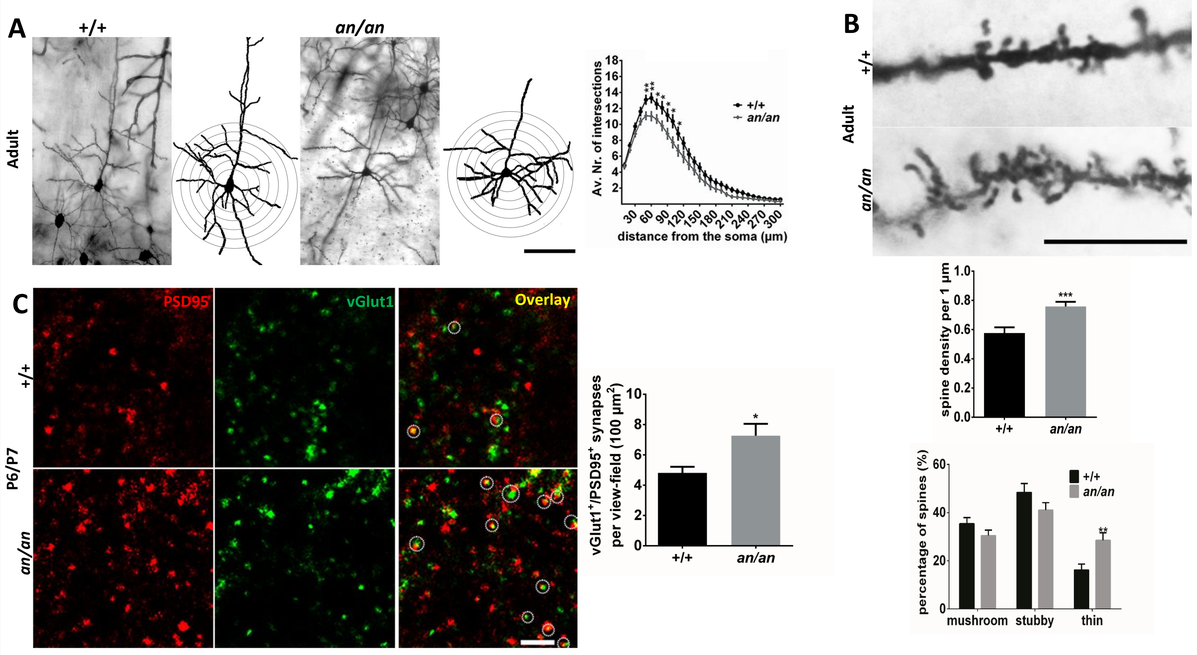
RESULTS

1. Cdk5rap2 is required for normal neocortical layers development



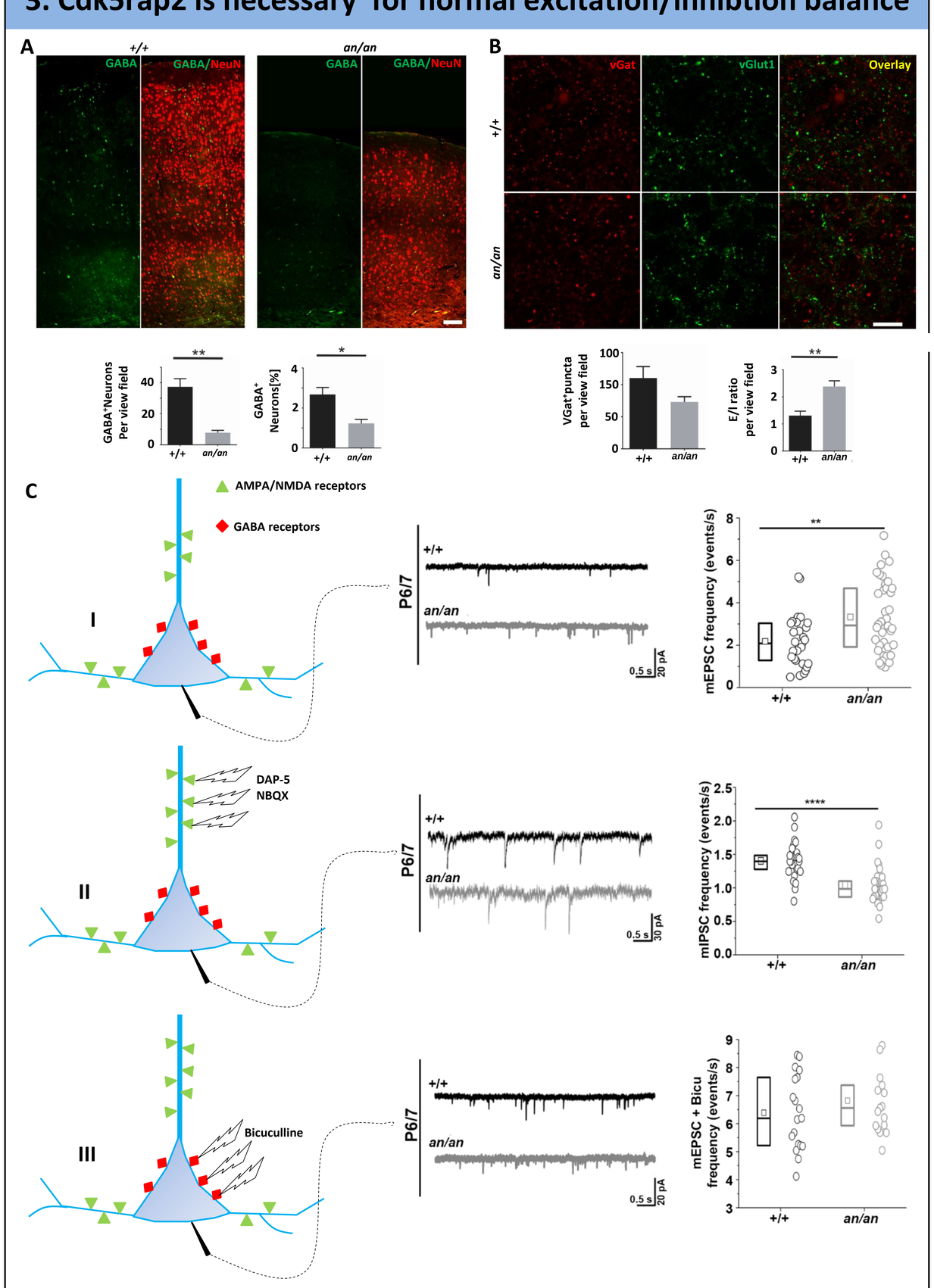
Microcephaly with pronounced cortical thinning and preserved neocortical layer organization despite reduced thickness of upper cortical layers in *an/an* mice. (A) Significant reduction of the neocortical area and parietal cortical thickness in *an/an* mice (n=7 animals/group, Nissl staining, DIC images, scale bars 500 µm). (B) While the Cux1⁺ upper layers and Ctip2⁺ deep layers are thinner, only the relative thickness of upper layers with respect to the total cortical thickness mice was reduced in an/an mice (n=7 animals/group, immunofluorescence images, scale bar 100 µm). Students' t test: *p<0.05, **p<0.01, ****p<0.0001.

2. Cdk5rap2 plays a role in normal dendritic and spine formation



Distinctive morphological characteristics of layer 2/3 pyramidal neurons from *an/an* mature neocortex. (A) Reduced dendritic complexity in photomicrographs / reconstructed neurons with less dendritic intersections 50-110 µm from the soma in *an/an* mice due to a reduction in apical and basal dendrites (n = 44 +/+ and 39 *an/an* neurons from 6 +/+ and 4 *an/an* animals, Golgi staining, DIC images,scale bar 100 µm). (B) Average spine density was increased with a larger proportion of thin-shaped immature spines in *an/an* mice (n = 410 +/+ and 373 *an/an* spines counted in 34 (+/+) and 30 (*an/an*) 20 µm long dendritic segments from 5 animals/group). (C) The number of VGlut1/PSD95 positive synapses (dotted circles) at layer 2/3 areas is increased in *an/an* mice (n = 18 images from 4 +/+ animals and 28 images from 5 *an/an* animals, confocal images, scale bar 2 µm). Students' t test: *p<0.05, **p<0.01, ***p<0.001.

3. Cdk5rap2 is necessary for normal excitation/inhibtion balance



Loss of GABAergic input adjusts excitatory drive in neocortical layer 2/3 pyramidal neurons of +/+ and *an/an* animals. (A) The total number of interneurons positive for GABA per view-field and the proportion of these cells in relation to total NeuN⁺ neurons per view-field is reduced in *an/an* mice. (n= 6 +/+ and 4 *an/an* animals, immunofluorescence images, scale bar 100 µm). (B) The trend towards reduced number of inhibitory synapses (VGat puncta) contributes to an increased E/I ratio at layer 2/3 areas in *an/an* mice (n = 15 images from 6 +/+ animals and 20 images from 6 *an/an* animals, confocal images, scale bar 10 µm). (C-I) sEPSCs recordings in neurons voltage clamped at -60 mV showing a trend towards increased in sEPSC frequency in neurons from *an/an* mice (n= 29 +/+ and 38 an/an neurons from 4 animals/group). (C-II) Scheme and example of mIPSCs traces recorded at -60 mV using equimolar Cl^- and blocking excitatory postsynaptic currents showing reduced mIPSCs frequency in *an/an* neuro (n = 30 +/+ and 32 *an/an* neurons from 3 animals/group). (C-III) When the inhibitory influence on neurons is prohibited, no significant changes in the frequency of mEPSC were detected between *+/+* and *an/an* littermates. Students' t test: *p<0.05, **p<0.01, ****p<0.0001.

METHODS

We used a combined longitudinal morphological, immunohistochemical and electrophysiological approach on neocortical layer 2/3 pyramidal neurons in *ex vivo* brain slice and primary neuronal culture preparations of *Cdk5rap2* mutant and wild-type mice. Animals were genotyped using standard PCR reactions specific for wild-type (+/+) and *Cdk5rap2* mutant mice (*an/an*). For Nissl staining, paraffin sections of P0 and adult brains were stained with 1% cresyl violet. For Golgi staining, brains were immersed in the impregnation solution in darkness at room temperature (RT) for 2 weeks, and transferred into tissue-protectant solution at 4 °C for 4 days. Brains were cut into 200 µm sections for dendritic complexity analysis and 100 µm sections for dendritic spine analysis. Immunostaining was performed using antibodies against Cux1 (layers 2-4), Ctip2 (layers 5-6), vGlut1 (excitatory-presynaptic), PSD95 (postsynaptic), vGat (inhibitory-presynaptic), GABA (interneuron marker), NeuN (neuronal marker). Electrophysiological investigations were done on *ex-vivo* brain slices as well as on primary neuronal cultures. DAP-5 and NBQX were used to inhibit AMPA/NMDA receptors and Bicuculline to inhibit GABA receptors.

CONCLUSIONS AND OUTLOOK

We demonstrate the critical role of Cdk5rap2 regarding morphogenesis and synaptic connectivity during mammalian development. Our studies show, that inhibitory signailing (GABA) is reduced in *an/an* Hertwig mice. Our findings indicate a developmental and persisting disturbance in balance of excitation - inhibition in the neocortex of *Cdk5rap2* mutant mice and hence putatively in MCPH3 patients. This places MCPH type microcephalies pathophysiologically in close proximity to other neurodevelopmental disorders such as neuropsychiatric diseases and intellectual disability. Further studies need to identify methanisms, why inhibitory signaling is lost and therapeutic strategies can be evolved with this knowledge.

Reference: Zaqout S, et al., Altered inhibition and excitation in neocortical circuits in congenital microcephaly. *Neurobiology of Disease* 2019;129:130-143.