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General Article

GENERAL ARTICLE

Site-specific abnormalities in the visual system of a mouse model of CDKL5 deficiency disorder

Leonardo Lupori[1,2,‡], Giulia Sagona[2,3,4,‡], Claudia Fuchs[5], Raffaele Mazziotti[2,3], Antonia Stefanov[2], Elena Putignano[2], Debora Napoli[1,2], Enrica Strettoi[2], Elisabetta Ciani[5] and Tommaso Pizzorusso[1,2,3,*,†]

[1]BIO@SNS Laboratory, Scuola Normale Superiore, Via Moruzzi 1, Pisa 56124, Italy [2]Institute of Neuroscience, National Research Council, Via Moruzzi 1, Pisa 56124, Italy [3]Department of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA University of Florence, Area San Salvi—Pad. 26, Florence 50135, Italy [4]Department of Developmental Neuroscience, IRCCS Stella Maris Foundation, Pisa 56128, Italy [5]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna 40126, Italy

*To whom correspondence should be addressed at: Istituto Neuroscienze Consiglio Nazionale delle Ricerche, Via Moruzzi 1, Pisa 56125, Italy. Tel: +39 0503153167; Fax: +39 0503153220; Email: tommaso.pizzorusso@in.cnr.it

Abstract

CDKL5 deficiency disorder (CDD) is a neurodevelopmental disorder characterized by a severe global developmental delay and early-onset seizures. Notably, patients show distinctive visual abnormalities often clinically diagnosed as cortical visual impairment. However, the involvement of cerebral cortical dysfunctions in the origin of the symptoms is poorly understood. CDD mouse models also display visual deficits, and cortical visual responses can be used as a robust biomarker in CDKL5 mutant mice. A deeper understanding of the circuits underlying the described visual deficits is essential for directing preclinical research and translational approaches. Here, we addressed this question in two ways: first, we performed an in-depth morphological analysis of the visual pathway, from the retina to the primary visual cortex (V1), of CDKL5 null mice. We found that the lack of CDKL5 produced no alteration in the organization of retinal circuits. Conversely, CDKL5 mutants showed reduced density and altered morphology of spines and decreased excitatory synapse marker PSD95 in the dorsal lateral geniculate nucleus and in V1. An increase in the inhibitory marker VGAT was selectively present in V1. Second, using a conditional CDKL5 knockout model, we showed that selective cortical deletion of CDKL5 from excitatory cells is sufficient to produce abnormalities of visual cortical responses, demonstrating that the normal function of cortical circuits is dependent on CDKL5. Intriguingly, these deficits were associated with morphological alterations of V1 excitatory and inhibitory synaptic contacts. In summary, this work proposes cortical circuit structure and function as a critically important target for
studying CDD.

†Tommaso Pizzorusso, http://orcid.org/0000-0001-5614-0668
‡The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.
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Introduction

In humans, mutations in CDKL5 cause a devastating disorder of neurodevelopment-designated CDKL5 deficiency disorder (CDD). Patients are characterized by a severe global developmental delay, early-onset seizures and a distinctive visual impairment (23,35) often clinically diagnosed as cortical visual impairment (CVI). CVI, also designated as cerebral visual impairment, is generally due to alterations involving the retrochiasmal visual tracts in the brain with non-existent or minimal ocular morbidity (18). However, little is known about the neural structures involved in CVI in CDD patients. During the past years, CDD mouse models have become available (1,14,24,41), paving the way to mechanistic and preclinical investigations on possible therapies. These studies have produced a plethora of preclinical data; however, the efficacy assessment of these potential treatments in a clinical trial critically depends on the availability of non-invasive biomarkers with high sensitivity. Unfortunately, these tools are still missing. Previous studies demonstrated that visual cortical responses assessed by visual evoked potential (VEP) recordings or intrinsic optical signal (IOS) imaging (a technique to dynamically image the activity-dependent blood oxygenation-dependent signal) can be used to monitor the progression of the symptoms in a CDD mouse model (22). Sensitivity analysis revealed that visual responses were able to correctly classify mice as mutant or wild-type (WT) with >93% accuracy and 92% sensitivity proving the robustness of this technique in phenotype assessment. Defects in sensory processing have also been reported using auditory evoked potentials (41). These observations raise the possibility that visual alterations could represent a biomarker of high-translational potential for CDD. To gain a deeper understanding of the circuits underlying visual deficits induced by CDKL5 deficiency, we characterized the morphofunctional defects present at different levels along the retino-cortical projection in CDKL5 mutant mice. Our data show that, although CDKL5 is expressed in the retina, its deletion does not alter any of its major neuronal populations. By contrast, neurons in the visual thalamic relay nucleus [the dorsal lateral geniculate nucleus (dLGN)] and in the visual cortex showed reduced spine density and PSD95 synaptic staining in CDKL5 germline knockout mice. To understand whether the impairment in cortical visual responses present in CDKL5 mutants originates from alterations occurring at the thalamic level, we used IOS imaging to record the amplitude of primary visual cortical activation in response to visual stimulation in conditional CDKL5 mutant mice with preserved CDKL5 expression in subcortical nuclei. We found that the selective deletion of CDKL5 from cortical excitatory and some glial cells is sufficient to produce abnormalities of visual cortical responses, demonstrating that CDKL5 plays an essential role in establishing or maintaining a normal function of cortical circuitry.

Results

CDKL5 null mice show normal retinal morphology

CDKL5 deletion results in structural and functional abnormalities in the visual cortex (22,27). In order to assess whether CDKL5 deletion also affects upstream structures of the visual system, we first analyzed the retina of mice with CDKL5 deletion ($CDKL5^{-/y}$) (1). We first performed a western blot analysis on adult male $CDKL5^{-/y}$ ($N=4$) and WT ($CDKL5^{+/y}$ $N=4$) retinal homogenates to demonstrate CDKL5 retinal expression. Our data (Fig. 1A) indicate that CDKL5 is expressed at the protein level in the retina, in accordance with RNA sequencing data showing CDKL5 in NRL-positive rods (16). No signal was observed at the expected molecular weight in the retina of CDKL5 null mice confirming the specificity of the CDKL5 antibody. This prompted us to assess the effect of the lack of CDKL5 on retinal organization. We stained retinal tissue of P28 male $CDKL5^{-/y}$ ($N=3$) and $CDKL5^{+/y}$ ($N=3$) mice with an extensive set of markers for diverse cell types and markers of connectivity. Staining for cone photoreceptors (cone arrestin positive cells) and rod outer segments (Rhodopsin positive cells) in retinal sections (Fig. 1B and C) revealed that the overall structural organization of photoreceptor cells is identical in the two experimental groups. We then proceeded to assess the total number of selected cell types (cones, cholinergic amacrine cells and ganglion cells) using whole-mount preparations. No statistical difference between $CDKL5^{-/y}$ and $CDKL5^{+/y}$ mice was present in the number of cone photoreceptors (cone arrestin positive cells; Fig. 1D–F; two-tailed Student's *t*-test $P=0.435$), retinal ganglion cells (RGCs; RNA-binding protein with multiple splicing; RNA-binding protein with multiple splicing (RBPMS) positive cells; Fig. 1G–I; two-tailed Student's *t*-test $P=0.098$) and cholinergic amacrine cells [GAD67 and choline acetyltransferase (ChAT) double positive cell] both in the inner nuclear layer (INL; Supplementary Material, Fig. S2C–F; two-tailed Student's *t*-test $P=0.782$) and in the ganglion cell layer (GCL; Supplementary Material, Fig. S2C–F; two-tailed Student's *t*-test $P=0.932$). To further characterize how the structural organization of the retina is influenced by the lack of CDKL5, we performed immunohistochemical experiments for specific markers of different retinal subpopulations on vertical sections of $CDKL5^{-/y}$ ($N=3$) and $CDKL5^{+/y}$ ($N=3$) retinas. This analysis reveals cellular morphology, layering and connectivity allowing an in-depth assessment of possible abnormalities. We investigated the following cell types: cholinergic amacrine cells (GAD67 and ChAT double positive cells; Supplementary Material, Fig. S2A and B), horizontal and amacrine cells (calbindin D positive cells) together with type 2 cone bipolar cells and axonal endings of type 6 cone bipolar cells (ZNP1/synaptotagmin staining; Supplementary Material, Fig. S1A and B). Rod bipolar cells [protein kinase Cα (PKCα) positive cells] were analyzed in combination with a marker of synaptic ribbons [C-terminal-binding protein 2 (CtBP2/RIBEYE) staining] in the outer and inner plexa of the retina (Supplementary Material, Fig. S1C and D). Finally, we labeled Müller glial cells (glutamine synthetase positive cells) in combination with a gap-junction subunit (Connexin36) abundant in the inner plexiform layer of the retina (Supplementary Material, Fig. S1E and F). We found no qualitative alterations in the morphology and distribution of any of these retinal markers. In summary, this series of experiments indicate that the lack of CDKL5 does not alter the morphological organization of retinal circuits.

$CDKL5^{-/y}$ mice show synaptic alterations in the lateral geniculate nucleus and in the primary visual cortex

CDKL5 is also expressed in the mouse thalamus (30). For this reason, we next asked whether the lack of CDKL5 could affect the circuits of the dLGN, the next station of the visual information pathway from the retina to the primary visual cortex (V1). Since CDKL5 is known to regulate spine formation and stability in the cortex and hippocampus (8,38), we assessed density and morphology of dendritic spines in Golgi-stained slices (Fig. 2A) of the dLGN of $CDKL5^{-/y}$ ($N=4$) and $CDKL5^{+/y}$ ($N=5$) control littermates. $CDKL5^{-/y}$ mice showed a reduction in spine density