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Research paper

Abnormally increased surface expression of AMPA receptors in the cerebellum, cortex and striatum of *Cln3*$^{-/-}$ mice

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HIGHLIGHTS

- We examined AMPA receptor surface expression in a mouse model of CLN3 disease.
- Surface levels of GluA1 and GluA2 were elevated in the cerebellum of Cln3$^{-/-}$ mice.
- Surface level of GluA2 was elevated in the cortex and striatum of Cln3$^{-/-}$ mice.
- These data suggest that CLN3 is involved in AMPA receptor cycling.

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ABSTRACT

Mutations in the *CLN3* gene cause a fatal neurodegenerative disorder, juvenile CLN3 disease. Exploring the cause of the motor coordination deficit in the *Cln3*$^{-/-}$ mouse model of the disease we have previously found that attenuation of AMPA receptor activity in 1-month-old *Cln3*$^{-/-}$ mice significantly improves their motor coordination [20]. To elucidate the mechanism of the abnormally increased AMPA receptor function in *Cln3*$^{-/-}$ mice, we examined the surface expression of AMPA receptors using surface cross-linking in brain slices from 1-month-old wild type (WT) and *Cln3*$^{-/-}$ mice. In surface cross-linked brain samples, Western blotting for AMPA receptor subunits revealed significantly increased surface levels of GluA1 and GluA2 in the cerebellum, and of GluA2 in the cortex and striatum of *Cln3*$^{-/-}$ mice as compared to WT mice. Expression levels of the GluA4 subunit were similar in the cerebellum of WT and *Cln3*$^{-/-}$ mice. While intracellular GluA1 levels in the WT and *Cln3*$^{-/-}$ cerebellum or cortex were similar, the intracellular expression of GluA1 in the *Cln3*$^{-/-}$ striatum was decreased to 56% of the WT level.

Our results show a prominent increase in AMPA receptor surface expression in the brain of *Cln3*$^{-/-}$ mice and suggest that CLN3 is involved in the regulation of AMPA receptor surface expression.

1. Introduction

Ion channel-forming, ionotropic receptors mediate the fast excitatory neurotransmission by glutamate in the mammalian central nervous system. Named after their specific agonists, ionotropic glutamate receptors are classified into 3 groups: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors [38]. AMPA receptors are heterotetramers formed by different combinations of four subunits (GluA1-4), and the subunit composition largely determines the functional properties of the receptor. For instance, the presence of the GluA2 subunit makes AMPA receptors calcium-impermeable [31]. Abnormal glutamate neurotransmission and glutamate receptor overactivation play a central role in the pathophysiology of a number of neurodegenerative disorders, e.g., Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease [6].

Neuronal ceroid lipofuscinoses, also called Batten disease, are a group of recessively inherited, fatal lysosomal storage disorders characterized by the intracellular accumulation of autofluorescent lipopigment and progressive neurodegeneration [32]. Batten disease mostly affects children. It is a rare disease with an estimated incidence of 1–2 in 50,000 live births in the US. Mutations of the *CLN3* gene cause the majority of the most prevalent, juvenile

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; NMDA, *N*-methyl-D-aspartate; BS^3, bis[sulfosuccinimidyl] suberate; ACSF, artificial cerebrospinal fluid.

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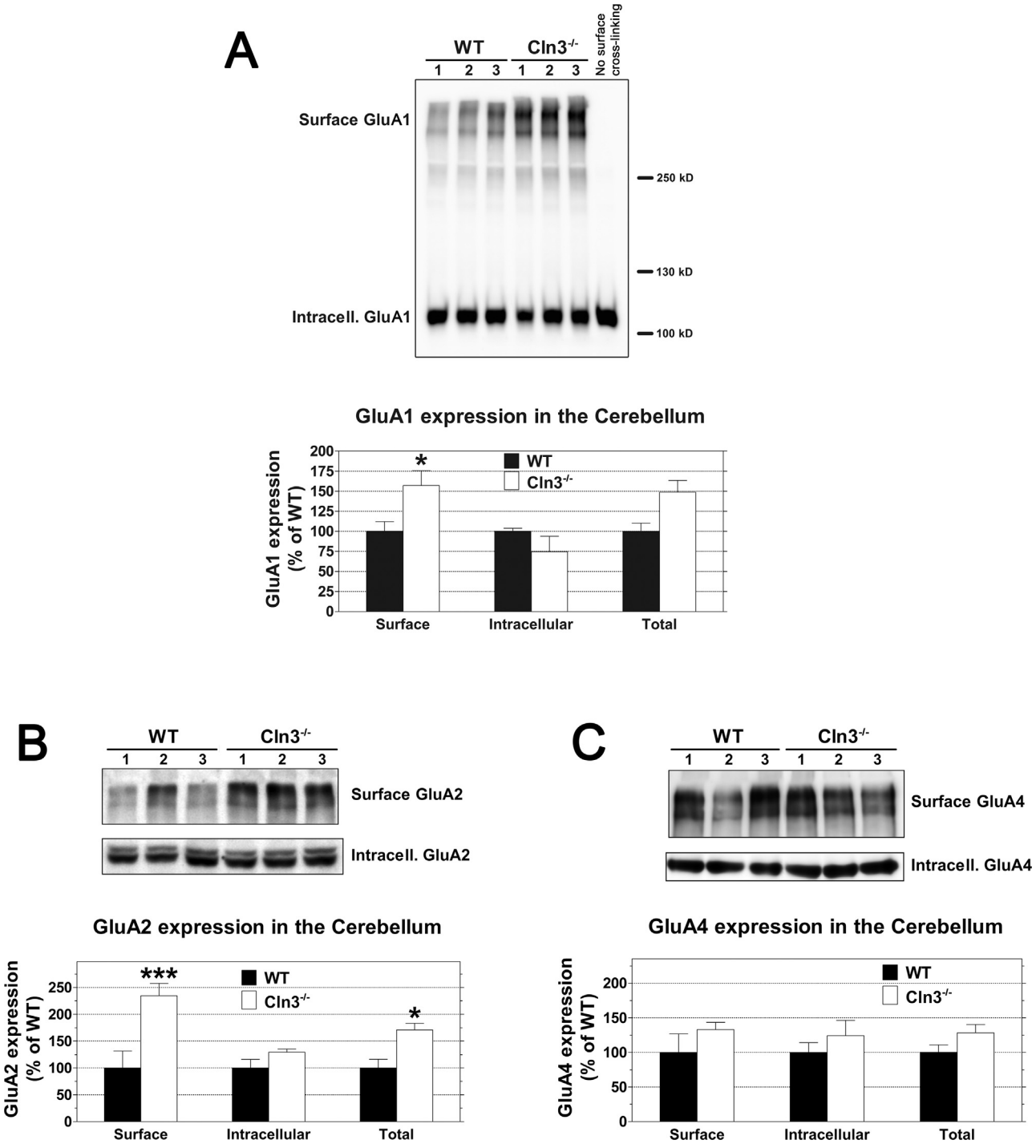

Fig. 1. AMPA receptor surface expression in the cerebellum of *Cln3*$^{-/-}$ mice. The surface and intracellular expression levels of the GluA1, GluA2 and GluA4 AMPA receptor subunits in acutely isolated cerebellar slices from 1-month-old WT and *Cln3*$^{-/-}$ male mice were determined by surface cross-linking and Western blotting. (A) GluA1 surface and intracellular expression in the cerebellum. The representative Western blot shows separation of surface GluA1 (in cross-linked, high-molecular-weight complexes) and intracellular GluA1. Notice the lack of surface bands in the cerebellar sample without surface cross-linking. The graph shows quantification of surface, intracellular, and total (surface + intracellular) GluA1 expression levels in surface cross-linked cerebellar samples from 7 WT and 8 *Cln3*$^{-/-}$ mice. Columns and bars represent mean ± SEM. (B) GluA2 surface and intracellular expression in the cerebellum. A representative Western blot and quantification of surface, intracellular, and total GluA2 expression levels are shown. Columns and bars represent mean ± SEM (5 WT and 5 *Cln3*$^{-/-}$ mice). (C) GluA4 surface and intracellular expression levels in the cerebellum of WT and *Cln3*$^{-/-}$ mice are similar. A representative Western blot and quantification of surface, intracellular, and total GluA4 expression levels are shown. Columns and bars represent mean ± SEM (5 WT and 5 *Cln3*$^{-/-}$ mice). The expression levels of GluA1, GluA2 and GluA4 were normalized to the total protein levels determined by Ponceau S staining. Statistical significance was determined by 2-way ANOVA with Bonferroni's post-test for pairwise multiple comparisons: $^{***}p < 0.001$, $^{*}p < 0.05$.

onset form of Batten disease, and this disorder is now called juvenile CLN3 disease to clearly identify the genetic cause and clinical form [11,41]. The disease begins between 4 and 10 years of age, and reaches its terminal stage in the late teens or early 20s. *CLN3* encodes a putative lysosomal transmembrane protein but the exact function of CLN3 and why *CLN3* mutations cause selective neurodegeneration are still unknown. Several studies indicated a role