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Age-dependent alterations in neuronal activity in the hippocampus and visual cortex in a mouse model of Juvenile Neuronal Ceroid Lipofuscinosis (CLN3)

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ABSTRACT

Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is a fatal lysosomal storage disease caused by autosomal recessive mutations in CLN3. JNCL is typified by progressive neurodegeneration that has been suggested to occur from excessive excitatory and impaired inhibitory synaptic input; however, no studies to date have directly evaluated neuronal function. To examine changes in neuronal activity with advancing disease, electrophysiological recordings were performed in the CA1 hippocampus (HPC) and visual cortex (VC) of acute brain slices from $Cln3^{\Delta ex7/8}$ mice at 1, 4, 8, and 12 months of age. Basic electrophysiological parameters, such as field excitatory post-synaptic potential (fePSP) and population spike (PS) amplitudes, were not altered in $Cln3^{\Delta ex7/8}$ CA1 and VC neurons at any age. However, fiber volley (FV) amplitudes were significantly increased in $Cln3^{\Delta ex7/8}$ neurons in the HPC at 1 month as well as layer II/III of the VC at 1 and 4 months, suggesting increased axonal excitability. In older $Cln3^{\Delta ex7/8}$ mice (8 and 12 months), FV amplitude in the CA1 HPC and VC reached levels that were equal to or significantly lower than WT animals. Significant alterations in the synaptic strength of $Cln3^{\Delta ex7/8}$ CA1 neurons were also linked to age-dependent changes in axonal responses. Additionally, paired-pulse and 12-pulse facilitation responses calculated from PS recordings were significantly decreased in the CA1 HPC and layer II/III of the VC of $Cln3^{\Delta ex7/8}$ mice at all ages, suggesting permanent alterations in neuronal short-term plasticity. Collectively, our study has identified novel age- and region-dependent alterations in axonal excitability as well as synaptic and non-synaptic neuronal activity in $Cln3^{\Delta ex7/8}$ mice during disease progression, which may inform neurodegenerative mechanisms in JNCL.

1. Introduction

Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), or juvenile Batten disease, is a lysosomal storage disorder caused by autosomal recessive loss-of-function mutations in CLN3. Clinical symptoms of JNCL first appear in children between 5 and 8 years of age, beginning with progressive visual deterioration leading to blindness, seizures, motor dysfunction, cognitive decline, and premature death by late teens to 20s (Anderson et al., 2013). The function of CLN3 remains unknown; however, modeling predicts a lipophilic transmembrane protein (Nugent et al., 2008; Ratajczak et al., 2014) that has been reported to influence several cellular processes, including autophagy, exo- and endocytosis, and cytosolic transport, as well as the function of mitochondria, endosomes, and lysosomes (Cotman and Staropoli, 2012; Phillips et al., 2005; Carcel-Trullols et al., 2015). JNCL is typified by lysosomal inclusions composed primarily of autofluorescent lipofuscin and mitochondrial ATP synthase subunit C in addition to other proteins and lipids (Fossale et al., 2004). Although CLN3 is ubiquitously expressed, neurons are the most vulnerable to CLN3 loss and show progressive accumulation of lysosomal inclusions. Neuronal death results in reduced cortical and hippocampal volume that is observed in JNCL patients (Autti et al., 2008; Tokola et al., 2014). Despite identifying CLN3 as the causative mutation for JNCL in 1995 (Lerner, 1995), there is currently no cure for the disease and available therapeutics only target disease symptoms.

Abbreviations: 12PF, 12-pulse facilitation; 12PR, 12-pulse ratio; ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; fePSP, field excitatory post-synaptic potential; FP, field potential; FV, fiber volley; GABA, γ-aminobityric acid; HPC, hippocampus; IC, incremental current; JNCL, Juvenile Neuronal Ceroid Lipofuscinosis; KO, knockout; NMDA, *N*-Methyl-D-aspartate; PPF, paired-pulse facilitation; PPR, paired-pulse ratio; PS, population spike; Py, pyramidal layer of the HPC; Rad, stratum radiatum; TTX, tetrodotoxin; WT, wild type.

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The $Cln3^{\Delta ex7/8}$ knock-in mouse was created to genetically model the 1.02 kb common deletion that occurs in approximately 85% of mutated CLN3 alleles (Cotman et al., 2002). Despite the fact that $Cln3^{\Delta ex7/8}$ mice exhibit many characteristics of JNCL, such as the progressive accumulation of autofluorescent inclusions and early glial activation (Cotman et al., 2002; Pontikis et al., 2005; Burkovetskaya et al., 2014) retinal pathology and neuronal loss occur relatively late in the disease process (i.e. 12–18 months) (Volz et al., 2014) and $Cln3^{\Delta ex7/8}$ animals do not exhibit gross evidence of seizures although they display a lower seizure threshold (Pontikis et al., 2005; Eliason et al., 2007). In our laboratory, $Cln3^{\Delta ex7/8}$ mice display significant motor dysfunction (accelerating rotarod) as early as 2 months of age (Bosch et al., 2016) and others have reported neurodevelopmental delays in these animals (Osorio et al., 2009). This suggests the existence of early neuronal pathology that significantly predates neuronal death at 12–18 months, which is supported by several studies in Cln3 mutant mice. First, high-resolution NMR metabolomics have identified a glutamate/γ-aminobityric acid (GABA) imbalance in JNCL, namely increased excitatory glutamate and reduced inhibitory GABA in 1–3 month old Cln3 knockout (KO) mice (Pears et al., 2005). Further magnifying perturbations in glutamate regulation, Cln3 mouse models as well as JNCL patients develop autoantibodies against glutamic acid decarboxylase 65, the enzyme responsible for glutamate to GABA conversion (Ramirez-Montealegre et al., 2005). A role for glutamate excitotoxicity has been shown in Cln3 KO mice, where pharmacological blockade of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or *N*-Methyl-D-aspartate (NMDA) receptors improved motor performance and attenuated glial activation (Kovacs et al., 2011; Kovacs et al., 2015). Our laboratory has provided additional evidence to support glutamate dysfunction in JNCL. Specifically, we have shown that glutamate excitatory amino acid transporters 1 and 2 are significantly decreased in $Cln3^{\Delta ex7/8}$ astrocytes in vivo (Burkovetskaya et al., 2014), which coincides with impaired glutamate uptake (Bosch and Kielian, manuscript in preparation). We have also reported significant decreases in glutamine synthetase expression in the $Cln3^{\Delta ex7/8}$ brain (Burkovetskaya et al., 2014), which can further contribute to glutamate dysfunction. Another group described perturbations in Ca^{2+} homeostasis in a $Cln3^{\Delta ex7/8}$ cerebellar cell line, demonstrating the vulnerability of $Cln3^{\Delta ex7/8}$ neurons to Ca^{2+}-induced cytotoxicity (Chang et al., 2007) and increased Ca^{2+}-mediated sensitivity to thapsigargin (An Haack et al., 2011; Chandrachud et al., 2015).

Collectively, although the currently available evidence suggests that neuronal activity is altered in $Cln3^{\Delta ex7/8}$ mice, neuronal network function has never been examined. Therefore, we performed a detailed analysis of evoked neuronal responses in the CA1 region of the hippocampus (HPC) and visual cortex (VC) using electrophysiological methods on acute brain slices of $Cln3^{\Delta ex7/8}$ and WT animals at 1, 4, 8, and 12 months of age. Our work provides the first evidence of age-dependent changes in CA1 HPC and VC neuronal activity during the course of JNCL progression. There is currently little evidence of cognitive disturbances in $Cln3^{\Delta ex7/8}$ mice, although progressive cognitive decline is a hallmark feature of JNCL in humans (Lamminranta et al., 2001; Kwon et al., 2011). In the current work, we provide the first electrophysiological evidence for alterations in short-term plasticity in the $Cln3^{\Delta ex7/8}$ brain, which may facilitate the future understanding of a role for CLN3 in cognitive function.

2. Materials and methods

2.1. Ethics statement

This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and complies with the ARRIVE guidelines. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center (Approval ID: 11-074-08-EP).

2.2. Mice

Male $Cln3^{\Delta ex7/8}$ knock-in mice (C57BL/6 background), which lack a 1.02 kb segment spanning exons 7 and 8 of CLN3, were used in this work (Cotman et al., 2002) with age- and sex-matched C57BL/6 wild type (WT) mice as controls (The Jackson Laboratory, Bar Harbor, ME). To analyze changes in neuronal activity during JNCL progression, animals were examined at 1, 4, 8, and 12 months of age (n = 4–10/group).

2.3. Brain slice preparation

$Cln3^{\Delta ex7/8}$ and WT mice were anesthetized using isoflurane and immediately sacrificed by decapitation. Brains were quickly removed and positioned in a Leica VT1000S vibrating-blade microtome chamber (Leica Microsystems, Germany) filled with ice-cold artificial cerebrospinal fluid (ACSF) formulated specifically for the sectioning and recovery steps during brain slice preparation (cutting/recovery ACSF, containing in mM: 92 NaCl, 30 $NaHCO_3$, 3 KCl, 10 $MgCl_2$, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 25 glucose, 20 HEPES, 2.5 ascorbic acid, 7.5 Na-pyruvate, 5 thiourea; pH 7.4, maintained by continuous bubbling with carbogen [95% O_2 and 5% CO_2]). The composition of cutting/recovery ACSF was based on a prior publication (Ting et al., 2014) as well as our own observations, which preserved neuron morphology and improved the quality of electrophysiological recordings in acute brain slices prepared from adult and aged mice. Upon sectioning, 300 μm thick coronal brain slices containing the HPC and VC were incubated in cutting/recovery ACSF for 30 min at 32 °C. Next, brain slices were transferred to a standard ACSF solution (mM): 124 NaCl, 26 $NaHCO_3$, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 10 glucose, 0.5 ascorbic acid, 1.5 Na-pyruvate, 1 thiourea; pH 7.4, maintained by continuous bubbling with carbogen [95% O_2 and 5% CO_2] at room temperature and were allowed to equilibrate for at least 1 h prior to electrophysiological recordings.

2.4. Electrophysiology

Evoked local field potential (FP) recordings in acute brain slices were performed in a submerged chamber (RC-27, Warner Instruments) continuously perfused with standard ACSF at a rate of ~2 ml/min at 30 °C. Tissue stimulation and electrophysiological recordings were performed using stainless steel and tungsten (0.8–1.2 MΩ impedance in ACSF at 1000 Hz) electrodes (FHC Inc. and RMI Inc., respectively) using a computer-controlled amplifier (Multiclamp 700B, Molecular Devices) connected to an isolated pulse stimulator (Models 2100, 2200, and 2300, A-M Systems). Analog signals from the amplifier were digitized at a 10 kHz sampling rate using a 16-bit resolution Digidata-1440A acquisition system and pClamp-10 (Molecular Devices). Amplifier filters were set from 3 Hz to 3 kHz. The acquisition parameters were selected to digitize not only FPs but also stimulation pulse artifacts, which were used as time stamps to detect and calculate primary FP parameters with custom-designed software used in our previous studies (ImpulswXE) (Karpuk et al., 2012; Karpuk et al., 2011).

For HPC recordings, FP responses were subdivided into three groups: 1) field excitatory post-synaptic potentials (fePSPs) recorded in the stratum radiatum of CA1 (Rad) to estimate the synaptic activity of CA1 pyramidal neurons (Fig. 1A); 2) population spikes (PSs) recorded in the pyramidal cell layer (Py) of the CA1 HPC to analyze neuron action potential activity (Fig. 1A); and 3) fiber volleys (FVs) simultaneously elicited with PSs or fePSPs to assess axonal conductivity of CA3 pyramidal neurons (Fig. 1A). In the CA1 region, a double-wired stimulation bipolar electrode was positioned at the border of the lacunosum moleculare and Rad layers. The recording microelectrode was placed in either the Rad approximately 300 μm away from the stimulation electrode for fePSP recordings, or about 300 μm from the tips of the stimulating electrodes for the Py (Fig. 1A). In the VC, all FPs were recorded with double microelectrodes located in layers II/III and V, whereas the