Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Increased excitability and compromised long-term potentiation in the neocortex of NPC1 $^{-/-}$ mice

Yosef Avchalumov[a], *Timo Kirschstein*[a], *Jan Lukas*[c], *Jiankai Luo*[c], *Andreas Wree*[b], *Arndt Rolfs*[c], *Rüdiger Köhling*[a,*]

[a]*Oscar Langendorff Institute of Physiology, University of Rostock, Germany*
[b]*Institute of Anatomy, University of Rostock, Germany*
[c]*Albrecht Kossel Institute for Neuroregeneration, University of Rostock, Germany*

ARTICLE INFO

Article history:
Accepted 8 January 2012
Available online 17 January 2012

Keywords:
Synaptic plasticity
Synaptic transmission
Corticostriatal
Schaffer collateral
CA1
Neocortex

ABSTRACT

Niemann–Pick type C1 (NPC1) disease is a neurodegenerative lysosomal storage disorder caused by mutations in the NPC1 gene which encodes a transmembrane protein of the acidic compartment. Albeit the NPC1$^{-/-}$ mouse is available serving as an appropriate animal model of the human disease, the precise function of this protein remains obscure. Here, we investigated the synaptic consequences of this disease and explored long-term potentiation (LTP) in slices taken from the hippocampal CA1 region, the dorsomedial striatum as well as the somatosensory neocortex in NPC1$^{-/-}$ mice using extracellular field potential recordings. We did not observe significant changes in synaptic excitability as well as LTP in the hippocampal CA1 region and the dorsomedial striatum of NPC1$^{-/-}$ mice when compared to wild-type littermates. However, neocortical excitability was significantly enhanced while LTP was abolished. These results suggest that at least in the somatosensory neocortex NPC1 protein is instrumental in synaptic function.

1. Introduction

Niemann–Pick type C1 (NPC1) disease is a neurodegenerative lysosomal storage disorder caused by mutations in the NPC1 gene that encodes a transmembrane protein of the acidic compartment. The precise function of NPC1 protein is largely unknown, but it has been suggested that it is important for the liberation of cholesterol out of the lysosome to other cellular sites such as endoplasmic reticulum and the plasma membrane (Bauer et al., 2002; Paul et al., 2004). Thus, NPC1 disease is typically associated with abnormal intracellular accumulation of cholesterol in a number of tissues which includes spleen and liver (Garver and Heidenreich, 2002; Sturley et al., 2004). The mutations in the NPC1 gene cause widespread neurological deficits, including ataxia, dystonia, seizures, dementia and deficits in cognitive function which eventually lead to premature death. Most neuropathological alterations in patients with NPC1 disease have been found in the basal ganglia, hippocampus, cortex, and cerebellum (Sturley et al., 2004).

Mice lacking the NPC1 gene (NPC1$^{-/-}$) are available (Pentchev et al., 1980) and represent an appropriate model to study the function of this protein. The accumulation of cholesterol and other fatty acids in the brain of NPC1$^{-/-}$ mice suggests that these lipids have a neuronal origin (Dietschy and Turley, 2002). Several studies have demonstrated that cerebellar Purkinje cells have abnormal axonal and dendritic morphologies which

* *Corresponding author at:* University of Rostock, Medical Faculty, Oscar Langendorff Institute of Physiology, Gertrudenstrasse 9, D-18057 Rostock, Germany. Fax: +49 381 494 8002.
E-mail address: ruediger.koehling@uni-rostock.de (R. Köhling).

0006-8993/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2012.01.019

often include swollen dendrites and decreased numbers of spines (Higashi et al., 1993; Sarna et al., 2003). Others have shown swollen axon hillocks, axonal swelling, and abnormal axonal branching in both Purkinje and pyramidal cells (March et al., 1997; Sarna et al., 2003; Zervas et al., 2001), all in all hence suggesting a neuronal phenotype.

Yet, although alterations in neuronal morphology in NPC1 disease are well described, the electrophysiological consequences of this pathology seem less clear. Only one intracellular study was performed in sensory-motor cortex, which, however, failed to detect significant differences in electrophysiological properties between neurons from NPC1$^{-/-}$ mice and control littermates (Deisz et al., 2005). This raises the question whether synaptic functions including synaptic excitability and plasticity are altered in NPC1$^{-/-}$ mice particularly in view of dystonic symptoms, seizures and cognitive deficits in NPC1 patients which are suggestive of synaptic alterations. Long-term potentiation (LTP) is a sustained increase in synaptic efficacy induced by high-frequency stimulation (Bliss and Collingridge, 1993). Multiple lines of evidence suggest an important link between memory and learning and synaptic plasticity (Malenka and Bear, 2004; Rumpel et al., 2005; Whitlock et al., 2006). Induction of LTP requires an increase of the intracellular Ca^{2+} concentration, mediated by Ca^{2+} influx into postsynaptic neurons through NMDA receptors (Artola and Singer, 1987). Several studies have demonstrated the role of cholesterol in synaptic plasticity (Frank et al., 2008; Koudinov and Koudinova, 2001; Parkinson et al., 2009). Since NPC1$^{-/-}$ mice represent an appropriate model of a metabolic disorder of cholesterol transport, the aim of this study was to determine whether or not synaptic transmission and synaptic plasticity are affected in NPC1$^{-/-}$ mice.

2. Results

2.1. *Corticostriatal LTP is preserved in NPC1$^{-/-}$ mice*

Since one of the prominent features of Niemann–Pick type C1 disease is dystonia, we asked whether NPC1 gene ablation might interfere with synaptic transmission and plasticity in the dorsomedial striatum. Recent reports have correlated altered synaptic plasticity in the dorsomedial striatum with several different animal models of dystonia (Kohling et al., 2004; Martella et al., 2009). As shown in Fig. 1A, the basal synaptic transmission assessed by an input–output analysis was unchanged in NPC1$^{-/-}$ mice when compared with wildtype littermates. Moreover, the paired-pulse ratio (PPR) was also not significantly different between these two groups (wildtype: 107±8%, n=16; NPC1$^{-/-}$: 92±15%, n=8; Fig. 1B, leftmost bars). Following a stable baseline recording of 20 min, a high-frequency stimulation protocol (HFS; 4 trains of 100 pulses at 100 Hz with an inter-train interval of 10 s) was used to induce LTP (Li et al., 2009). In wildtype mice, robust LTP was obtained with a mean relative field potential slope of 124±10% (n=16, $p<0.05$ versus pre-HFS baseline; open symbols in Fig. 1C). Interestingly, LTP could also be induced in NPC1$^{-/-}$ mice (133±9%, n=8, $p<0.05$ versus pre-HFS baseline; closed symbols in Fig. 1C), and no significant difference was found between these two groups. Moreover, the PPR did not show any differences following LTP induction, either (Fig. 1B, rightmost bars). Hence, from this set of experiments we concluded that ablation of the NPC1 protein may rather not play a major role in the regulation of synaptic plasticity in the dorsomedial striatum.

2.2. *Hippocampal CA1-LTP is also preserved in NPC1$^{-/-}$ mice*

LTP is one of the key features within the hippocampal network and is considered a correlate of cognitive functions. Thus, our next step was to explore synaptic function and plasticity in the CA1 region. Basal synaptic transmission, was not altered in NPC1$^{-/-}$ mice when compared to wildtype littermates (Fig. 2A), and the paired-pulse ratio (PPR) was also unchanged by the genetic disruption of NPC1 protein (wildtype: 103±12%, n=11; NPC1$^{-/-}$: 116±29%, n=6; Fig. 2B, leftmost bars). We again induced LTP with tetanic stimulation (2 trains of 100 stimuli at 100 Hz with an inter-train interval of 30 s) yielding a robust potentiation of the relative field potential slope which was 193±27% in wildtype mice (n=16, $p<0.05$ versus pre-HFS baseline; open symbols in Fig. 2C) and 171±16% in NPC1$^{-/-}$ mice (n=5, $p<0.05$ versus pre-HFS baseline; closed symbols in Fig. 2C). Again, there was no significant difference between these two groups, and the PPR did not detect any differences following LTP induction (Fig. 2B, rightmost bars). Like in the dorsomedial striatum, our data do not suggest a major role for the NPC1 protein in the regulation of synaptic plasticity in the hippocampal CA1 region.

2.3. *Neocortical LTP is dramatically compromised in NPC1$^{-/-}$ mice*

Since clinically, NPC1 patients also show symptoms suggesting neocortical involvement, we next investigated LTP in the somatosensory neocortex. Here, we recorded field potentials from the neocortical layer 2/3 while the stimulation was performed in layer 6 or between layer 6 and white matter. In contrast to corticostriatal and hippocampal synapses, neocortical synapses from NPC1$^{-/-}$ mice showed a significantly enhanced input–output relationship compared to the wildtype (Fig. 3A) while the PPR analysis again did not reveal any significant differences between these two groups (wildtype: 108±7%, n=10; NPC1$^{-/-}$: 101±10%, n=9; Fig. 3B, leftmost bars). Since neocortical synapses of NPC1$^{-/-}$ mice appeared to be more excitable than controls, it was intriguing to study their propensity to undergo synaptic plasticity. Following 20 min of stable baseline recording, we employed theta-burst stimulations (TBS; 10 trains of 4 stimuli at 100 Hz with an inter-train interval of 200 ms) (Khaleghi et al., 2009). Theta-burst stimulation induced significant LTP in wildtype mice (136±10%, n=14, $p<0.01$ versus pre-TBS baseline, open symbols in Fig. 3C). In NPC1$^{-/-}$ mice, however, LTP was almost abolished (107±6%, n=11, $p<0.05$ versus wildtype; closed symbols in Fig. 3C). These data suggest that the loss of neocortical synaptic plasticity in NPC1$^{-/-}$ mice might be due to an increase of network excitability, which leads to saturation of synaptic responses, and thereby infringes synaptic plasticity. Hence, we conclude that the NPC1 protein plays an important role in the regulation of synaptic plasticity, and can be instrumental in regulation of network excitability — at least at neocortical