Increased excitability and compromised long-term potentiation in the neocortex of NPC1−/− mice

Yosef Avchalumov, Timo Kirschstein, Jan Lukas, Jiankai Luo, Andreas Wree, Arndt Rolfs, Rüdiger Köhling

PII: S0006-8993(12)00057-1
Reference: BRES 42027

To appear in: Brain Research

Accepted date: 8 January 2012

Please cite this article as: Yosef Avchalumov, Timo Kirschstein, Jan Lukas, Jiankai Luo, Andreas Wree, Arndt Rolfs, Rüdiger Köhling, Increased excitability and compromised long-term potentiation in the neocortex of NPC1−/− mice, Brain Research (2012), doi: 10.1016/j.brainres.2012.01.019

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Increased excitability and compromised long-term potentiation in the neocortex of NPC1\(^{-/-}\) mice

Yosef Avchalumov\(^1\), Timo Kirschstein\(^1\), Jan Lukas\(^3\), Jiankai Luo\(^3\), Andreas Wree\(^2\), Arndt Rolfs\(^3\), Rüdiger Köhling\(^1\)

\(^1\) Oscar Langendorff Institute of Physiology, University of Rostock
\(^2\) Institute of Anatomy, University of Rostock
\(^3\) Albrecht Kossel Institute for Neuroregeneration, University of Rostock

Word count:
- Abstract: 149 words
- Introduction: 455 words
- Discussion: 746 words

Corresponding Author:
Prof. Dr. Rüdiger Köhling
University of Rostock
Medical Faculty
Oscar Langendorff Institute of Physiology
Gertrudenstrasse 9
D-18057 Rostock
Germany

Tel.: +49 381 494 8000
Fax: +49 381 494 8002
E-mail: ruediger.koehling@uni-rostock.de
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 wildtype 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.

Key words

Synaptic plasticity, synaptic transmission, corticostriatal, Schaffer collateral, CA1, neocortex
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 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\textsuperscript{+/−} 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\textsuperscript{+/−} 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\textsuperscript{+/−}: 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\textsuperscript{+/−} 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<sup>−/−</sup>: 101 ± 10%, n=9; Fig. 3B, leftmost bars). Since neocortical synapses of NPC1<sup>−/−</sup> 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<sup>−/−</sup> 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<sup>−/−</sup> 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 synapses. The PPR analysis at baseline and following LTP induction did not show any significant differences implicating that the effect of the NPC1 protein on synaptic function was postsynaptic (Fig. 3B, rightmost bars).
3. Discussion

Niemann-Pick type C (NPC) disease is an inherited and complex disorder due to the inactivation of NPC1 or NPC2 protein (Carstea et al., 1997). At the cellular level, the disorder is a result of dysfunctional trafficking 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). There has been an increasing interest in the effect of NPC disease on neuronal function in the brain. Clinically, the disease causes widespread neurological deficits, including seizures, dystonia, ataxia, dementia which ultimately lead to neurodegeneration and premature death. In the present study, we investigated whether synaptic transmission and plasticity are affected in NPC1-/- mice. We found that synaptic transmission and plasticity were unaltered in the hippocampal CA1 region and in the dorsomedial striatum of NPC1-/- mice. In contrast, we found that synaptic plasticity in neocortical synapses from NPC1-deficient tissue was compromised, and at the same time, neocortical network excitability was significantly enhanced relative to control littermates.

What is the impact of an elevated cholesterol level in the brain? Due to the blood-brain barrier, very little systemic cholesterol will enter the brain. Rather, the central nervous system relies upon endogenously synthesized cholesterol. High levels of cholesterol cause alterations in cell morphology and function (Sturley et al., 2004), e.g. elongated swellings at axon hillocks referred to as meganeurites (Purpura and Suzuki, 1976) or swollen dendrites in cortical pyramidal cells (Zervas et al., 2001). Another prominent feature of NPC disease is the occurrence of neurofibrillary tangles in many parts of the brain, including basal ganglia cortex, hippocampus, hypothalamus (Love et al., 1995). Thus, it is an intriguing question how elevated
cholesterol levels might affect synaptic transmission and plasticity in the brain. Among all brain areas, the hippocampus has been the most extensively studied region with respect to synaptic plasticity such as long-term potentiation (LTP). Following the induction of LTP in the hippocampal CA1 region, new spines appear on the postsynaptic dendrites, whereas in the control situation, where LTP was blocked, spine growth did not occur (Engert and Bonhoeffer, 1999). On the contrary, low-frequency stimulation at these synapses led to a marked shrinkage of dendritic spines, which was reversed by subsequent high-frequency stimulation (Zhou et al., 2004). Interestingly, (Hering et al., 2003) reported that cholesterol depletion leads to instability of surface AMPA receptors and to the gradual loss of excitatory and inhibitory synapses and dendritic spines in the CA1 region of the hippocampus. These findings might suggest an enhanced stability of surface AMPA receptors in the NPC1<sup>−/−</sup> mouse leading to a facilitation of LTP. In our hands, LTP was unchanged in the striatum and the hippocampus, but impaired in the neocortex. However, at least in the neocortex, network excitability was significantly increased, which is consistent with an increase of excitatory synapses and spines as well as enhanced stability of surface AMPA receptors in the NPC1-deficient tissue. This increase of network excitability could, if saturating, infringe synaptic plasticity in neocortex.

Token together, this suggests that cholesterol in neurons could maintain synapses and spines as well, and disruption of cholesterol metabolism or lysosomal cholesterol levels might lead to distortion of synaptic plasticity in the neocortex. Another way by which high levels of cholesterol might disrupt synaptic plasticity in neocortex is through the ion channels and other proteins associated with synapses. Recent evidence suggests that cholesterol inhibits P-type calcium channels and thereby
transmitter release (Sparrow et al., 1999; Taverna et al., 2004). Several studies have shown that cholesterol and other lipid rafts are important for regulation of exocytosis, membrane fusion, and neurotransmitter release through several exocytotic machinery molecules, such as SNAP-25 and VAMP2 (Chamberlain et al., 2001; Hering et al., 2003). (Mitter et al., 2003) have demonstrated that at the biochemical level neurons from NPC1 mice have significantly reduced amounts of the synaptophysin/synaptobrevin complex. Synaptophysin binds to synaptobrevin to promote the formation of functional SNARE complexes which is required for regulating synaptic transmission and synaptic plasticity (Pozzo-Miller et al., 1999). This reduction of the synaptophysin/synaptobrevin complex might also lead to a downregulation of LTP in the neocortex of NPC1<sup>−/−</sup> mice.

In summary, we found that synaptic plasticity and synaptic transmission in various brain areas were differentially affected in NPC1<sup>−/−</sup> mice. The loss of the NPC1 protein compromised long-term potentiation in the neocortex which was accompanied by an enhanced excitability of these synapses. In contrast, LTP and synaptic transmission were preserved in the hippocampal CA1 region, and in the dorsomedial striatum.
4. Experimental procedure

4.1. Animals and slice preparation

The present experiments were carried out in NPC1⁻/⁻ mice (Pentchev et al., 1980) and their corresponding wildtype littermates (BALB/cNctr-Npc1⁻/⁻, The Jackson Laboratory, Bar Harbor, Maine, USA; 35-40 days old) in accordance with the German guidelines for the use of experimental animals. All mice were kept under controlled and constant environmental conditions. Following deep anesthesia with diethylether, the animals were decapitated and the brains were quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 26 NaHCO₃, 4 KCl, 1.25 NaHPO₄, 2 CaCl₂, 1.3 MgCl₂ and 10 glucose.

Since clinical symptoms in NPC1 disease include dystonia, seizures and cognitive deficit, suggesting involvement of striatum, neocortex and hippocampus, we studied synaptic function in NPC1⁻/⁻ mice, using slices from dorsomedial striatum, hippocampus, and somatosensory neocortex. For the experiments in the dorsomedial striatum, angulated slices were used (Kohling et al., 2004). To achieve this, the brain was trimmed on the dorsal side at an angle of approximately 140° from the horizontal and glued to a vibratome on that side (Integraslice 7550 mm, Campden Instruments Loughborough, United Kingdom). From this slanted tissue block, angulated slices (500 µm thickness) were cut which contained the motor cortex and entire striatum, with connections between these two regions still intact. For the experiments on other brain regions, transverse hippocampal slices (400 µm thickness), and coronal slices of the somatosensory neocortex (520 µm thickness) were prepared. Two slices were immediately transferred to the interface-type
recording chamber (BSC-HT, Harvard Apparatus, Holliston, USA; perfusion rate of 2 ml/min) and incubated at room temperature for at least 1-1.5 hours and then for another 1-1.5 hours at 32-33°C (controlled by TC-10, npi electronic, Tamm, Germany). The rest of the slices were stored in a submersion-type bath filled with ACSF. Thus, the recordings were started after at least 2.5-3 hours of incubation.

4.2. Electrophysiological recordings

Evoked field potentials were recorded with pipettes made from borosilicate glass capillaries (GB150-8P, Science Products, Hofheim am Taunus, Germany) and filled with ACSF (tip resistance of 2-3 MΩ) from the dorsomedial striatum, the CA1 stratum radiatum and neocortical layers 2-3 in slices taken from NPC1-/- mice and their wildtype littermates, respectively. Stimulation was delivered using thin bipolar platinum wires connected to a stimulus isolator (ISO-STIM 01D, npi electronic) at a baseline rate of 0.033 Hz (controlled by Master-8, A.M.P.I., Jerusalem, Israel). The stimulation sites of the afferent fibers were corticostriatal fibers, stimulated in white matter between the cortex and the striatum, Schaffer collateral fibers, stimulated within the stratum radiatum, and neocortical afferents, stimulated between the white matter and neocortical layer 6 or within layer 6.

For the input-output characterization of the evoked potentials, the stimulus intensity was increased stepwise until reaching saturating responses. Then, stimulus strength was set at 50% of saturating intensity. Paired-pulse plasticity was tested at an interstimulus interval of 40 ms, and the ratio of the second to the first amplitude of the field potential was calculated. Following 20 minutes of stable baseline, long-term potentiation (LTP) was induced with several paradigms: corticostriatal LTP was
induced by 4 trains of 100 pulses at 100 Hz with inter-train intervals of 10 s (Li et al., 2009). The hippocampal CA1-LTP induction protocol consisted of 2 trains of 100 pulses at 100 Hz with an inter-train interval of 30 s. For neocortical LTP, a theta-burst stimulation (TBS) protocol was used consisting of 10 trains of 4 pulses at 100 Hz which were delivered at 200 ms intervals (Khaleghi et al., 2009). For LTP recordings in the neocortex, MgCl$_2$ was reduced to 1.0 mM.

The recordings were amplified and digitized (Micro1401, CED, Cambridge Electronic Design, Cambridge, UK) using Signal 2.03 software (CED, Cambridge, UK). The field potential slopes were measured and expressed as the means ± SEM relative to the mean slope of the baseline response. The LTP level for each experiment was calculated as the mean field potential slope 55-60 min after LTP induction relative to baseline values. The statistical analysis was performed with SigmaStat software (Chicago, IL, USA) using the Student’s t-test, and significant differences were indicated by asterisks (*p<0.05; **p<0.01).
Figure legends

Fig. 1. Corticostriatal LTP is preserved in NPC1<sup>-/-</sup> mice.

(A) Basal synaptic transmission (input-output relationship) was not altered in NPC1<sup>-/-</sup> mice (closed symbols) compared with wildtype littermates (open symbols).

(B) The paired-pulse ratio did not show any significant differences between wildtype and NPC1<sup>-/-</sup> mice – neither at baseline nor following LTP induction.

(C) The time course of relative field potential slopes following high-frequency stimulation (indicated by the gray arrow) revealed no significant difference in the level of LTP between wildtype (open symbols) and NPC1<sup>-/-</sup> mice (closed symbols). Representative recordings were taken from time-points 1 (baseline) and 2 (55-60 minutes after HFS). Traces were superimposed to illustrate differences in amplitudes and slopes.

Fig. 2. Hippocampal CA1-LTP is also preserved in NPC1<sup>-/-</sup> mice.

(A) Basal synaptic transmission (input-output relationship) was not altered in NPC1<sup>-/-</sup> mice (closed symbols) compared with wildtype littermates (open symbols).

(B) The paired-pulse ratio did not show any significant differences between wildtype and NPC1<sup>-/-</sup> mice – neither at baseline nor following LTP induction.

(C) The time course of relative field potential slopes following high-frequency stimulation (indicated by the gray arrow) revealed no significant difference in the level of LTP between wildtype (open symbols) and NPC1<sup>-/-</sup> mice (closed symbols). Representative recordings were taken from time-points 1 (baseline) and 2 (55-60
minutes after HFS). Traces were superimposed to illustrate differences in amplitudes and slopes.

**Fig. 3. Neocortical LTP is dramatically compromised in NPC1⁻/⁻ mice.**

(A) Basal synaptic transmission (input-output relationship) was significantly increased in NPC1⁻/⁻ mice (closed symbols) compared to wildtype littermates (open symbols).

(B) The paired-pulse ratio did not show any significant differences between wildtype and NPC1⁻/⁻ mice – neither at baseline nor following LTP induction.

(C) The time course of relative field potential slopes following theta-burst stimulation (indicated by the gray arrow) revealed a significant difference in the level of LTP between wildtype (open symbols) and NPC1⁻/⁻ mice (closed symbols). Representative recordings were taken from time-points 1 (baseline) and 2 (55-60 minutes after TBS). Traces were superimposed to illustrate differences in amplitudes and slopes.
References


Parkinson, P. F., Kannangara, T. S., Eadie, B. D., Burgess, B. L., Wellington, C. L., Christie, B. R., 2009. Cognition, learning behaviour and hippocampal synaptic plasticity are not disrupted in mice over-expressing the cholesterol transporter ABCG1. Lipids Health Dis. 8, 5-


Taverna, E., Saba, E., Rowe, J., Francolini, M., Clementi, F., Rosa, P., 2004. Role of lipid microdomains in P/Q-type calcium channel (Cav2.1) clustering and function in presynaptic membranes. J. Biol. Chem. 279, 5127-5134.


Fig. 1

(A) Field potential slope (V/s) vs. stimulus intensity (V).

(B) Paired-pulse ratio (% baseline) for wildtype and NPC1<sup>-/-</sup> mice.

(C) Weldtype and NPC1<sup>-/-</sup> mouse responses to high-frequency stimulation (4 x 100 stimuli at 100 Hz, 10 s apart).
Fig. 2
Fig. 3
Highlights:

- NPC1<sup>−/−</sup> mouse, modeling Niemann-Pick type C1 disease, was investigated in vitro
- Changes in network excitability and synaptic plasticity were assessed
- Three different regions were investigated: hippocampus, striate body, and neocortex
- No changes were seen in hippocampus and striatum
- In neocortex, excitability was significantly enhanced while LTP was abolished