Niemann-Pick type C disease reveals a link between lysosomal cholesterol and PtdIns(4,5)P2 that regulates neuronal excitability

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Niemann-Pick Type C Disease Reveals a Link between Lysosomal Cholesterol and PtdIns(4,5)P_2 That Regulates Neuronal Excitability

Graphical Abstract

Highlights

- Loss of NPC1 function causes neurons to become hyperexcitable
- Intrinsic hyperexcitability is triggered by reduced KCNQ2/3 current
- Decrease of KCNQ2/3 current in NPC1 disease is caused by a reduction in PtdIns(4,5)P_2
- Loss of PtdIns(4,5)P_2 in NPC1 disease occurs due to upregulation of the ABCA1 transporter

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In Brief

NPC1 disease is a neurodegenerative disorder that occurs due to mutations in the lysosomal NPC1 cholesterol transporter. Vivas et al. define steps in the pathogenic cascade, downstream of lysosomal cholesterol accumulation, that lead to hyperexcitability in NPC1 disease neurons.

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These pathways are particularly relevant for maintaining brain and other transcription factors (Laplante and Sabatini, 2012). The mammalian target of rapamycin complex 1 (mTORC1) and signaling organelles that can mediate signal transduction via and recycling, lysosomes are now known to be highly dynamic. Once simply thought of as acidic centers for degradation, lysosomes are now known to be highly dynamic. Emphasizes the crucial role that lysosomes play in neurodegeneration.

Neurodegeneration is a common feature of many neurological disorders. Cell biological, genetic, and in vivo work increasingly emphasizes the crucial role that lysosomes play in neurodegeneration. Once simply thought of as acidic centers for degradation and recycling, lysosomes are now known to be highly dynamic signaling organelles that can mediate signal transduction via the mammalian target of rapamycin complex 1 (mTORC1) and regulate gene expression via transcription factor EB (TFEB) and other transcription factors (Laplante and Sabatini, 2012). These pathways are particularly relevant for maintaining brain homeostasis, as dysfunction of the endolysosomal and autophagic pathways has been associated with common neurodegenerative diseases, such as Alzheimer and Parkinson (Fraldi et al., 2016; Laplante and Sabatini, 2012), and lysosomal storage disorders—a group of inherited disorders characterized by the intralysosomal buildup of partially degraded metabolites, including cholesterol (Castellano et al., 2017; Schulze and Sandhoff, 2011).

Increasingly, dysregulation of cholesterol homeostasis is proposed as a contributing factor in the development of neurodegenerative disorders (Abdel-Khalik et al., 2017; Chang et al., 2016; Di Paolo and Kim, 2011; Eriksson et al., 2017). A main source of neuronal cholesterol is the internalization of cholesterol that is synthesized by glia. This import of cholesterol is predominantly mediated by astrocyte-derived apolipoprotein E (ApoE)-containing lipoprotein particles (Boyles et al., 1989; Herz and Bock, 2002). These lipoproteins are internalized via clathrin-mediated endocytosis and delivered to late lysosomal compartments where acidic lipases liberate cholesterol. The free cholesterol is transferred by the luminal Niemann-Pick type C2 (NPC2) protein to the membrane-bound Niemann-Pick type C1 (NPC1) protein for export to the endoplasmic reticulum (ER) by sterol transfer proteins (e.g., ORP5, ORP1L) at membrane contact sites between the ER and the lysosome (Du et al., 2011; Karten et al., 2009; Li et al., 2016; Luo et al., 2017; Zhao and Ridgway, 2017). The importance of the NPC1 protein in regulating the transfer of cholesterol to the ER is underscored by the severity of the fatal Niemann-Pick type C1 (NPC1) neurodegenerative disease. This autosomal recessive lysosomal storage disorder most commonly occurs due to a single I1061T substitution that results in misfolding of the NPC1 protein and subsequent targeting for ER-associated degradation (Gelsthorpe et al., 2008). The reduction in functional NPC1 protein results in massive luminal accumulation of cholesterol in lysosomes and cellular changes in cholesterol homeostasis (Millard et al., 2000). Neurologically, NPC1 patients typically exhibit progressive impairment of motor and intellectual function, before succumbing to the disease, tragically within the first 2 decades of life (Vanier, 1999). Despite the commonality of altered cholesterol metabolism and compromised neuronal function in neurodegenerative disorders, the molecular mechanisms that link cellular changes in cholesterol with alterations in neuronal excitability are absent. Thus, this monogenetic disease, with such profound alterations in cholesterol metabolism, allows us to...
test the hypothesis that altered lysosomal cholesterol metabolism can change the intrinsic electrical excitability of neurons (Figure 1A).

Neuronal excitability depends on the proper function of ion channels whose activities can be tuned by plasma membrane (PM) lipids, including cholesterol and the minor phospholipid, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂). PtdIns(4,5)P₂ regulates upward of 100 ion channels and transporters (Hilgemann et al., 2001; Hille et al., 2015), while cholesterol commonly suppresses the activity of several types of channels, including inwardly rectifying K⁺ channels (Romanenko et al., 2004), Ca²⁺-sensitive K⁺ channels (Bukiya et al., 2011;...
Crowley et al., 2003; Purcell et al., 2011), and voltage-gated K⁺ channels (Abi-Char et al., 2007; Hajdú et al., 2003). Despite separate biosynthetic pathways, the metabolism of cholesterol and phosphoinositoles is not as distinct as previously thought. The transport of cholesterol between intracellular membranes at membrane contact sites uses phosphoinositides as the driving force to transport cholesterol (Mesmin et al., 2013). Another link between cholesterol and phosphoinositide metabolism involves the ABCA1 cholesterol transporter, which has been reported as being a PtdIns(4,5)₂ floppase (exports PtdIns(4,5)₂ out of the cell) (Gulshan et al., 2016). Hence, alterations in the cholesterol content of organelle membranes could dysregulate ion channel activity directly or indirectly by modifying PtdIns(4,5)₂.

Here, we find that both pharmacological inhibition of NPC1 and its genetic mutation in an established transgenic animal model of the disease render neurons hyperexcitable. Furthermore, we discover that reduced KCNQ2/3 current density underlies this electrical hyperexcitability, which is mediated by a significant reduction in the abundance of PM PtdIns(4,5)₂. The molecular mechanism that leads to a reduction in PtdIns(4,5)₂ in NPC1 disease is a sterol regulatory element-binding protein (SREBP)-dependent upregulation of ABCA1. These data support the hypothesis that the regulated efflux of cholesterol from lysosomal membranes via NPC1 continually aids in the regulation of neuronal excitability and reveals a link between lysosomal cholesterol and phosphoinositide metabolism in NPC1 disease. This link could have broad implications for other neurodegenerative disorders.

RESULTS
Pharmacological Inhibition of NPC1 Increases Neuronal Excitability
NPC1 disease cells exhibit a characteristic accumulation of cholesterol in lysosomes (Gelsthorpe et al., 2008; Millard et al., 2000; Yu et al., 2005). This cellular phenotype has become useful in the diagnosis of NPC1 disease since it is readily identifiable following staining with the naturally fluorescent polyene antibiotic, filipin (Vanier and Latour, 2015). Figure 1B presents confocal micrographs of filipin-stained fibroblasts isolated from healthy and NPC1 patients carrying the most prevalent mutation (NPC1I1061T) (Millat et al., 1999; Park et al., 2003). Analysis of these images revealed that NPC1I1061T fibroblasts have larger cholesterol-containing vesicles (Figure 1C), with the median vesicle size of healthy fibroblasts being 0.22 μm² (Q1 = 0.13 μm² and Q3 = 0.39 μm²) and NPC1I1061T fibroblasts being 0.38 μm² (Q1 = 0.18 μm² and Q3 = 0.75 μm²). To mimic the cholesterol accumulation phenotype of NPC1 disease in isolated neurons, we used the well-characterized NPC1 inhibitor U18666A (U18; 1 μM). This cationic amphiphile directly binds to the sterol-sensing domain of the NPC1 protein (Lu et al., 2015) and blocks the efflux of cholesterol from lysosomes (Lange et al., 2000), thereby mimicking NPC1 disease. Cultured sympathetic (Figures 1D and 1E) or hippocampal (Figure S1A) neurons were treated overnight in the presence of U18 and stained with filipin. Similar to NPC1I1061T patient fibroblasts, U18 treatment induced the accumulation of cholesterol within vesicles (Figures 1D and 1E). While the median vesicle size of neurons cultured without U18 (control) was 0.03 μm² (Q1 = 0.01 μm² and Q3 = 0.12 μm²), the median vesicle size of neurons cultured in the presence of U18 was 0.11 μm² (Q1 = 0.02 μm² and Q3 = 0.49 μm²). Thus, treatment of neurons with U18 mimics the accumulation of cholesterol observed in fibroblasts from NPC1I1061T patients and represents a pharmacological tool to dissect the role of lysosomal cholesterol efflux in regulating neuronal function.

To test whether U18-induced cholesterol accumulation alters neuronal excitability, action potentials (APs) were recorded in current-clamp mode using the perforated-patch configuration. Figure 1F shows recordings from a representative neuron cultured in the absence of U18 (control). Here, injecting 40 pA elicits a solitary AP despite the depolarizing stimulus being applied for 1 s (black trace). This typical firing pattern of sympathetic neurons corresponds to the cell adapting to the stimulus (spike adaptation) (Brown and Passmore, 2009; Zaika et al., 2006). In striking contrast, the same stimulus induced the firing of 9 APs in a neuron cultured in the presence of U18 (Figure 1F, U18, black trace). We interpreted this result as a loss of spike adaptation. From these data it is also noticeable that the neuron cultured in the presence of U18 fired more APs per unit of injected current (e.g., 20 pA, red trace) compared to the control neuron. To quantify changes in excitability, we measured the following: (1) the resting membrane potential (V_m), (2) the amount of current injected necessary to elicit at least 1 AP (loosely called here rheobase), and (3) the number of APs fired with increasing current injections. Neurons cultured in the presence of U18 had a 9 mV more depolarized V_m (Figure 1G, control: −59 ± 2 mV, U18: −50 ± 2 mV), required less current injection to induce AP firing (Figure 1H, control: 37 ± 5 pA, U18: 17 ± 4 pA), and fired more APs per picoamperes of current injected (Figure 1I). These U18-mediated alterations in AP firing could also be recapitulated in cultured hippocampal neurons (Figures S1B and S1C). In conclusion, inhibiting NPC1-mediated cholesterol egress from the lysosome leads to neurons becoming more excitable.

NPC1I1061T Disease Neurons Are Hyperexcitable
Overnight application of U18 has been used extensively to investigate cellular changes in NPC1 disease. Despite its utility in doing so, this inhibition is acute relative to the chronic changes that occur during disease development in patients. Accordingly, the changes in electrical activity described above could represent transient changes in excitability observed when NPC1 is inhibited for hours; but over longer periods, neurons may adapt to restore their excitability. Therefore, to test whether neurons were also hyperexcitable in conditions closer to those of patients suffering from NPC1 disease (Figure 2A), we isolated neurons from a recently developed NPC1I1061T knockin murine model (Praggastis et al., 2015). Figure 2B shows micrographs of filipin-stained neurons isolated from NPC1I1061T mice compared to littermate WT controls. Similar to patient fibroblasts and U18-treated neurons, NPC1I1061T neurons exhibit an enhanced accumulation of cholesterol within vesicular structures, with the distribution of vesicle size shifted toward larger vesicles in NPC1I1061T neurons. While the median vesicle size in neurons...
from WT mice was 0.01 μm² (Q1 = 0.01 μm² and Q3 = 0.08 μm²), the median vesicle size in neurons from NPC1 I1061T mice was 0.12 μm² (Q1 = 0.02 μm² and Q3 = 0.34 μm²). Next, we measured AP firing in WT and NPC1 I1061T neurons. Figure 2D contrasts the electrical responses from representative neurons of each group. Comparable to what was observed following NPC1 inhibition, a 40-pA current injection induced the firing of a solitary AP in WT neurons, whereas the same injection stimulus produced 9 APs from an NPC1 I1061T neuron. Analysis of neuronal electrical properties revealed that NPC1 I1061T neurons had an 8-mV more depolarized Vrest (Figure 2E, WT: −56 ± 1 mV, n = 20; NPC1 I1061T: −48 ± 2 mV), required less current to induce AP firing (Figure 2F, WT: 29 ± 4 pA; NPC1 I1061T: 14 ± 2 pA), and fired a greater number of APs per picoampere of current injected (Figure 2G, 100 pA; WT: 4 ± 1 AP; NPC1 I1061T: 16 ± 2 APs). These results show that the most prevalent NPC1 disease-causing mutation renders neurons hyperexcitable, suggesting a common mechanism that relates lysosomal cholesterol transport to the ability of neurons to send and receive electrical inputs.

Loss of NPC1 Function Reduces KCNQ2/3 Current ($I_{\text{KCNQ2/3}}$)

Based on the significant changes in neuronal firing behavior, we hypothesized that KCNQ2/3 voltage-gated potassium channel (Kv 7.2/7.3) current density may be altered in NPC1 disease (Figure 3A). Our rationale follows: (1) KCNQ2/3 channels activate at negative potentials (~ −65 mV) and thus help establish Vrest, (2) KCNQ2/3 channels open in response to depolarization to repolarize the membrane potential, and (3) KCNQ2/3 channels participate in spike frequency adaptation (Brown and Passmore, 2009). Thus, reduced $I_{\text{KCNQ2/3}}$ is expected to depolarize Vrest, increase intrinsic electrical excitability, and decrease spike frequency adaptation—all electrophysiological consequences observed in NPC1 disease neurons. Consequently, we measured $I_{\text{KCNQ2/3}}$ densities from U18-treated, NPC1 I1061T, and NPC1 I1061T neurons. $I_{\text{KCNQ2/3}}$ density was smaller following the inhibition of NPC1 (Figures 3B and 3C, 1.8 ± 0.3 pA/pF) compared to control neurons (5.9 ± 0.8 pA/pF). This reduction in $I_{\text{KCNQ2/3}}$ density was not observed following a 2-min acute application of the inhibitor, ruling out a direct inhibition or block of the channel (Figure S2).
and 3G, WT: 6.8 ± 0.8 pA/pF; NPC1−/−: 2.9 ± 0.4 pA/pF). Consequently, inhibition, deletion, or disease-causing mutation of the NPC1 cholesterol transporter reduces IKCNQ2/3 density and offer a potential mechanism underlying the hyperexcitability phenotype in NPC1 disease.

**Opening IKCNQ2/3 Channels Rescues NPC1<sup>11061T</sup> Neuron Excitability**

Following the premise that the reduction of IKCNQ2/3 leads to a hyperexcitable state in neurons with non-functional NPC1, we predicted that the application of retigabine, a KCNQ2/3 channel opener (Gunthorpe et al., 2012), would make them less excitable (Figure 4A). Bath application of 10 μM retigabine quickly and reversibly restored the normal firing behavior of U18-treated neurons (Figures 4B–4E), with fewer APs recorded per picocoulomb of current injected (100 pA; U18 treated: 19 ± 1 AP; with retigabine: 3 ± 2 APs; washout: 18 ± 1 APs; n = 8, p < 0.0001 for U18 versus retigabine; p = 0.5 for U18 versus washout; and p < 0.0001 for retigabine versus washout). Retigabine was also effective at abrogating the hyperexcited state of NPC1<sup>11061T</sup> neurons (Figure 4F, 100 pA; NPC1<sup>11061T</sup>; 16 ± 2 APs; NPC1<sup>11061T</sup> + retigabine: 8 ± 3). These results support the hypothesis that a reduction in IKCNQ2/3 density is responsible for the enhanced excitability observed in NPC1 disease neurons.

**PM PtdIns(4,5)P<sub>2</sub> Is Reduced in NPC1 Disease**

We next asked what mechanism underlies the reduction in IKCNQ2/3. First, we ruled out the possibility that KCNQ channel abundance was altered in NPC1 disease (Figure S3). Then, we tested the hypothesis that PtdIns(4,5)P<sub>2</sub> is altered in NPC1 disease. PtdIns(4,5)P<sub>2</sub> is the most abundant phosphoinositide at the PM and is absolutely required for KCNQ2/3 channel function (Suh and Hille, 2002; Zhang et al., 2003); hence, any reductions in PtdIns(4,5)P<sub>2</sub> could lead to reduced IKCNQ2/3 (Figure 5A). To determine whether PtdIns(4,5)P<sub>2</sub> is altered in NPC1 disease, we took two approaches: expression of a fluorescently tagged, genetically encoded PtdIns(4,5)P<sub>2</sub> biosensor (yellow fluorescent protein [YFP]-pleckstrin homology domain [PH] PLC<sub>β</sub>1) and ultra-high pressure liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). We chose these two complementary approaches because UPLC-MS/MS is extremely quantitative but does not distinguish specific PtdIns<sub>2</sub> regioisomers (i.e., PtdIns(3,5)P<sub>2</sub> versus PtdIns(4,5)P<sub>2</sub>), whereas the imaging approach is semiquantitative but offers spatial information and is excellent at differentiating specific regioisomers. Analysis of healthy and NPC1<sup>11061T</sup> patient fibroblasts transfected with YFP-PH<sub>PLC</sub> revealed a decrease in the ratio of fluorescence intensity at the PM compared to the cytoplasm (Cyt) in NPC1<sup>11061T</sup> patient fibroblasts (Figures 5B and 5C; WT: 4.0 ± 0.2, n = 21; NPC1<sup>11061T</sup>: 2.8 ± 0.2). In addition, MS showed a 50% reduction in total PtdIns<sub>2</sub> in NPC1<sup>11061T</sup> fibroblasts (Figure 5D; WT: 2.3 ± 10<sup>6</sup> ± 0.6 ± 10<sup>6</sup>; NPC1<sup>11061T</sup>; 1.2 ± 10<sup>5</sup> ± 0.8 ± 10<sup>5</sup>). To further test this hypothesis, we also measured PtdIns<sub>2</sub> levels from three different brain regions (hippocampus, cortex, and cerebellum) of NPC1<sup>11061T</sup> mice and found similar reductions in PtdIns<sub>2</sub> across each region (Figures 5E–5H), with total PtdIns<sub>2</sub> reduced by 66% (Figure 5I; WT: 6.6 × 10<sup>7</sup> ± 0.9 × 10<sup>7</sup>; NPC1<sup>11061T</sup> = 2.5 × 10<sup>7</sup> ± 0.7 × 10<sup>7</sup>). These results indicate
that PtdIns(4,5)P₂ is reduced in NPC1 disease and offers an underlying mechanism for the reduction of I_{KCNQ2/3} and neuron hyperexcitability.

**Exogenous Application of PtdIns(4,5)P₂ Rescues Both I_{KCNQ2/3} and Excitability**

Following the premise that neurons lacking functional NPC1 have less PtdIns(4,5)P₂ and consequently reduced I_{KCNQ2/3} and increased excitability, we next asked whether exogenous dialysis of PtdIns(4,5)P₂ would increase I_{KCNQ2/3} and rescue excitability in U18-treated neurons (Figure 6A). Patch-clamp electrophysiology was performed in whole-cell mode with pipettes filled with a standard internal solution or a solution supplemented with 30 μM diC8-PtdIns(4,5)P₂. Figure 6B shows representative recordings of I_{KCNQ2/3} from control neurons patched with or without diC8-PtdIns(4,5)P₂. The addition of diC8-PtdIns(4,5)P₂ to the cell through the patch pipette did not change the current density in control neurons (Figure 6D, without diC8-PtdIns(4,5)P₂: 10.9 ± 2.0 pA/pF; with diC8-PtdIns(4,5)P₂: 7.8 ± 1.5 pA/pF). However, the addition of diC8-PtdIns(4,5)P₂ significantly increased I_{KCNQ2/3} density in neurons cultured in the presence of U18 (8.4 ± 1.3 pA/pF) compared to neurons cultured in the presence of U18 alone (2.4 ± 0.9 pA/pF; Figures 6C and 6D). I_{KCNQ2/3} density was not statistically different between control neurons and U18-treated neurons following the application of diC8-PtdIns(4,5)P₂, suggesting that the exogenous addition of PtdIns(4,5)P₂ rescues I_{KCNQ2/3} in neurons lacking functional NPC1. Finally, we tested whether the addition of 30 μM diC8-PtdIns(4,5)P₂ rescues the excitability of neurons with inhibited NPC1. Figure 6E shows representative current-clamp recordings of U18 neurons treated with or without diC8-PtdIns(4,5)P₂. Note that the addition of diC8-PtdIns(4,5)P₂ to neurons caused fewer APs to fire following a 40 pA current injection (1 AP, black trace) compared to a neuron cultured in the presence of U18 but patched without diC8-PtdIns(4,5)P₂ (9 APs, black trace). In fact, neurons patched with diC8-PtdIns(4,5)P₂ fired fewer APs with every current injection tested, reaching a maximum of 5 ± 2 APs when neurons were stimulated with 100 pA current injection (n = 8), compared to 13 ± 3 APs when neurons were patched without PtdIns(4,5)P₂ (Figure 6F).

In conclusion, exogenous application of diC8-PtdIns(4,5)P₂ to neurons lacking functional NPC1 is enough to rescue the hyperexcitable phenotype, supporting the hypothesis that these neurons have less PtdIns(4,5)P₂ and that the reduction of this lipid is the underlying mechanism of hyperexcitability in NPC1 disease.

**Upregulation of ABCA1 Reduces PtdIns(4,5)P₂, Leading to Hyperexcitability in NPC1 Disease**

Finally, we sought to determine the molecular link between the accumulation of cholesterol in the lysosome and the reduction of PtdIns(4,5)P₂ at the PM. A striking consequence of disrupting NPC1-dependent cholesterol efflux is the activation of SREBP, a transcription factor not only controls cholesterol synthesis through an end product feedback mechanism but it also controls many other membrane lipids (Brown and Goldstein, 1997). This transcription factor not only controls cholesterol synthesis through an end product feedback mechanism but it also controls many other physiological processes. For instance, the overexpression of SREBP in myocytes changes the expression of ~1,500 genes.
Figure 5. Plasma Membrane PtdIns(4,5)P$_2$ Is Reduced in NPC1 Disease

(A) Schematic of the hypothesis: decreased KCNQ2/3 density is due to reduced PM PtdIns(4,5)P$_2$.

(B) Top: diagram illustrating PH$_{PLC\delta1}$ as a PtdIns(4,5)P$_2$ biosensor. Bottom: inverted confocal micrographs of healthy (left) and NPC1$^{I1061T}$ (right) patient fibroblasts transfectected with PH$_{PLC\delta1}$. Insets show reduced intensity of PH$_{PLC\delta1}$ at the PM of NPC1$^{I1061T}$ patient fibroblasts.

(C) Comparison of the ratio of PH$_{PLC\delta1}$ fluorescence intensity at the PM to the cytoplasm (Cyt) in healthy (n = 21) and NPC1$^{I1061T}$ fibroblasts (n = 28, p = 0.0001).

(D) UPLC-MS/MS PtInsP$_2$ measurements from healthy and NPC1$^{I1061T}$ patient fibroblasts (n = 5 independent cultures, p < 0.0001).

(E) Mouse brain regions used for experiments: blue, cortex; orange, hippocampus; and green, cerebellum.

(F) Profile of the m/z ratio for the most abundant PtInsP$_2$ isofrom 38:4.

(G) Comparison of elution profiles of 38:4 PtInsP$_2$ in WT and NPC1$^{I1061T}$ mice from each brain region (n = 3 mice).

(H) Comparison of the ratio of total PtInsP$_2$ in NPC1$^{I1061T}$ to WT mice across brain regions. Total PtInsP$_2$ includes all isofroms of PtInsP$_2$ (e.g., 38:4, 36:2, 34:1).

(I) Summary of UPLC-MS/MS PtInsP$_2$ measurements from WT and NPC1$^{I1061T}$ brains (n = 3 animal, p = 0.03).

See also Figure S3.
Considering the significant number of protein-coding genes under the control of SREBP, we tested whether crucial enzymes related to PtdIns(4,5)P2 metabolism are altered in NPC1 disease (Figure 7A). To begin, we verified that the activation of SREBP, independent of NPC1 function, reduced PM PtdIns(4,5)P2 (Figure S4). Next, determination of the protein abundances of PIP5K1A (phosphatidylinositol 4-phosphate 5-kinase type 1A), SYNJ1 (Synaptojanin 1), and PLCb1 (phospholipase Cb1) revealed no significant difference between healthy and NPC1 I1061T (Figures 7B and 7C). Therefore, we ruled out the misregulation of any of these important proteins as a possible mechanism for the reduction of PtdIns(4,5)P2 in NPC1 disease.

Given that phosphoinositide lipid kinases or lipid phosphatases do not appear to underlie the reduction in PM PtdIns(4,5)P2, we focused our attention on proteins that are key regulators of the transport of both cholesterol and phosphoinositide metabolism. To this end, we tested whether several known phosphoinositide transfer proteins (ORP2, ORPL1, ORP5/8) or the ABCA1 (ATP-binding cassette transporter A1) transporter are differentially expressed in NPC1 disease. Oxysterol-binding protein (OSBP)-related proteins are transfer proteins that transfer cholesterol at membrane contact sites through the counter-transport of PtdIns4P and PtdIns(4,5)P2 (Ghai et al., 2017; Mesmin et al., 2013; Wang et al., 2019; Zhao and Ridgway, 2017), whereas ABCA1 (ATP-binding cassette transporter A1) is a transmembrane protein, which plays a major role in lipid homeostasis by regulating cholesterol efflux from the cell. In addition, ABCA1 has been recently reported to exhibit PtdIns(4,5)P2 floppase activity, moving PtdIns(4,5)P2 from the inner leaflet to the outer leaflet of the PM to mediate Apo1-dependent binding during nascent high-density lipoprotein (HDL) assembly (Gulshan et al., 2016). Determination of protein abundances revealed that the average fold change of ORP2 was 1.35 ± 0.05 (n = 3, p = 0.0004), ORPL1 was 1.33 ± 0.06 (n = 4, p = 0.002), ORP8 was 1.16 ± 0.25 (n = 4, p = 0.5), and ABCA1 was 3.3 ± 0.3 (n = 4,
**A**

PtIns4P → PtIns(4,5)P₂ → PIP5K1A → SYNJ1 → PLCβ1 → IP₃ + DAG

**B**

Healthy NPC1¹⁰⁶¹T

- PIP5K1A
- SYNJ1
- PLCβ1

**C**

Fold change relative to WT

- p = 0.005
- p = -20 mV
- p = -60 mV
- NPC1I₁₀₆¹T
- probucol

**D**

WT Normal PtIns(4,5)P₂

- ABCA1 Up-regulation
- PM Cyt
- Less PtIns(4,5)P₂

**E**

Healthy Healthy + LXR agonist NPC1¹⁰⁶¹T

- Ratio PH₃⊙₀₁ PM/Cyt
- p = 0.0001

**F**

Fold change relative to WT

- p = 0.0001
- Healthy Healthy + LXR agonist
- NPC1¹⁰⁶¹T

**G**

WT ABCA1 overexpression

- Ratio PH₃⊙₀₁ PM/Cyt
- p = 0.0001

**H**

Fold change relative to WT

- p = 0.0001
- ABCA1

**I**

Healthy NPC1¹⁰⁶¹T

- Ratio PH₃⊙₀₁ PM/Cyt

**J**

NPC neuron

- Hyper-excitable
- Less excitable

- NPC1
- KCNQ2/3

**K**

WT NPC¹⁰⁶¹T

- NPC¹⁰⁶¹T + probucol

- Δ = 10 pA
- 500 ms
- 100 mV

**L**

- # Action Potentials
- Current (pA)
- probucol
DISCUSSION

Here, we show for the first time that both inhibition and disease-causing mutation of the lysosomal cholesterol transporter, NPC1, render neurons hyperexcitable, in that neurons have a more depolarized $V_{\text{rest}}$, less current is required to induce them to fire APs, and they lose the ability to complete spike adaptation. Changes in excitability result from reduced PM PtdIns(4,5)$P_2$ and the subsequent reduction of KCNQ2/3 channel function. Furthermore, the molecular mechanism that links cholesterol accumulation and PtdIns(4,5)$P_2$ reduction is the upregulation of ABCA1. Based on these data, we propose that NPC1 disease is a phosphoinositide-deficient disease and that ABCA1 links lysosomal cholesterol efflux with PM phosphoinositide levels to tune neuronal excitability.

PtdIns(4,5)$P_2$ Deficiency Underlies Neuronal Hyperexcitability in NPC1 Disease

Like many monogenetic disorders, despite the seemingly non-descriptive nature of a single mutation, the mechanisms of NPC1 pathogenesis are complicated. For NPC1 disease, >1,500 genes (Rome et al., 2008) and ~300 proteins (Rauniyar et al., 2015) are differentially regulated. At the cellular level, these alterations manifest as defects in vesicular trafficking, mTOR signaling (Castellano et al., 2017), lysosomal calcium handling (Lloyd-Evans et al., 2008), and lipid localization (Lloyd-Evans et al., 2008; Praggastis et al., 2015). We report for the first time hyperexcitability in NPC1 disease. Evidence presented in this study suggests that the mechanism underlying this enhanced excitability is a reduction in $I_{\text{KCNQ2/3}}$ mediated by a decline in PtdIns(4,5)$P_2$. Could other lipids altered in NPC1 disease, such as cholesterol or sphingolipids, account for the reduction in $I_{\text{KCNQ2/3}}$? For cholesterol, KCNQ2/3 channels are reported as being negatively regulated by PM cholesterol (Lee et al., 2010). In cells lacking functional NPC1, there is a described reduction in the amount of cholesterol in the endofacial leaflet of the PM (Maekawa and Fairn, 2015). Thus, decreased PM cholesterol in NPC1 disease...
would disinhibit KCNQ2/3 channels and result in more current, which is the opposite of our observations. Another lipid that accumulates in lysosomes in NPC1 disease is sphingosine-1-phosphate (S1P) (Praggastis et al., 2015). Some reports show that KCNQ2/3 channel activity can be weakly stimulated (increase in channel open probability [Po] to 0.16) by a high concentration (100 μM) of S1P (Telezhkin et al., 2012). In contrast, a lower concentration (10 μM) of PtdIns(4,5)P₂ is enough to activate KCNQ2/3 channels with a Po of 0.54. Therefore, if S1P is reduced in the PM of NPC1 disease neurons, it would be anticipated that the differences in affinity that KCNQ2/3 has for each lipid would mean that any S1P effects would be secondary to PtdIns(4,5)P₂-dependent alterations. In conclusion, decreases in either cholesterol or S1P appear unlikely to account for the reductions of I KCNQ2/3 in NPC1 disease.

**Cholesterol and PtdIns(4,5)P₂ Dysregulation in Neurodegenerative Diseases**

We present evidence linking the disruption of lysosomal cholesterol efflux with decreases in PtdIns(4,5)P₂-dependent KCNQ2/3 channel activity, leading to aberrant neuronal activity. At the molecular level, our data suggest that the cholesterol transporter and PtdIns(4,5)P₂ floppase, ABCA1, is responsible for the decline in PtdIns(4,5)P₂ that consequently modifies the electrical properties of NPC1 disease neurons. Fibroblasts from patients with Tangier disease, a disease caused by mutations in ABCA1, transfer 50% less PtdIns than fibroblasts from healthy patients (von Eckardstein et al., 1998), while mutations in ABCA1 have been associated with a higher risk of Alzheimer disease (Nordestgaard et al., 2015). Thus, there is a precedent for the involvement of ABCA1 as a potential contributing factor in neurodegeneration. Is there evidence in the literature to suggest that other neurodegenerative diseases have either dysfunction of KCNQ2/3 channel activity or altered levels of PtdIns(4,5)P₂? In fact, there is accumulating evidence that mutations in the genes encoding KCNQ2/3 subunits or PtdIns(4,5)P₂ metabolizing enzymes lead to neuropathies similar to those in NPC1 disease. Mutations in KCNQ2/3 cause the hyperexcitability of neurons, leading to epileptic phenotypes in neonatal epilepsy (Jentsch, 2000; Watanabe et al., 2000), Huntington disease (Cao et al., 2015), and the bulb form of amyotrophic lateral sclerosis (ALS) (Ghezzi et al., 2018). Opening KCNQ2/3 channels with retigabine protects motoneurons against the excitotoxicity characteristic of this form of ALS (Ghezzi et al., 2018). Further, mutations in the enzymes responsible for PtdIns(4,5)P₂ synthesis are associated with Alzheimer disease (Zhu et al., 2015), Parkinson disease (Cao et al., 2017), and Friedreich ataxia (Bayot et al., 2013), another rare neurodegenerative disease. These data support the idea that maintaining PtdIns(4,5)P₂ levels in neurons is essential for normal brain function and that dysfunction of KCNQ2/3 channel activity may be a contributing factor to the progression of neurodegenerative diseases. It is important to note that the relationship between enhanced neuronal excitability and neurodegeneration in NPC1 disease is correlative at this point. Given the fundamental importance of regulated ion channel function and Ca²⁺ homeostasis for neuron health and the Ca²⁺ hypothesis of neurodegeneration, which posits that altered Ca²⁺ dynamics contribute to neuropathology (Berridge, 2010), we hypothesize that alterations in the excitability alluded to above would facilitate enhanced Ca²⁺ influx into cerebellar neurons, potentially leading to their degeneration in NPC1 disease. Future experiments are needed to establish causation between hyperexcitability and cell death in NPC1 disease.

To conclude, mutations within the NPC1 protein lead to a poorly understood sequence of events that end in neurodegeneration and premature death. We have defined steps in the pathogenic cascade of NPC1 disease, namely upregulation of ABCA1 leading to a decrease in PM PtdIns(4,5)P₂ and I KCNQ2/3, which produces hyperexcitability in neurons. Thus, NPC1 disease is not only a cholesterol storage disease but also a phosphoinositide-deficient disorder with a hyperexcitability phenotype.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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  - Electrophysiological Recordings
  - Protein extraction and abundance determination
  - Measuring PtdIns(4,5)P₂
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.04.099.

**ACKNOWLEDGMENTS**

We thank Drs. Fernando Santana, Manuel Navedo, and Claudia Moreno and members of the Dickson and Dixon laboratories for helpful comments and discussions on the manuscript; Dale Whittington, Dr. Alexis Traynor-Kaplan, and the other members of the Washington University School of Pharmacy Mass Spectrometry Center for help and advice, and Elizabeth Quasebarth for help and guidance on the NPC1I1061T animal model. This work was supported by R01GM127513 and University of California funds (to E.J.D.), Pharmacology T32 training award T32GM099608 (to S.A.T.), AHA grant 15SDG25560035 (to R.E.D.), and NIH R01HL06773 (to D.S.O.).

**AUTHOR CONTRIBUTIONS**

O.V. and E.J.D. designed the experiments. O.V. collected and analyzed the electrophysiology and imaging data. O.V. and S.A.T. collected and analyzed the western blot data. E.J.D. collected and analyzed the MS data. O.V. and E.J.D. wrote the manuscript, with input from all of the authors.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.
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Genet.

pean descent and correlates with a classic juvenile phenotype. Am. J. Hum.


Yu, W., Ko, M., Yanagisawa, K., and Michikawa, M. (2005). Neurodegeneration in heterozygous Niemann-Pick type C1 (NPC1) mouse: implication of het-

eryzogous NPC1 mutations being a risk for tauopathy. J. Biol. Chem. 280, 27296–27302.


## STAR METHODS

### KEY RESOURCES TABLE

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Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Eamonn J. Dickson (ejdickson@ucdavis.edu).

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

### Murine models of NPC1 disease

NPC1 knock-out (NPC1<sup>−/−</sup>, RRID: IMSR_JAX:003092), NPC1<sup>I1061T</sup> knock-in (Praggastis et al., 2015), and wild-type (WT) mice from a C57BL/6 background were kindly provided by Daniel Ory (Washington University Saint Louis). Male C57BL/6 WT mice used for experiments involving U18666A (U18, SIGMA, Cat # 662015) were purchased from the Jackson Laboratory (RRID: IMSR_JAX:000664). All animals were kept in an animal facility with controlled conditions and were given standard chow and water ad libitum. The animal handling protocol was approved by the University of California Institutional Animal Care and Use Committee. To genotype NPC1<sup>I1061T</sup> mice, we extracted DNA and amplified an NPC1 allele with the following primers: forward (5' -tgatctgcacacttggaaccgag-3') and reverse (5' -cactgccttgagcagcatctcag-3'). WT allele was identified by a 200 bp fragment, whereas the knock-in allele was identified by a 234 bp fragment. Heterozygous mice, containing both 200 bp and 234 bp, were not used in this study. Experiments using either knock-out or knock-in animals were conducted using both males and females. Knock-out animals were used at around 70 days old. Knock-in animals were used at around 90 days old.

### Cell lines

Fibroblast cell lines from a healthy male (GM05659, RRID: CVCL_7434) and a male patient with a homozygous mutation in NPC1<sup>I1061T</sup> (GM18453, RRID: CVCL_DA78, from the NIGMS repository) were purchased from the Coriell Institute. Fibroblasts were grown in Eagle's Minimum Essential Medium (MEM) with Earle's salts and non-essential amino acids (SIGMA, Cat # M5650) supplemented with 2 mM L-glutamine (GIBCO, Cat # 25030-081), 15% non-inactivated fetal bovine serum (GIBCO, Cat # 26140-079) and 0.2% penicillin/streptomycin (GIBCO, Cat # 15140-122), passaged twice a week, and incubated in 5% CO<sub>2</sub> at 37°C.

HeLa cells stably expressing ABCA1-GFP were provided by Dr. Alan Remaley, NHLBI, Bethesda, MD. HeLa cells were grown in DMEM (GIBCO, Cat # 11995-065) supplemented with 10% non-inactivated fetal bovine serum and 0.2% penicillin/streptomycin, passaged twice a week, and incubated in 5% CO<sub>2</sub> at 37°C. Expression of ABCA1-GFP HeLa cells was induced by adding 150 μg/ml geneticin (GIBCO, Cat # 10131-035) and 200 μg/ml Hygromycin (Invitrogen, Cat # 10687010).

**METHOD DETAILS**

### Neuron isolation

Neurons from superior cervical ganglion (SCG) were prepared from 10 to 16 week-old female or male mice by enzymatic digestion following a standardized protocol for rats (Vivas et al., 2013) but reducing the amount of enzymes. Briefly, mice were anesthetized with pentobarbital sodium (VETONE, Cat # 501016) before extraction of the SCG. SCG were cleaned and sliced into 4 pieces. This tissue was transferred to a tube containing 10 U/ml papain (SIGMA, Cat # 4762) in Hank’s solution and kept at 37°C for 6 min, then it was transferred to a solution containing 0.33 mg/ml collagenase type II (Worthington, Cat # LS004176) and 2.5 mg/ml dispase (GIBCO, Cat # 0479) for up to 60 min. Every 20 min the tissue was pipetted to dissociated into individual cells. After obtaining a homogeneous cell suspension, cells were washed with DMEM followed by centrifugation at 180 g for 3 min. Washing step was repeated 3 times. Isolated neurons were plated on poly-L-lysine (MW > 300,000, SIGMA, Cat # P5899) coated glass coverslips and incubated in 5% CO<sub>2</sub> at 37°C in DMEM supplemented with 10% FBS and 0.2% penicillin/streptomycin. Neurons were used up to 24 h after plating.

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Filipin Staining and Super-resolution Imaging
Cells washed with PBS were fixed with a mix of 3% paraformaldehyde (Electron Microscopy Sciences, Cat # 15710) and 0.1% glutaraldehyde (SIGMA, Cat # G7651) for 10 min at 21 °C. Following a 5-minute incubation with sodium borohydrate (10 mM, SIGMA, Cat #, 213462), cells were stained with 3 mg/ml filipin (SIGMA, Cat # F9765) for 2 h at room temperature. Cells were imaged in PBS. During the filipin staining process cells were protected from unnecessary light exposure. Cells were imaged using a Zeiss880 AiryScan microscope (axial resolution of 120 nm) by exciting with a 405 nm LED. This imaging mode allowed us to resolve cholesterol-containing vesicles.

Electrophysiological Recordings
All electrophysiological recordings were performed at room temperature. Voltage responses were recorded using perforated-patch configuration (except Figure 6) in the current-clamp mode, whereas KCNQ2/3 current was recorded using the whole-cell configuration in voltage clamp mode. To isolate KCNQ2/3 tail currents, the voltage was held at −20 mV, before stepping to −60 mV. The amplitude of KCNQ2/3 currents was measured from the resulting currents relative to the current at the end of the pulse (current close to 0 pA). We used an Axopatch 200B amplifier coupled with an Axon Digidata 1550B data acquisition board (Molecular Devices Electrophysiology) to acquire the electrical signals. Patch pipettes had a resistance of 2 – 6 MΩ. Liquid junction potential of 4 mV was calculated using the pCLAMP 10 software and was not corrected for, hence the Vrest reported in the Results section is in fact 4 mV more negative (around −63 mV in WT SCG neurons). Voltage responses were sampled at 5 KHz, whereas currents were sampled at 2 KHz. For current recordings, cell capacitance was cancelled out and series resistances of < 10 MΩ were compensated by 60%. Due to remaining series resistance, voltage error is expected to be < 4 mV. The bath solution (Ringer’s solution) contained 150 mM NaCl, 2.5 mM KCl, 2 mM CaCl_2, 1 mM MgCl_2, 10 mM HEPES, and 8 mM glucose, adjusted to pH 7.4 with NaOH. The internal solution used to fill the patch pipettes contained 175 mM KCl, 1 mM MgCl_2, 5 mM HEPES, 0.1 mM K_2BAPTA, 3 mM Na_2ATP, and 0.1 mM Na_2GTP, adjusted to pH 7.2 with KOH. For current-clamp recordings, 30 μM amphotericin B (SIGMA, Cat # A4888) was added to the internal solution to facilitate electrical access to the cell. The bath solution was perfused at 2 ml/minute, permitting solution exchange surrounding the recording cell with a time constant of 4 s. DiC8-PtdIns(4,5)P_2 (30 μM, Avanti Polar Lipids, Cat # 850185) was added in the internal solution.

Protein extraction and abundance determination
Protein from cell cultures, SCG, or brain tissue was harvested in RIPA buffer (Thermo Scientific, Cat # 89900) with Complete, Mini, EDTA-free protease inhibitor cocktail (Roche, Cat # 11836170001) for 15 min at 4 °C. For SCG and brain tissue, protein samples were sonicated using a bath sonicator at 4 °C. Postnuclear supernatant was isolated by centrifuging for 20 minutes at 13,600 g at 4 °C. Protein concentration was quantified with a plate reader using the Pierce BCA protein assay kit (Thermo Scientific, Cat # 23225). Protein samples were resolved in 4%–12% Bis-Tris gels under reducing conditions. Proteins were transferred onto nitrocellulose membranes (Life Technologies, Cat # LC2000) using the Mini-Bolt system (Thermo Scientific, Cat # A25977). Membranes were blotted using rabbit monoclonal anti-NPC1 (Abcam, Cat # ab134113, RRID: AB_2734695, 1:1,000), rabbit anti-KCNQ2/2 (Abcam, Cat # ab22897, RRID: AB_775890, 1:500), mouse anti-ABCA1 (Abcam, Cat # ab18180, RRID: AB_444302, 1:1000), rabbit anti-PIP5K1A (Cell Signaling Technology, Cat # 9693, RRID: AB_216498, 1:500), rabbit anti-SYNJ1 (SIGMA, Cat # HPA011916, RRID: AB_1857692, 1:500), rabbit anti-PLCβ1 (Abcam, Cat # ab182359, 1:1000), and mouse anti-β-actin (Thermo Scientific, Cat # MA1-91399, RRID: AB_2273656, 1:10,000). Blotted bands were detected using fluorescent secondary antibodies goat anti-rabbit 680RD (P/N 926-68071, LI_COR biosciences, RRID: AB_10956389, 1:10,000) and goat anti-mouse 800CW (P/N 925-32210, LI_COR biosciences, RRID: AB_2687825, 1:20,000). Imagej was used to calculate fluorescence density of each band. The abundance of NPC1 or KCNQ2 from NPC11T061T or U18-treated tissue was reported as normalized to β-actin and relative to the abundance in WT or control tissue.

Measuring PtdIns(4,5)P_2
To measure PtdIns(4,5)P_2 in fibroblasts, a plasmid for PHPLCβ1 tagged with YFP was transfected (0.2 μg DNA) using Lipofectamine LTX (Invitrogen, Cat # 11686-027). Imaging of this biosensor was performed 24 h post-transfection using a spinning disk confocal microscope (Andor W-1 spinning disk). To measure PtdIns(4,5)P_2 from fibroblasts and brain samples by mass spectrometry, lipids were extracted with butanol and chloroform, as described previously (Traynor-Kaplan et al., 2017). A PtdIns(4,5)P_2 standard was added 17:0, 20:4 PtdIns(4,5)P_2 (Avanti Lipids, Cat # LM-1904) to all biological samples. Samples were neutralized by methylation and infused with sodium formate and analyzed using a Waters XEVO TQ-S MS/MS in multiple reaction monitoring mode (MRM) using electrospray and positive ion mode. Elution profiles plotting the intensity (arbitrary units) as a function of elution time (minutes) were analyzed to determine relative changes in PtdInsP_2 levels between samples. Total integrated areas under peaks from samples and standards were quantified using MassLynx software (Waters, RRID: SCR_014271). Peak areas of PIP_2 from the biological sample were normalized to the synthetic standard and further corrected for tissue amount using total protein.
QUANTIFICATION AND STATISTICAL ANALYSIS

We used IGOR Pro (IGOR Software, WaveMetrics, RRID: SCR_000325), Excel (Microsoft), and Prism (GraphPad, RRID: SCR_002798) to analyze data. ImageJ (RRID: SCR_003070) was used to process images. Data were collected from independent experiments from at least three mice and are presented as Mean ± SEM. For the super-resolution data of vesicle size, the median, first (Q1), and third (Q3) quartiles instead of the mean was used given that the data were not normally distributed (Normality test in Prism). A non-parametric statistical test (Mann-Whitney Wilcoxon) was used to test for statistical significance between vesicle size in different conditions. All other statistical analyses presented in this study were performed using parametric Student’s t test, considering p values < 0.05 as statistical significance. The number of cells used for each experiment is detailed in each figure legend.
Supplemental Information

Niemann-Pick Type C Disease Reveals a Link between Lysosomal Cholesterol and PtdIns(4,5)P₂ That Regulates Neuronal Excitability

Oscar Vivas, Scott A. Tiscione, Rose E. Dixon, Daniel S. Ory, and Eamonn J. Dickson
Supplemental Information

Figure S1. Inhibition of NPC1 increases hippocampal neuron excitability. Related to Figure 1. A. Inverted confocal micrographs of control (left) and U18-treated (right) hippocampal neurons fixed and stained with filipin. Insets highlight accumulation of filipin within vesicle structures. B. Current-clamp recordings of spontaneous firing from control (left) and U18-treated (right) hippocampal neurons. C. Quantification of spontaneous action potential (AP) firing frequency. Note that U18-treated neurons fire APs more frequently.
Figure S2. U18 does not inhibit $I_{KCNQ2/3}$ acutely. Related to Figure 3. A. Representative $I_{KCNQ2/3}$ current recordings before (left, black trace) and 2 min after (right, red trace) U18 application. Inset shows the protocol used to isolate $I_{KCNQ2/3}$. B. Time course of normalized $I_{KCNQ2/3}$ amplitude. Purple bar indicates the time of application of U18 (1 μM).
Figure S3. Inhibition or mutation of NPC1 does not alter KCNQ channel abundance. Related to Figure 5. **A.** Schematic of the hypothesis: there are fewer KCNQ2/3 channels in neurons with non-functional NPC1. **B.** Inverted confocal micrographs of neurons fixed and stained with filipin show accumulation of cholesterol in neurons isolated from ganglia cultured overnight with 1 μM U18. **C.** Comparison of normalized cumulative frequencies of vesicle size between control (black, 18 cells, 1015 vesicles) and U18-treated neurons (red, 30 cells, 1185 vesicles, * = p < 0.0005, Mann-Whitney Wilcoxon test). **D.** Blot stained for KCNQ2 and β-actin from ganglia treated without or with U18. **E.** Summary of KCNQ2 abundance in neurons treated with U18 relative to control (n = 3 biological replicates, each biological replicate from four to six ganglia, p = 0.7). **F.** Blots stained for NPC1, KCNQ2, and β-actin from brain tissue collected from NPC1^{11061T} and WT littermate mice. Three brain regions were analyzed separately (Cx = cortex, Hp = hippocampus, Cb = cerebellum). **G.** Summary of NPC1 and KCNQ2 abundance in neurons from NPC1^{11061T} mice relative to WT (n = 4 biological replicates).
Figure S4. Overexpressing SREBP decreases PM PtdIns(4,5)P$_2$ independently of NPC1 function. Related to Figure 7. A. Control tsA-201 cell transfected with PH$_{PLC \delta 1}$. B. Same as A, only with SREBP also overexpressed. C. Line scans taken from the yellow lines in A and B. D. Quantification of PH$_{PLC \delta 1}$ ratio from control and SREBP expressing tsA-201 cells. Note that smaller PH$_{PLC \delta 1}$ ratio suggests less PtdIns(4,5)P$_2$ in cells overexpressing SREBP.
Figure S5. Mutation of NPC1 mildly upregulates some lipid transfer proteins. Related to Figure 7. A. Blots stained for ORP2, ORPL1, and ORP8 from healthy and NPC1^{11061T} fibroblasts. B. Summary of fold change of protein abundance in NPC1^{11061T} relative to healthy fibroblasts. Average fold change of ORP2 is 1.35 ± 0.05, n = 3, p = 0.0004. Average fold change of ORPL1 is 1.33 ± 0.06, n = 4, p = 0.002. Average fold change of ORP8 is 1.16 ± 0.25, n = 4, p = 0.5. N number represents different cell cultures.
Figure S6. Activating LXR in hippocampal neurons decreases PM PtdIns(4,5)P$_2$ independently of NPC1 function. Related to Figure 7. A. Left: wild-type hippocampal neuron transfected with PH$_{PLC\delta1}$. Right: magnified image from white ROI in A. B. Same as A, only treated with LXR agonist, T0901317 (500 nM) overnight. C. Line scans taken from the yellow lines in A and B. D. Quantification of PH$_{PLC\delta1}$ ratio from control and LXR-treated neurons. Note that smaller PH$_{PLC\delta1}$ ratio suggests less PtdIns(4,5)P$_2$ in hippocampal neurons treated with LXR agonist.
Figure S7. ABCA1 siRNA rescues hyperexcitability phenotype from UA-treated neurons. Related to Figure 7. A. Quantification of resting membrane potential from UA-treated (left), UA-treated neurons transfected with siRNA against ABCA1 (middle), and neurons transfected with siRNA against ABCA1 (right). Note that knocking down ABCA1 significantly hyperpolarizes the RMP of neurons. B. Representative examples of spontaneous firing from hippocampal neurons. C. Comparison of $I_{\text{KCNQ2/3}}$ density from tsA-201 cells transiently transfected with KCNQ2/3 channels and cultured without U18 (left), with U18 (middle), and with UA and transfected with siRNA against ABCA1 (right). Note that knocking down ABCA1 increases KCNQ2/3 current density to control levels. D. Representative recordings of $I_{\text{KCNQ2/3}}$ from tsA-201 cells. Dotted lines represent 0 pA current.