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Decreased Estradiol Release from Astrocytes Contributes to the Neurodegeneration in a Mouse Model of Niemann-Pick Disease Type C

GANG CHEN,[1,2] HAI-MIN LI,[1] YI-REN CHEN,[2] XIAO-SONG GU,[2]* AND SHUMIN DUAN[1]*
[1]*Institute of Neuroscience and Key Laboratory of Neurobiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China*
[2]*Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Nantong, Jiangsu, China*

KEY WORDS
Niemann-Pick disease type C; astrocyte; estradiol; neuron-glia interaction; neurodegenerative disease

ABSTRACT
Niemann-Pick disease type C (NPC) is a deadly neurodegenerative disease often caused by mutation in a gene called NPC1, which results in the accumulation of unesterified cholesterol and glycosphingolipids in the endosomal–lysosomal system. Most studies on the mechanisms of neurodegeneration in NPC have focused on neurons. However, the possibility also exists that NPC1 affects neuronal functions indirectly by acting on other cells that are intimately interacting with neurons. In this study, using a heterotypic neuron-glia coculture system, we found that wild-type neurons cultured on a layer of NPC1−/− astrocytes showed decreased neurite growth compared with those cultured on wild-type astrocytes. RT-PCR and immunohistochemical assessments showed significantly lower expression of neurosteroid enzymes and StAR (steroidogenic acute regulatory protein) in NPC1−/− astrocyte cultures than in wild-type cultures. Furthermore, a reduced level of estradiol was measured from both astrocyte culture medium and whole brains from NPC1−/− mice. Administration of 17β-estradiol to neonatal NPC1−/− mice significantly delayed the onset of neurological symptoms, increased Purkinje cell survival, and extended the animals' life span. Our findings suggest that astrocyte dysfunction contributes to the neurodegeneration of NPC and estradiol treatment may be useful in ameliorating progression of the disease.

INTRODUCTION

Niemann-Pick disease type C (NPC) is an autosomal recessive neurodegenerative disorder characterized by intracellular accumulation of unesterified cholesterol and gangliosides within the endosomal–lysosomal system (Carstea et al., 1997; Walkley and Suzuki, 2004). About 95% of cases of human NPC are caused by mutations of the NPC1 gene. The neuropathology associated with NPC1 dysfunction is characterized by distended neurons, an accumulation of lipid storage bodies, the presence of dendritic and axonal abnormalities, and eventual neuronal loss (Sturley et al., 2004). To date, although great progress has been made in characterizing the biochemical and genetic defects in this disease, the pathways that lead from these defects to neurodegeneration are inadequately understood.

NPC1−/− disease is a defect in every cell in the body with respect to cholesterol flux. Although all neurons in the brain appear to accumulate lipids in this disorder, the most striking and well-documented histological change in NPC1−/− mice is the progressive loss of cerebellar Purkinje cells (PCs). However, stereotactic cell counting revealed that glia in the corpus callosum have a greater early reduction in number than PCs (48% for glia *versus* 13% for PCs, at 3 weeks) (German et al., 2001a). Furthermore, from the detection of NPC1 protein in astrocytic processes (Patel et al., 1999), NPC1 appears to play a role in vesicular trafficking in astrocytes (Suzuki et al., 2003), and immunohistochemical staining of microglia and astroglia shows a morbid state in NPC1−/− mice (Baudry et al., 2003; German et al., 2001a,b; 2002). These results indicate that glial cells are a major target for pathology in NPC1−/− mice.

Astrocytes, which constitute about one-half of the cells in the human brain, have been long recognized as structural and metabolic support for neurons (Ullian et al., 2004). Given the importance of neural-glial interactions in the nervous system, glial dysfunction has been implicated in "noncell-autonomous" degeneration in many neurological diseases. Studies of amyotrophic lateral sclerosis, Alzheimer disease, frontotemporal dementia, multiple system atrophy, prion disease, and the polyglutamine disease spinocerebellar ataxia also implicate glial cells, including astrocytes, oligodendrocytes, microglia, and Bergmann glia, in the neurodegenerative processes (Clement et al., 2003; Custer et al., 2006; Forman et al., 2005; McGeer et al., 1993; Raeber et al., 1997; Yazawa et al., 2005). However, whether glial cells contribute to the neuropathology of NPC1−/− disease remains unknown. In this article, we used a heterotypic neuron-glia coculture system (wild-type neurons cultured on a

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*Correspondence to: S. Duan. E-mail: shumin@ion.ac.cn or X.-S. Gu. E-mail: neurongu@public.nt.js.cn

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layer of wild-type or NPC1−/− astrocytes) to answer this question.

A recent study demonstrated that neurosteroid synthesis is reduced in NPC1−/− mice and administration of allopregnanolone increases life span and neuronal survival (Griffin et al., 2004). However, the cell type involved in this abnormal neurosteroidogenic capacity was not identified. Neurosteroidogenesis is accomplished by complex contributions from almost all cell types in the brain. Astrocytes appear to be the most active cells because they produce pregnenolone, progesterone, dehydroepiandrosterone, androstenedione, testosterone, estrone, and estradiol (Garcia-Segura and Melcangi, 2006; Zwain and Yen, 1999). Combined with their numerical density, astrocytes are considered the primary source of neurosteroids in the brain. Neurosteroids are synthesized from cholesterol in the CNS by means of a series of enzymatic processes, using the pathway "cholesterol → pregnenolone → dehydroepiandrosterone → androstenedione → testosterone → estradiol" (Kawato, 2004). Since decreases in pregnenolone, allopregnanolone, and testosterone synthesis in NPC1−/− mice have been reported (Griffin et al., 2004; Roff et al., 1993), the synthesis of other neurosteroids such as estradiol may also be disrupted. Experimental and clinical studies have indicated that estradiol may influence memory, cognition, postural stability, fine motor skills, mood, and affectivity (Garcia-Segura and Melcangi, 2006; MacLusky et al., 2005; Yankova et al., 2001). In addition, estradiol is protective against neurodegeneration and brain injury (Henderson, 2006; Miller et al., 2001; Tsang et al., 2000) and estrogen depletion in the brain is a significant risk factor for developing Alzheimer's disease (Yue et al., 2005). In this study, we found that estradiol content was decreased both in pure NPC1−/− astrocyte culture medium and in NPC1−/− mouse brain, and *in vivo* studies showed that estradiol replacement therapy alleviated the symptoms of the NPC1−/− mouse phenotype. Combined with the results from heterotypic neuron-glia coculture, our findings suggest that astrocytes play an important role in the neurodegeneration of NPC and estradiol treatment may be useful in ameliorating the progression of the disease.

MATERIALS AND METHODS

All chemicals were from Sigma (St. Louis, MO) unless otherwise noted.

BALB/c NPC1^{NIH} Mice and Genotype Analysis

The use and care of animals followed the guidelines of the Shanghai Institutes for Biological Sciences Animal Research Advisory Committee. A breeding pair of BALB/c NPC1^{NIH} mice were obtained from Jackson Laboratories (Bar Harbor, ME). These mice were bred to produce normal (NPC1+/+), heterozygous (NPC1+/−), and homozygous affected (NPC1−/−) mice. DNA was isolated from the tail tips of each mouse and PCR was performed at the NPC1 locus using primer pairs as previously described (Loftus et al., 1997).

Cell Culture

Primary cultures of astrocytes were prepared as described previously (Yang et al., 2003). Briefly, astrocytes were prepared from cerebral cortex of NPC1−/− or wild-type mice on postnatal day (P) 0, and cultured in a 75 cm^2 flask in MEM (Invitrogen) with 10% FBS (Gibco). After reaching confluence, the cells were shaken and replated on poly-D-lysine-coated glass coverslips or six-well plates. Astrocytes formed a confluent layer 2–3 days after replating and were ready for neuron coculture. Cortex from NPC1−/− or wild-type mice at P0 were dissociated and plated on a layer of primary astrocytes (wild-type or NPC1−/−) and maintained in DMEM medium (Invitrogen) containing 10% FBS and 10% F-12 (Invitrogen) for 24 h, then treated with cytosine arabinoside (10 μM) to kill proliferating cells and subsequently maintained in Neurobasal medium (Gibco) with 2% B27 supplement (Life Technologies, Gaithersburg, MD) and 0.25% glutamine. In some experiments, 1 nM anastrozole (AstraZeneca Pharmaceuticals LP), 1 nM tamoxifen, or 10 nM 17β-estradiol were added.

RT-PCR Analysis

RT-PCR analysis of steroidogenic enzymes was performed as described (Zwain and Yen, 1999). Briefly, pure cultured NPC1−/− or wild-type astrocytes were homogenized in 1 mL Trizol reagent (Invitrogen) and RNA was isolated according to the manufacturer's instructions. Total RNA (5 μg) was reverse transcribed in independent duplicate reactions using Oligo$(dT)_{20}$ primer and SuperScript III reverse transciptase (Invitrogen). One tenth of the RT reaction was used as a template for amplification by PCR using PrimeSTAR HS DNA polymerase (Takara Biotechnology). Amplification was performed in a GeneAmp PCR system 2700 (Applied Biosystems) with the following cycling parameters: initial activation of the PrimeSTAR HS DNA polymerase at 94°C for 10 min, denaturation at 94°C for 45 s, annealing at 52.5, 55, or 60°C (optimal conditions were determined for each set of primers) for 45 s, and extension at 72°C for 1 min. A total of 35 cycles was used, followed by a 10-min final extension at 72°C. The specific sense and antisense oligonucleotide PCR primers were as follows: for cytochrome P450 side-chain cleavage (size: 342 bp), 5′-acttccggtacttgggcttt-3′ and 5′-acctgggcaggtaatcacag-3′; for 17a-hydroxylase/C17-20-lyase (360 bp), 5′-gtcacggtgggagacatctt-3′ and 5′-cataaaccgatctggctggt-3′; for 17β-hydroxysteroid dehydrogenase (346 bp), 5′-gttat gagcaagccctgagc-3′ and 5′-ttgtcctgacccggtttatc-3′; for cytochrome P450 aromatase (344 bp), 5′-aacacgtctgg tctcctgct-3′ and 5′-caccgtaagcaactgggttt-3′; for 3α-hydroxysteroid dehydrogenase (333 bp), 5′-aagctttggagcactttcca-

3′ and 5′-atggcattctacctggttgc-3′; for 20α-hydroxysteroid dehydrogenase (335 bp), 5′-cgaagctttggtcaacttcc-3′ and 5′-atggcattctacctggttgc-3′; for 5α-reductase type 1 (308 bp), 5′-gaagcagggagcaatgagtc-3′ and 5′-tagcccaaaggaagagcaaa-3′; for 5α-reductase type 2 (388 bp), 5′-acttaacctggtggctggtg-3′ and 5′-cagtttggagacacccgttt-3′; for the steroidogenic acute regulatory protein (356 bp), 5′-gttcctcgctacgttcaagc-3′ and 5′-gaaacaccttgcccacatct-3′; and for actin (367 bp), 5′-actgggacgacatggaaaag-3′ and 5′-gaaggaatagccacgctcag-3′. PCR products were resolved on 2% agarose gel, stained with ethidium bromide, and visualized under UV light.

Filipin, Masson Trichrome, and Golgi Staining

Filipin staining followed the method described previously (Deisz et al., 2005). Briefly, astrocytes were fixed for 10 min at room temperature in 4% paraformaldehyde, then incubated with 100 μg/mL filipin in PBS containing 1% BSA for 1 h at room temperature. The cells were examined with a cool-CCD camera (pixel size 6.7 μm, PCO SensiCam, PCO, Germany). Masson trichrome and Golgi staining followed the protocols published on the IHC World Website.

Immunostaining

Cultured cells or cerebellar slices were fixed with 4% paraformaldehyde and permeabilized with 0.01% Triton, then treatment with 10% BSA. Samples were stained overnight at 4°C with one or two of the following antibodies: mouse anti-βIII-tubulin (1:1,000; Promega), rabbit anti-calbindin (1:1,000; Chemicon), rabbit anti-StAR (1:100; Abcam). After washing to remove excess primary antibodies, the samples were incubated for 1 h at room temperature with fluorescence-conjugated secondary antibodies (Jackson Immunoresearch, West Grove, PA): anti-mouse-IgG-FITC, anti-rabbit-IgG-Cy^5, or anti-rabbit-IgG-FITC. Excess antibody was removed and cells were imaged using a confocal microscope (Olympus Fluoview 500 IX71).

Morphological Analysis

Cocultured neurons maintained for 1, 2, or 3 days in the presence or absence of 1 nM anastrozole, 1 nM tamoxifen, or 10 nM 17β-estradiol were immunostained with anti-βIII tubulin. The ratio of neurite number/cell and total neurite length/cell were measured using MetaMorph software (Universal Imaging, West Chester, PA). The length and the number of neuritis of PCs revealed by Golgi staining were measured using ImageJ software (National InstituteS of Health, USA).

Cholesterol and apoE Quantitation

Confluent NPC1−/− or wild-type astrocytes were washed three times with HBSS, then serum free DMEM was added and cells were incubated for 3 days. The medium was collected and centrifuged at 1,000*g* for 10 min to remove cell debris. The supernatant were used for cholesterol analysis and apoE immunoblotting. The Amplex Red cholesterol assay kit (Molecular Probes) was used to assess cholesterol content in astrocyte culture medium. Fluorescence was measured with a Packard FluoroCount fluorometer with a 571/585-nm filter set. For apoE immunoblotting assay, the quantity of media used for SDS-PAGE analyses was normalized to equivalent quantities of proteins in the corresponding cell lysates, subjected to 12% SDS-PAGE under reducing conditions, and electroblotted to nitrocellulose membranes. The membranes were blocked and incubated with mouse monoclonal apoE (at 1:1,000 dilution). The antibody was visualized with horseradish peroxidase-conjugated anti-mouse IgG (1:2,000) by using the ECL kit (Pierce).

Neurosteroid Quantification

Neurosteroid concentrations were measured as described previously (Amateau et al., 2004). Briefly, culture medium conditioned by NPC1−/− or wild-type astrocytes was collected at 5 DIV after replating for measurement of estradiol and progesterone content. Whole brains from NPC1−/− or wild-type mice (P60) rinsed in cold saline were homogenized in RIPA buffer by mechanical trituration. After keeping on ice for 30 min, lysates were centrifuged at 14,000*g* and 4°C for 5 min, and the supernatants were used for measurement. Neurosteroid levels were determined by a competitive EIA method as described previously (Hira et al., 1990). The procedures for estradiol and progesterone followed the instructions in the Microwell Estradiol EIA kit (sensitivity 10 pg/mL; Syntron Bioresearch) and the Microwell Progesterone EIA kit (sensitivity 200 pg/mL; Syntron Bioresearch). Protein levels of cultured astrocytes and brains were quantified using a bicinchoninic acid assay (Beyotime Biotechnology, Haimen, China) at the same time.

Measurement of Plasma Liver Enzyme Activities

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (U/L) were measured by a commercial laboratory.

Estradiol Treatment

NPC1−/− mice received subcutaneous injections of 2 mg/kg or 20 mg/kg of 17β-estradiol in corn oil weekly from P7; the concentrations of 17β-estradiol were 10 ng/10 μL or 100 ng/10 μL.

Rota-Rod Performance Test

This test measures motor coordination and balance (Zausinger et al., 2000). The apparatus consisted of a rotating rod with a diameter of 3 cm and a nonslippery

surface. The apparatus was turned on at the minimum speed, the mice were positioned on the rotating rod, and acceleration started immediately until 10 rpm was reached. Three testing sessions were performed at 5 min intervals. Results were expressed as the longest time that a mouse remained on the treadmill. Mice were tested every 5 days from P30.

Statistical Analysis

Data are presented as mean ± SEM. Statistical comparisons were assessed with an ANOVA test or paired *t*-test with SPSS software; $P < 0.05$ was taken as significant.

RESULTS

Inhibited Outgrowth in Neurons Co-cultured with NPC1−/− Astrocytes

To determine whether astrocytes from NPC1−/− mice also accumulate unesterified cholesterol, astrocytes derived from wild-type and NPC1−/− mice were stained with filipin, a dye that labels unesterified cholesterol. The filipin stain revealed an intracellular, punctate pattern of intense fluorescence in astrocytes from NPC1−/− mice, whereas in wild-type astrocytes the fluorescence intensity was weak (Figs. 1A,B).

Astrocytes secrete many factors that support neuronal structure and function (Christopherson et al., 2005; Haydon, 2001; Mauch et al., 2001; Yang et al., 2003; Zhang et al., 2003). To verify whether astrocytes from NPC1−/− mice play similar supportive roles, cortical neurons from P0 wild-type mice were plated on a layer of primary astrocytes derived from wild-type or NPC1−/− mice. Staining of neuronal marker βIII-tubulin showed that while neuritic extension was evident at 48 h in those on the layer of wild-type astrocytes, it was impaired in those on the layer of NPC1−/− astrocytes (Figs. 1C,D). In addition, time-dependent analysis indicated that neuritic extension and sprouting were significantly suppressed by the NPC1−/− astrocyte layer, compared with the wild-type layer (Figs. 1E,F). Furthermore, when NPC1−/− neurons were co-cultured with wild-type astrocytes, the neurite length and the neurite number observed at 72 h (171.17 ± 10.93 μm/cell and 2.91 ± 0.09/cell, respectively) were significantly increased ($P < 0.01$ and $P < 0.05$, respectively, *t*-test, $n = 150$), as compared with the same NPC1−/− neurons cultured with NPC1−/− astrocytes (151.57 ± 5.98 μm/cell and 2.78 ± 0.07/cell, respectively). These resultes indicate that NPC1−/− astrocytes are not able to supply sufficient neurotrophic factors for neuronal growth.

Release of Cholesterol and apoE from Astrocytes was not Affected by NPC1 Deficiency

Defective trafficking of intracellular cholesterol is a major characteristic in NPC1−/− disease. Since astrocytes-derived cholesterol has been reported to play important role in neuronal outgrowth, synaptogenesis, and synaptic plasticity (Hayashi et al., 2004; Koudinov and Koudinov, 2001; Mauch et al., 2001), it is possible that a defect in cholesterol and/or lipoprotein production by NPC1−/− astrocytes contribute to the impaired support for neuronal outgrowth. To determine whether or not the release of cholesterol depended on NPC1 genotype, we compared the release of cholesterol from wild-type and NPC1−/− astrocyte cultures. The amount of cholesterol in the medium of NPC1-/- astrocytes was not significantly different from that in wild-type astrocytes

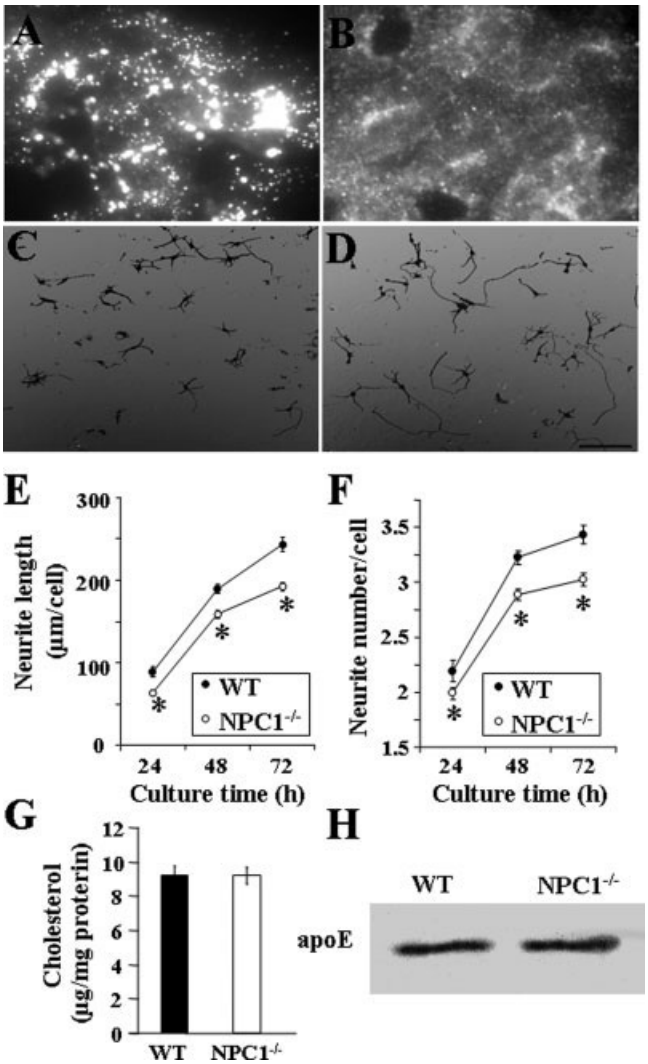

Fig. 1. Accumulation of unesterified cholesterol and impaired supporting function for neuronal outgrowth in NPC1−/− astrocyte culture. (**A**, **B**) Astrocytes isolated from NPC1−/− (A) and wild-type (B) stained with filipin at 7 DIV. (**C**, **D**) Wild-type (WT) neurons cocultured with NPC1−/− astrocytes (C) and wild-type astrocytes (D) at 48 h. Neurons stained with βIII-tubulin. Bar, 10 μm. (**E**, **F**) Summary of the average total neurite length (E) and neurite number (F) in two different cultures. $n = 400$ cells for each group. "*" $P < 0.05$, as compared with wild-type group. (**G**) The cholesterol concentration in the cultured medium collected from wild-type (WT) and NPC1−/− astrocyte cultures. Results are means ± SEM for three independent experiments. (**H**) The example immunoblot of secreted apoE in the cultured medium of wild-type (WT) and NPC1−/− astrocyte cultures. The data are representative of three independent experiments with similar results.

(Fig. 1G). Furthermore, the amounts of apoE in the medium of NPC1−/− astrocyte cultures estimated by western-blot was also not significantly different from that in wild-type astrocyte cultures (Fig. 1H). These results were consistent with the previous reports (Karten et al., 2005; Mutka et al., 2004; Suresh et al., 1998).

Estradiol Deficiency in Culture Medium of NPC1−/− Astrocytes

A noteworthy advance in NPC research was the finding that the NPC1−/− mouse contains substantially lower neurosteroid than the wild-type does (Gevry et al., 2004; Griffin et al., 2004; Roff et al., 1993). Since astrocytes are a major source of neurosteroid, we next examined whether NPC1−/− astrocytes are deficient in neurosteroid synthesis. The gene expression of various neurosteroid synthetic enzymes was determined by RT-PCR. A dramatic decrease in 17a-hydroxylase/C17-20-lyase (P450c) and the steroidogenic acute regulatory protein (StAR) and a moderate decrease in cytochrome P450 aromatase (P450a) were found in astrocyte cultures derived from NPC1−/− mice (Fig. 2A) Furthermore, immunostaining of StAR, a key cholesterol transport protein required for the synthesis of pregnenolone, also showed a significant decreased expression in NPC1−/− astrocyte cultures (Fig. 2B). These results suggest that neurosteroid synthesis is disrupted in NPC1−/− astrocytes.

Because the P450a is the rate-limiting enzyme in estrogen synthesis, we then measured the estradiol in medium conditioned by wild-type and NPC1−/− astrocytes. We found that the estradiol content in NPC1−/− astrocyte medium was 72% of that in wild-type medium (Fig. 2C), suggesting that estradiol deficiency in the culture medium of NPC1−/− astrocytes may be responsible for their abnormal role in supporting neuronal outgrowth. To explore this possibility, we added tamoxifen, an estrogen receptor antagonist, and anastrozole, a specific inhibitor of P450a, to the culture medium and used 17β-estradiol as a positive control. The difference in neurite outgrowth between NPC1−/− and wild-type cultures disappeared after adding tamoxifen, anastrozole, or 17β-estradiol (Figs. 2D,E), consistent with the idea that estradiol deficiency in NPC1−/− astrocytes results in inhibited neuronal outgrowth. Furthermore, application of 17β-estradiol significantly increased the neurite length in the wild-type group (Fig. 2D), suggesting that the basal levels of estradiol in wild-type cultures may be not saturated in stimulating neurite growth.

Estradiol Treatment Improved Behavioral Deficiencies in NPC1−/− Mice

We found that the content of progesterone and estradiol in the whole brain were significantly decreased in NPC1−/− mice (Fig. 3A). We thus next verified whether replacement of 17β-estradiol can alleviate some neurodegenerative features in NPC1−/− mice. As shown in Figs. 3B and C, two dose of replacement of 17β-estradiol significantly increased the lifespan of NPC1−/− mice. The untreated NPC1−/− mice had their maximal body weight approximately at 40 days (15.7 ± 2.1 g) and begin to loss their weight quickly afterward (Fig. 3D). Two doses of treatment with 17β-estradiol delayed the time starting to loss weight (50 days in treated mice *vs.* 45 days in untreated group) and attenuated the severity of weight loss estimated during 45–55 days. Furthermore, the locomotor function and motor coordination of NPC1−/− mice, estimated by the rota-rod test, were significantly improved by the treatment with 17β-estradiol (Fig. 3E). Tremors were first detected at 62.3 ± 0.4 days in 17β-estradiol-treated mice, significantly delayed as compared with 51.2 ± 0.5 days in untreated mice ($P < 0.01$, $n = 15$). There was no difference in survival, locomotor function, or motor coordination between male and female mice receiving 17β-estradiol treatment (data not shown). Thus, 17β-estradiol treatment significanly improved behavioral deficiencies in NPC1−/− mice.

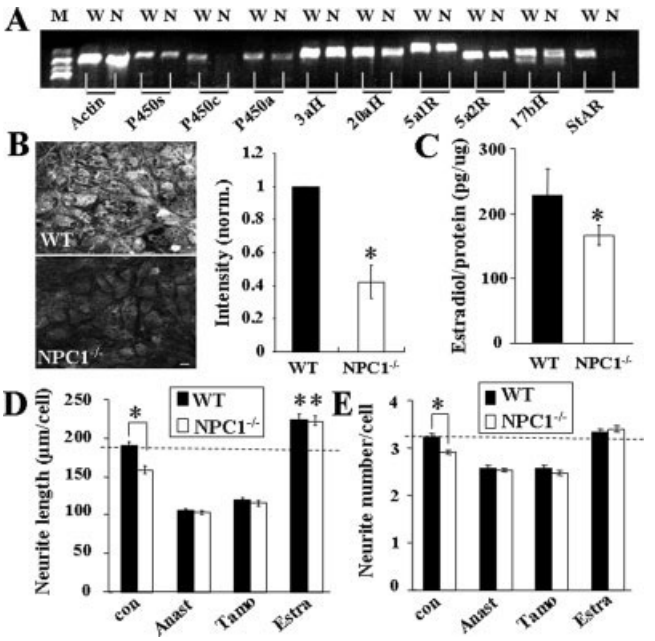

Fig. 2. Deficiency in estradiol synthesis in NPC1−/− astrocyte culture contributes to the impaired neurite growth. (**A**) RT-PCR analysis of steroidogenic enzymes, cytochrome P450 side-chain cleavage (P450s), 17a-hydroxylase/C17-20-lyase (P450c), cytochrome P450 aromatase (P450a), 3α-hydroxysteroid dehydrogenase (3αH), 20α-hydroxysteroid dehydrogenase (20αH), 5α-reductase type 1 (5α1R), 5α-reductase type 2 (5α2R), 17β-hydroxysteroid dehydrogenase (17βH), and the steroidogenic acute regulatory protein (StAR) in astrocyte cultures from the cerebral cortex of the neonatal wild-type (W) and NPC1−/− (N) brain. M is a DNA size marker. (**B**) Left, immunostaining of StAR in the two types of astrocyte cultures. Bar, 10 μm. Right, average fluorescence intensity of immunostaining StAR in NPC1−/− astrocytes was normalized with those in wild-type ($n = 4$ experiments for each group). "*" $P < 0.05$, as compared with wild-type group. (**C**) Elisa measurement of estradiol content in astrocyte culture medium conditioned by the two groups ($n = 5$ experiments for each group). "*" $P < 0.05$, as compared with wild-type group. (**D**,**E**) Summary of average total neurite length (E) and neurite number (**F**) of neurons co-cultured with wild-type (WT) and NPC1−/− astrocytes under different treatments. Con, untreated; Anast, anastrozole; Tamo, tamoxifen; Estra, 17β-estradiol. $n = 200$ cells for each group. Broken line represents the averaged value obtained from untreated neurons co-cultured with wild-type astrocytes. "*" $P < 0.05$, as compared with wild-type group.

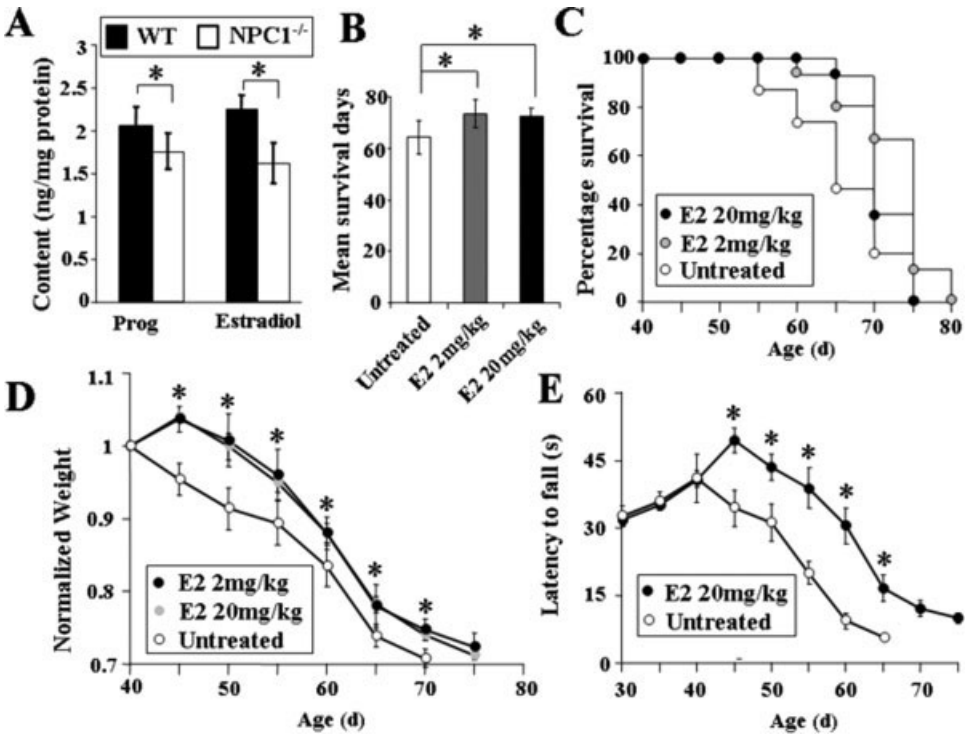

Fig. 3. Effects of 17β-estradiol (E2) injections on progression of NPC1−/− symptoms. (**A**) Elisa measurement of progesterone and estradiol content in wild-type and NPC1−/− mouse brain (n = 6 mice for each group). "*" $P < 0.05$, as compared with wild-type group. (**B**) Average lifespan of NPC1−/− mice with or without 17β-estradiol (E2) treatment (2 mg/kg or 20 mg/kg, subcutaneous injection weekly from P7). N = 15 mice for each group "*" $P < 0.05$, as compared with untreated group (Oneway ANOVA test). (**C**) Kaplan-Meier survival curves for different group mice as shown in (B). (**D**) Normalized body weight loss in NPC1−/− mice with or without 17β-estradiol treatment. Data were normalized with the body weight of the same mouse at 40 days of age. "*" $P < 0.05$, as compared with untreated group at the same age. (**E**) Rota-rod test assessed for NPC1−/− mice with or without 17β-estradiol treatment. "*" $P < 0.05$, as compared with untreated group at the same age.

Estradiol Promoted Purkinje Cell Survival

Cerebellar neuronal loss was a prominent phenomenon in NPC1−/− mice. We found that 17β-estradiol (20 mg/kg, weekly from P7) treatment improved the survival of cerebellar neurons. Untreated NPC1−/− mice had fewer Purkinje cells (PCs) (identified as anti-calbindin positive immunostaining, Figs. 4A,B) and granule cell (identified as calbindin-negative and βIII-tubulin-positive immunostaining, Fig. 4B) than wild-type mice, with the greatest loss of PCs occurring in cerebellar lobes 1–7 (Fig. 4C). Estradiol treatment was most effective in promoting PCs survival in lobes 3–9 ($P < 0.05$ or $P < 0.01$; Fig. 4C). Furthermore, Golgi staining showed that the survived PCs in NPC1−/− mice had shorter neurite and fewer branches than wild-type PCs did, while 17β-estradiol treatment partially rescued the deficiency in the neurite outgrowth (Fig. 5).

Estradiol Treatment had no Effect on the Liver Disorder in NPC1−/− Mice

Although estradiol treatment in NPC1−/− mice significantly rescued cerebellar neuronal loss and outgrowth deficiency (Figs. 4 and 5) and improved locomotor function and motor coordination (Fig. 3E), it only increased life longevity moderately (Figs. 3B,C). Because pathological change in the liver of NPC1−/− mice is another lethal factor, we then determined whether estradiol treatment rescued the liver disorder in NPC1−/− mice. We found that treatment with 17β-estradiol (20 mg/kg, weekly from P7) did not prevent the increases in the relative liver weight (Fig. 6A) or the plasma level of liver enzyme activity of ALT (Fig. 6B) and AST (Fig. 6C) in the NPC1−/− mice estimated at either 40 or 60 days of age. Moreover, histological examination of the liver by Masson staining at 60 days of age did not reveal significant improvement of pathological changes in NPC1−/− mice treated with estradiol (Figs. 6D,E). Large numbers of foamy macrophages were seen within the livers from both untreated and estradiol-treated NPC1−/− mice. Above all, these evidences indicated that estradiol treatment had no effect on liver pathology in NPC1−/− mice.

DISCUSSION

In most neurodegenerative diseases, neuronal loss can be attributed to cell-autonomous *versus* noncell-autonomous factors; however, for NPC1−/− disease, there have been inconsistent statements about the reasons for

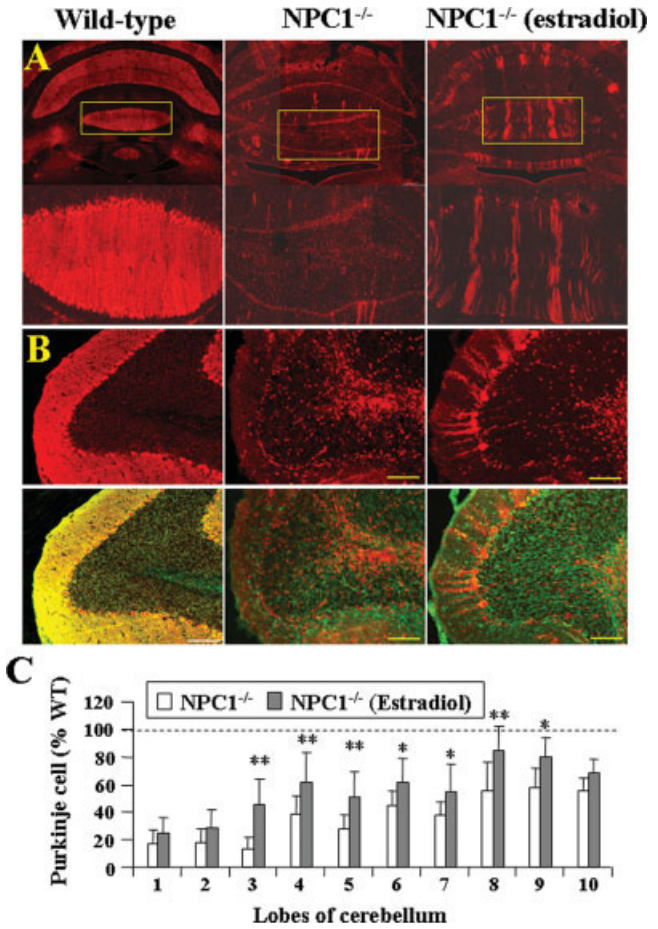

Fig. 4. Cerebellar histology examined at P60 from wild-type, NPC1−/− mice with or without treatment of 17β-estradiol (20 mg/kg, weekly from P7). (**A**) Immunostaining with calbindin (Purkinje cell marker, red). Enlargements of the boxed regions are shown in lower panels. Scale bars, 100 μm. (**B**) Immunostaining with calbindin (red, upper) and βIII-tubulin (neuronal marker, green). Scale bars, 100 μm. (**C**) Number of calbindin-immunopositive neurons determined in each cerebellar lobe in wild-type, NPC1−/−, and NPC1−/− mice treated with 17β-estradiol. All Purkinje cells in five sections at least 100 μm apart were counted for each mouse. Data are presented as the percentage of the number of Purkinje cells in wild-type mice (the broken line). $N = 10$ sections from two mice in each group, $^{*}P < 0.05$; $^{**}P < 0.01$.

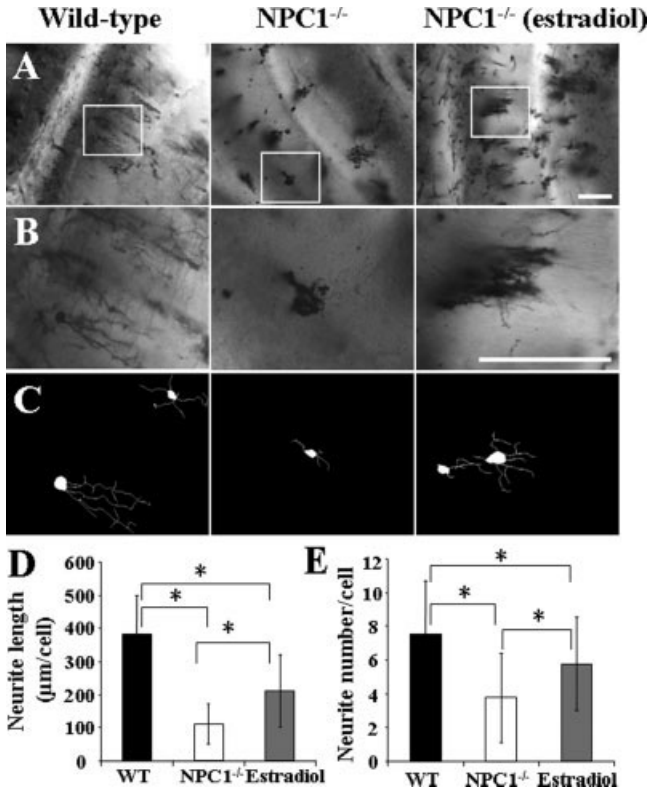

Fig. 5. Neurite outgrowth of cerebellar Purkinje cells revealed by Golgi staining at P60 in NPC1−/− mice with or without treatment of 17β-estradiol (20 mg/kg, weekly from P7). (**A**, **B**) Example images of Golgi staining of Purkinje cells in wild type and NPC1−/− mice. Note the shorter neurites and fewer branches of the survived Purkinje cells in NPC1−/− mice and the partial rescue of the abnormal morphology by treatment with 17β-estradiol. Enlargements of the boxed regions in (A) are shown in B. Scale bars, 100 μm. (**C**) The projection images showed by Golgi staining in B were semi-automatically traced and quantified with NIH ImageJ using the NeuronJ plugin. (**D**, **E**) Summary of the averaged total neurite length and neurite number of Purkinje cells as analyzed in (C) ($n = 100$ cells, 10 sections from two mice in each group, $^{*}P < 0.05$).

neuronal death. Ko et al. constructed genetically mosaic mice in which some cells had mutant NPC1 and some had normal NPC1 function. In the cerebella of these mosaic mice, PCs lacking NPC1 continued to die even when surrounded by normal cells, while normal PCs appeared unaffected by their partially mutant surroundings. From these findings, it was concluded that the neurodegeneration is due to a problem within the PCs themselves and not due to a lack of supporting factors provided by other cells or an extrinsic toxic or inflammatory insult (Ko et al., 2005). However, another study found that the loss of PCs was prevented in NPC1−/−, Tg(NPC1) mice, in which the expression of a wild-type murine NPC1 gene under the control of the mouse prion promoter was introduced into NPC1−/− mice (Loftus et al., 2002). Since PCs lack transgene expression of mouse prion promoter construct (Garden et al., 2002; La Spada et al., 2001; Schilling et al., 1999), the improved PC loss in NPC1−/−, Tg(NPC1) mice under the control of the mouse prion promoter (Loftus et al., 2002) suggests involvement of noncell-autonomous causes for neuronal death in NPC1−/− disease. Consistent with this idea, we found that dysfunction in astrocytes is involved in the neurodegeneration in this disease.

We found that StAR expression was markedly decreased in NPC1−/− mouse astrocytes (Fig. 2B). StAR is a key element in the rate-limiting step of steroid hormone biosynthesis. It regulates cholesterol delivery to the P450scc enzyme located in the inner mitochondrial membrane (King et al., 2002), which converts cholesterol to pregnenolone, a precursor of steroids (Lambeth et al., 1987). Mutations in the StAR gene have been shown to underlie lipoid congenital adrenal hyperplasia, a disorder leading to a dramatic congenital deficiency in all steroid hormones (Cherradi et al., 2001; Lin et al., 1995). In contrast, mitochondria are known to be important for sterol metabolism (Miller, 1995) and mitochondrial dysfunction in NPC1−/− mouse astrocytes has also been

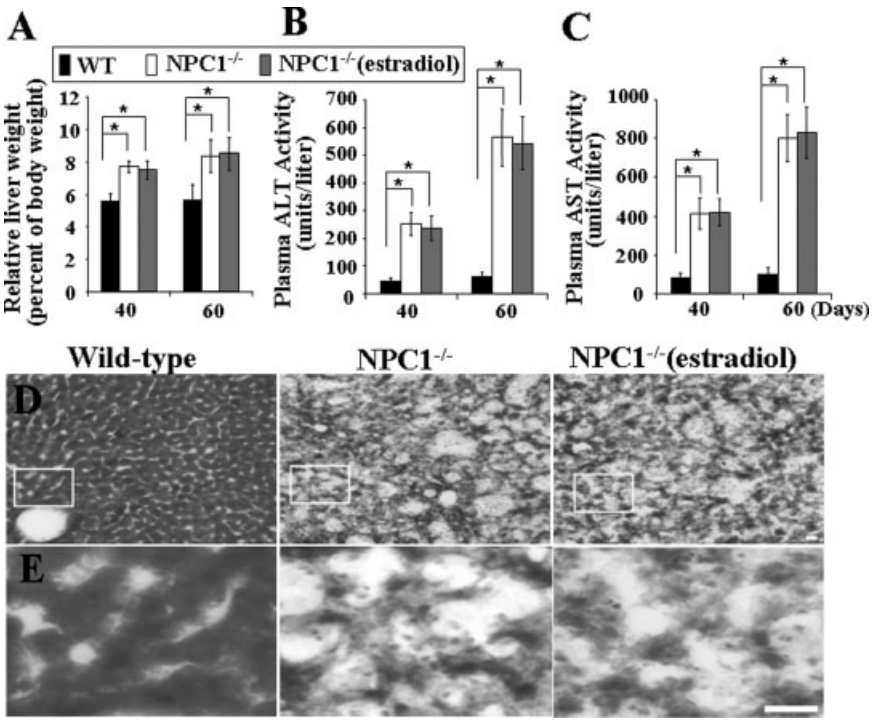

Fig. 6. Lack of effects of 17β-estradiol (20 mg/kg, weekly from P7) treatment on liver disorder in NPC1−/− mice. (**A**) Summary of average relative liver weights in wild-type, NPC1−/−, and NPC1−/− mice treated with 17β-estradiol at 40 and 60 days of age (n = 6 in each group, 3 mice for each time-point). "*" $P < 0.05$, as compared with wild-type group. (**B**, **C**) The averaged plasma levels of liver enzyme activity of ALT (alanine aminotransferase, B) and AST (aspartate aminotransferase, C) measured in the same groups of mice shown in A. "*" $P < 0.05$, as compared with wild-type group. (**D**, **E**) Histological examination of the liver at 60 days of age. Masson staining showed that large numbers of foamy macrophages were seen within the livers from both untreated and treated NPC1−/− mice. Enlargements of the boxed regions in D are shown in E. Bar, 25 μm.

reported (Huang et al., 2006; Yu et al., 2005). Consistent with these reports, we found the estradiol content decreased 28% in medium conditioned by NPC1−/− mouse astrocytes. The content of progesterone and estradiol in the NPC1−/− mouse brain were also decreased 15% and 29% respectively.

Estradiol exerts widespread and important regulatory functions in the brain. We found that decreased estradiol secretion by astrocytes from NPC1−/− mice was the crucial factor in impairment of support for neuronal outgrowth (Fig. 1). Thus, in the presence of 17β-estradiol, P450a inhibitor anastrozole, or estrogen receptor antagonist tamoxifen, no significant difference was observed for the neurite growth rate between neurons co-cultured with wild type astrocytes and those with NPC1−/− astrocytes (Figs. 2D,E), indicating that decreased estradiol secretion from astrocytes contributes to the development of NPC1−/− disease.

An assessment of the clinical phenotype of NPC1−/− mice and the rota-rod test revealed that estradiol treatment markedly delayed weight lost, tremors and ataxia (Fig. 3). Histochemical staining showed that estradiol treatment not only rescued PCs from death but also maintained their normal morphology (Figs. 4 and 5). We found surprisingly that although the clinical phenotypes were significantly improved, survival was only extended moderately. It is possible that other lethal pathological changes are not ameliorated by this treatment. More recent clinical information suggests that serious liver, spleen, and pulmonary disease are also important in NPC1−/− disease, as in the phenotypes of Niemann-Pick A and B (Beltroy et al., 2005; Erickson et al., 2005; Schofer et al., 1998). Liver plays the key role in the clearance of circulating cholesterol carried in lipoproteins and so manifests the highest rate of sterol accumulation in both the human and mouse with a mutation in NPC1 (Goldin et al., 1992; Xie et al., 1999). NPC1−/− mice develop significant hepatomegaly that reaches 8% of body weight at 5–6 weeks of age. This increase in liver size is associated with a linear increase in cholesterol content and with accumulation of amorphous cellular inclusions in both hepatocytes and macrophages and can be fatal before the onset of neurodegeneration (Beltroy et al., 2005; Erickson et al., 2005; Loftus et al., 2002). Our result that estradiol treatment did not ameliorate the liver pathogenesis in NPC1−/− disease (Fig. 6) may explain the limited effect of estradiol treatment on the survive length of NPC1−/− mice.

In summary, we found that astrocytes play an important role in the development of NPC1−/− disease and estradiol replacement therapy can ameliorate the NPC1−/− phenotype in the brain. Since NPC1−/− disease is a syndrome that is not only deleterious to brain, but also particularly pronounced in liver and spleen, effective treatment approaches should also consider how to restore function in organs other than the brain.

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REFERENCES

Amateau SK, Alt JJ, Stamps CL, McCarthy MM. 2004. Brain estradiol content in newborn rats: sex differences, regional heterogeneity, and possible de novo synthesis by the female telencephalon. Endocrinology 145:2906–2917.

Baudry M, Yao Y, Simmons D, Liu J, Bi X. 2003. Postnatal development of inflammation in a murine model of Niemann-Pick type C disease: immunohistochemical observations of microglia and astroglia. Exp Neurol 184:887–903.

Beltroy EP, Richardson JA, Horton JD, Turley SD, Dietschy JM. 2005. Cholesterol accumulation and liver cell death in mice with Niemann-Pick type C disease. Hepatology 42:886–893.

Carstea ED, Morris JA, Coleman KG, Loftus SK, Zhang D, Cummings C, Gu J, Rosenfeld MA, Pavan WJ, Krizman DB, Nagle J, Polymeropoulos MH, Sturley SL, Ioannou YA, Higgins ME, Comly M, Cooney A, Brown A, Kaneski CR, Blanchette-Mackie EJ, Dwyer NK, Neufeld EB, Chang TY, Liscum L, Strauss JF 3rd, Ohno K, Zeigler M, Carmi R, Sokol J, Markie D, O'Neill RR, van Diggelen OP, Elleder M, Patterson MC, Brady RO, Vanier MT, Pentchev PG, Tagle DA. 1997. Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. Science 277:228–231.

Cherradi N, Capponi AM, Gaillard RC, Pralong FP. 2001. Decreased expression of steroidogenic acute regulatory protein: a novel mechanism participating in the leptin-induced inhibition of glucocorticoid biosynthesis. Endocrinology 142:3302–3308.

Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA. 2005. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120:421–433.

Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ, Brown RH Jr, Julien JP, Goldstein LS, Cleveland DW. 2003. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 302:113–117.

Custer SK, Garden GA, Gill N, Rueb U, Libby RT, Schultz C, Guyenet SJ, Deller T, Westrum LE, Sopher BL, La Spada AR. 2006. Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport. Nat Neurosci 9:1302–1311.

Deisz RA, Meske V, Treiber-Held S, Albert F, Ohm TG. 2005. Pathological cholesterol metabolism fails to modify electrophysiological properties of afflicted neurones in Niemann-Pick disease type C. Neuroscience 130:867–873.

Erickson RP, Bhattacharyya A, Hunter RJ, Heidenreich RA, Cherrington NJ. 2005. Liver disease with altered bile acid transport in Niemann-Pick C mice on a high fat, 1% cholesterol diet. Am J Physiol 289:G300–G307.

Forman MS, Lal D, Zhang B, Dabir DV, Swanson E, Lee VM, Trojanowski JQ. 2005. Transgenic mouse model of tau pathology in astrocytes leading to nervous system degeneration. J Neurosci 25:3539–3550.

Garcia-Segura LM, Melcangi RC. 2006. Steroids and glial cell function. Glia 54:485–498.

Garden GA, Libby RT, Fu YH, Kinoshita Y, Huang J, Possin DE, Smith AC, Martinez RA, Fine GC, Grote SK, Ware CB, Einum DD, Morrison RS, Ptacek LJ, Sopher BL, La Spada AR. 2002. Polyglutamine-expanded ataxin-7 promotes non-cell-autonomous purkinje cell degeneration and displays proteolytic cleavage in ataxic transgenic mice. J Neurosci 22:4897–4905.

German DC, Liang CL, Song T, Yazdani U, Xie C, Dietschy JM. 2002. Neurodegeneration in the Niemann-Pick C mouse: glial involvement. Neuroscience 109:437–450.

German DC, Quintero EM, Liang CL, Ng B, Punia S, Xie C, Dietschy JM. 2001a. Selective neurodegeneration, without neurofibrillary tangles, in a mouse model of Niemann-Pick C disease. J Comp Neurol 433:415–425.

German DC, Quintero EM, Liang C, Xie C, Dietschy JM. 2001b. Degeneration of neurons and glia in the Niemann-Pick C mouse is unrelated to the low-density lipoprotein receptor. Neuroscience 105:999–1005.

Gevry NY, Lopes FL, Ledoux S, Murphy BD. 2004. Aberrant intracellular cholesterol transport disrupts pituitary and ovarian function. Mol Endocrinol 18:1778–1786.

Goldin E, Roff CF, Miller SP, Rodriguez-Lafrasse C, Vanier MT, Brady RO, Pentchev PG. 1992. Type C Niemann-Pick disease: a murine model of the lysosomal cholesterol lipidosis accumulates sphingosine and sphinganine in liver. Biochim Biophys Acta 1127:303–311.

Griffin LD, Gong W, Verot L, Mellon SH. 2004. Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. Nat Med 10:704–711.

Hayashi H, Campenot RB, Vance DE, Vance JE. 2004. Glial lipoproteins stimulate axon growth of central nervous system neurons in compartmented cultures. J Biol Chem 279:14009–14015.

Haydon PG. 2001. GLIA: listening and talking to the synapse. Nat. Rev. Neurosci 2:185–193.

Henderson VW. 2006. Estrogen-containing hormone therapy and Alzheimer's disease risk: understanding discrepant inferences from observational and experimental research. Neuroscience 138:1031–1039.

Hira PR, Bahr GM, Shweiki HM, Behbehani K. 1990. An enzyme-linked immunosorbent assay using an arc 5 antigen for the diagnosis of cystic hydatid disease. Ann Trop Med Parasitol 84:157–162.

Huang Z, Hou Q, Cheung NS, Li QT. 2006. Neuronal cell death caused by inhibition of intracellular cholesterol trafficking is caspase dependent and associated with activation of the mitochondrial apoptosis pathway. J Neurochem 97:280–291.

Karten B, Hayashi H, Francis GA, Campenot RB, Vance DE, Vance JE. 2005. Generation and function of astroglial lipoproteins from Niemann-Pick type C1-deficient mice. Biochem J 387:779–788.

Kawato S. 2004. Endocrine disrupters as disrupters of brain function: a neurosteroid viewpoint. Environ Sci 11:1–14.

King SR, Manna PR, Ishii T, Syapin PJ, Ginsberg SD, Wilson K, Walsh LP, Parker KL, Stocco DM, Smith RG, Lamb DJ. 2002. An essential component in steroid synthesis, the steroidogenic acute regulatory protein, is expressed in discrete regions of the brain. J Neurosci 10613–10620.

Ko DC, Milenkovic L, Beier SM, Manuel H, Buchanan J, Scott MP. 2005. Cell-autonomous death of cerebellar purkinje neurons with autophagy in Niemann-Pick type C disease. PLoS Genet 1:81–95.

Koudinov AR, Koudinova NV. 2001. Essential role for cholesterol in synaptic plasticity and neuronal degeneration. FASEB J 15:1858–1860.

La Spada AR, Fu YH, Sopher BL, Libby RT, Wang X, Li LY, Einum DD, Huang J, Possin DE, Smith AC, Martinez RA, Koszdin KL, Treuting PM, Ware CB, Hurley JB, Ptacek LJ, Chen S. 2001. Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. Neuron 31:913–927.

Lambeth JD, Xu XX, Glover M. 1987. Cholesterol sulfate inhibits adrenal mitochondrial cholesterol side chain cleavage at a site distinct from cytochrome P-450scc. Evidence for an intramitochondrial cholesterol translocator. J Biol Chem 262:9181–9188.

Lin D, Sugawara T, Strauss JF 3rd, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL. 1995. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 267:1828–1831.

Loftus SK, Erickson RP, Walkley SU, Bryant MA, Incao A, Heidenreich RA, Pavan WJ. 2002. Rescue of neurodegeneration in Niemann-Pick C mice by a prion-promoter-driven Npc1 cDNA transgene. Hum Mol Genet 11:3107–3114.

Loftus SK, Morris JA, Carstea ED, Gu JZ, Cummings C, Brown A, Ellison J, Ohno K, Rosenfeld MA, Tagle DA, Pentchev PG, Pavan WJ. 1997. Murine model of Niemann-Pick C disease: mutation in a cholesterol homeostasis gene. Science 277:232–235.

MacLusky NJ, Luine VN, Hajszan T, Leranth C. 2005. The 17alpha and 17beta isomers of estradiol both induce rapid spine synapse formation in the CA1 hippocampal subfield of ovariectomized female rats. Endocrinology 146:287–293.

Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A, Pfrieger FW. 2001. CNS synaptogenesis promoted by glia-derived cholesterol. Science 294:1354–1357.

McGeer PL, Kawamata T, Walker DG, Akiyama H, Tooyama I, McGeer EG. 1993. Microglia in degenerative neurological disease. Glia 7:84–92.

Miller MM, Monjan AA, Buckholtz NS. 2001. Estrogen replacement therapy for the potential treatment or prevention of Alzheimer's disease. Ann N Y Acad Sci 949:223–234.

Miller WL. 1995. Mitochondrial specificity of the early steps in steroidogenesis, J Steroid Biochem Mol Biol 55:607–616.

Mutka AL, Lusa S, Linder MD, Jokitalo E, Kopra O, Jauhiainen M, Ikonen E. 2004. Secretion of sterols and the NPC2 protein from primary astrocytes. J Biol Chem 279:48654–48662.

Patel SC, Suresh S, Kumar U, Hu CY, Cooney A, Blanchette-Mackie EJ, Neufeld EB, Patel RC, Brady RO, Patel YC, Pentchev PG, Ong WY. 1999. Localization of Niemann-Pick C1 protein in astrocytes: implications for neuronal degeneration in Niemann- Pick type C disease. Proc Natl Acad Sci USA 96:1657–1662.

Raeber AJ, Race RE, Brandner S, Priola SA, Sailer A, Bessen RA, Mucke L, Manson J, Aguzzi A, Oldstone MB, Weissmann C, Chesebro B. 1997. Astrocyte-specific expression of hamster prion protein (PrP) renders PrP knockout mice susceptible to hamster scrapie. EMBO J 16:6057–6065.

Roff CF, Goldin E, Comly ME, Blanchette-Mackie J, Cooney A, Brady RO, Pentchev PG. 1992. Niemann-Pick type-C disease: deficient intracellular transport of exogenously derived cholesterol. Am J Med. Genet 42:593–598.

Schilling G, Wood JD, Duan K, Slunt HH, Gonzales V, Yamada M, Cooper JK, Margolis RL, Jenkins NA, Copeland NG, Takahashi H, Tsuji S, Price DL, Borchelt DR, Ross CA. 1999. Nuclear accumulation of truncated atrophin-1 fragments in a transgenic mouse model of DRPLA. Neuron 24:275–286.

Schofer O, Mischo B, Puschel W, Harzer K, Vanier MT. 1998. Early-lethal pulmonary form of Niemann-Pick type C disease belonging to a

second, rare genetic complementation group. Eur J Pediatr 157:45–49.
Sturley SL, Patterson MC, Balch W, Liscum L. 2004. The pathophysiology and mechanisms of NP-C disease. Biochim Biophys Acta 1685:83–87.
Suresh S, Yan Z, Patel RC, Patel YC, Patel SC. 1998. Cellular cholesterol storage in the Niemann-Pick disease type C mouse is associated with increased expression and defective processing of apolipoprotein D. J Neurochem 70:242–251.
Suzuki H, Sakiyama T, Harada N, Abe M, Tadokoro M. 2003. Pathologic changes of glial cells in murine model of Niemann-Pick disease type C: immunohistochemical, lectin-histochemical and ultrastructural observations. Pediatr Int 45:1–4.
Tsang KL, Ho SL, Lo SK. 2000. Estrogen improves motor disability in Parkinsonian postmenopausal women with motor fluctuations. Neurology 254:2292–2298.
Ullian EM, Christopherson KS, Barres BA. 2004. Role for glia in synaptogenesis. Glia 47:209–216.
Walkley SU, Suzuki K. 2004. Consequences of NPC1 and NPC2 loss of function in mammalian neurons. Biochim Biophys Acta 1685:48–62.
Xie C, Turley SD, Pentchev PG, Dietschy JM. 1999. Cholesterol balance and metabolism in mice with loss of function of Niemann-Pick C protein. Am J Physiol 276:E336–E344.
Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, Poo M, Duan S. 2003. Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. Proc Natl Acad Sci USA 100:15194–15199.
Yankova M, Hart SA, Woolley CS. 2001. Estrogen increases synaptic connectivity between single presynaptic inputs and multiple postsynaptic CA1 pyramidal cells: a serial electron-microscopic study. Proc Natl Acad Sci USA 98:3525–3530.
Yazawa I, Giasson BI, Sasaki R, Zhang B, Joyce S, Uryu K, Trojanowski JQ, Lee VM. 2005. Mouse model of multiple system atrophy alpha-synuclein expression in oligodendrocytes causes glial and neuronal degeneration. Neuron 45:847–859.
Yu W, Gong JS, Ko M, Garver WS, Yanagisawa K, Michikawa M. 2005. Altered cholesterol metabolism in Niemann-Pick type C1 mouse brains affects mitochondrial function. J Biol Chem 280:11731–11739.
Yue X, Lu M, Lancaster T, Cao P, Honda S, Staufenbiel M, Harada N, Zhong Z, Shen Y, Li R. 2005. Brain estrogen deficiency accelerates Abeta plaque formation in an Alzheimer's disease animal model. Proc Natl Acad Sci USA 102:19198–19203.
Zausinger S, Hungerhuber E, Baethmann A, Reulen H, Schmid-Elsaesser R. 2000. Neurological impairment in rats after transient middle cerebral artery occlusion: a comparative study under various treatment paradigms. Brain Res 863:94–105.
Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL, Wu CP, Poo MM, Duan S. 2003. ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. Neuron 40:971–982.
Zwain IH, Yen SS. 1999. Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. Endocrinology 140:3843–3852.