Effects of short-term aerobic exercise in a mouse model of Niemann-Pick type C disease on synaptic and muscle plasticity

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Abstract

Background. Physical exercise can reduce the risk of developing chronic diseases and slow the onset of neurodegenerative diseases. Since it has not been assessed which kind of training protocol might positively modulate both synaptic and muscular plasticity in neurodegenerative diseases, we studied in a mouse model of Niemann Pick type C disease, a model of minimal Alzheimer’s Disease, the effect of a short term protocol.

Methods. We evaluated the effect of a short term, aerobic uniform exercise training on synaptic and muscle plasticity in three different mice groups: WT controls, NPC1+/− and NPC1−/− animals. The results were compared with those obtained in the sedentary respective groups. We analyzed the effects on synaptic plasticity by in vitro extracellular recordings in hippocampal mouse slices; moreover hippocampal and muscle tissue morphological structure have been investigated by transmission electron microscopy; to highlight any structural and functional changes due to training.

Results. The results indicate a rescue of long-term potentiation in homozygous but not in heterozygous mice slices and an induction of neuronal plasticity, observed by morphological analysis, both in homozygous and in heterozygous trained mice.

Conclusions. Hence this protocol is adequate to improve long term potentiation (LTP) impairment and counteract muscular deterioration in homozygous mice.

INTRODUCTION

Niemann Pick type C disease (NPCD) is a rare recessive genetic neurovisceral disorder, determined by NPC1 and/or NPC2 gene mutation. The loss of function of one of the two proteins encoded by these genes causes an endo-lysosomal storage of unesterified cholesterol and other lipids, such as sphingomyelin and gangliosides, GM2 and GM3 [1]. The ubiquitous cellular cholesterol infarction is also present in the central nervous system, although to a lesser extent compared to other tissues, while the accumulation of gangliosides GM2 and GM3 seems predominant at the cerebral level [2].

The neuropathological features of NPC1 brains are characterized by the loss of neurons such as Purkinje cells, hyper-phosphorylated tau, and neurofibrillary tangle formation with the accumulation of lipid storage bodies and the presence of dendritic and axonal abnormalities [3-7].

This pathological condition is characterized by a broad spectrum of symptoms and disorders, including hepatosplenomegaly, seizures, cerebellar ataxia, dystonia, up to cognitive disabilities and dementia. In a previous study, we have reported that synaptic plasticity phenomena, involved in learning and memory processes, are affected in homozygous (NPC1−/−) mice, a well-established mouse model for the NPC disease [8].

Heterozygous carriers of NPC1 mutations are not assumed to develop any neurological symptoms during their entire life span though, recently, the occurrence of this mutation in human adults, who also present a parkinsonism syndrome, has been reported [9].

Intermediate abnormalities in terms of cholesterol

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metabolism have been shown in non-neuronal tissues or cells from heterozygous (NPC1+/-) mice [10,11]. Moreover, it has been demonstrated that NPC1+/- mice exhibit intermediate dysfunction in the mitochondria of the brain [12], but until now no study has been carried on about learning and memory processes.

Scientific evidences previously reported that physical activity plays an important role in reducing morbidity and mortality of cardiovascular and respiratory diseases, improving physical performance and quality of life [13,14].

Physical exercise, furthermore, can significantly reduce the risk of developing other chronic diseases, such as obesity, osteoporosis, diabetes, stress, depression and slow the onset of neurodegenerative diseases, although most of the mechanisms underlying the benefit of exercise are still unknown [15-20]. We demonstrated previously [21] that short term aerobic exercise exerts positive effects inducing weight augmentation, strength endurance and aerobic endurance increase as well as potentiation of motor coordination in adult Wild Type (WT) mice. Moreover, augmentation in synaptic plasticity was observed. These lines of evidence have led us to examine whether in NPC1+/- mice aerobic exercise might counteract synaptic deficits.

Hence aim of this study is to assess: i) whether in NPC1+/- mice an impairment of LTP processes might occur as well as in NPC1-/- mice; ii) if short term aerobic physical exercise can ameliorate both structural and functional parameters in the hippocampus and in the anterior leg muscle of adult male NPC1+/- and in NPC1-/- mice.

MATERIALS AND METHODS

Animals

We employed animals from a colony of NPC1-/- mice that we established in our animal house and that represent a well-known experimental model of NPCD, since they display most of the clinical features of the disease including cognitive deficits. Breeding pairs of BALB/c/Ncr-Npc1m1IN/J (Stock Number: 003092) mice were purchased from Jackson Laboratories (Bar Harbor, MA, USA). This strain contains a spontaneous mutation in the NPC1 locus [22]. Animals were bred and maintained according to Italian Animal Care Committee rules. The health status of animals was daily monitored by resident veterinarians and experimenters. In particular, we have periodically monitored the housing conditions of animals, in terms of air/min replacement, humidity and temperature, providing for the constant environmental enrichment of the cages with food and water. During the training period, we evaluated the physical condition of animals, taking into consideration the weight, the state of the coat and skin, and bodily functions such as breathing. Moreover, from the behavioural point of view, we have daily verified the possible onset of stereotypy and postural alterations.

Heterozygous mice were bred and the genotypes of offspring animals were determined as indicated by Jackson Laboratories in genotyping protocols database by polymerase chain reaction (PCR) [22]. Twenty-four male mice thirty days old were used according to the EU Directive 2010/63/EU for animal experiments: eight were heterozygous, eight were homozygous and eight age-matched WT served as controls. For each of these three groups, four animals were submitted to a training protocol until the age of 53 days old, while the other four, untrained, were used as a sedentary control group.

Behavioural training

A Rotarod (for mice. Cat N° 47600, Ugo Basile srl, Italy,) was employed for the training. Biological adaptation was induced by administering an aerobic workout on the base of a Continuous Uniform protocol (CU). This was done at the rate of 9 laps for minute (RPM) for a duration of 30 minutes for each training session, covering a volume of 270 laps. The protocol was carried out three times a week from the thirtieth to the fifty-third day of life for a total of three weeks.

The choice to train NPC1/- mice at 9 RPM has been taken after recording data related to the falls through an Incremental Test performed only by WT mice that did not follow the training protocol. The incremental test, as previously reported [23], allowed to describe speed zones on the RotaRod: low intensity (7-14 RPM), average intensity (14-18 RPM), high intensity (18 -28 RPM) and very high intensity (30 RPM and over).

Extracellular recordings in mouse hippocampal slices

The animals belonging to the six different groups were sacrificed after three weeks of training. All efforts were made to minimize the number of animals used and their suffering. The hippocampal slices were prepared according to conventional procedures [8]. Some of the slices obtained were transferred into an interface tissue chamber constantly perfused by a flow of 1.2 ml/min of ACSF and humidified gas (95% O2 , 5% CO2 ) at 32-34 °C (pH 7.4) (preparation for the electrophysiological analysis). Other slices were immediately fixed, after sampling, with 2.5% glutaraldehyde in phosphate-buffered saline preparation (PBS) (pH 7.4), and kept at 4 °C for at least 48 hours (preparation for the ultra-structural analysis by electron microscopy).

Extracellular recordings of the population spikes (PSs) were made in the stratum pyramidale of the CA1 subfield, with glass microelectrodes filled with 2 M NaCl (resistance 5-10 MΩ). Orthodromic stimuli (10-500 mA, 20-90 ms, 0.1 Hz) were delivered through a platinum electrode placed in the stratum radiatum (Schaffer collaterals). The test stimulus intensity of 50-ms square pulses was adjusted to give a PS of 2-4 mV at 0.03 Hz. The PS amplitude, measured every minute, corresponds to an average of 6 recordings/min. After recording stable signals (20-30 min), a tetanic stimulation (100 Hz, 1 s) was delivered to induce the long term potentiation (LTP) at the same stimulus intensity used for the baseline responses. Signals were acquired, digitized, and stored using a personal computer with standard acquisition software (Axon, Foster City, CA, USA.). Signal was fed to a computer interface (Digidata 1440A, Axon Instruments, Foster City, CA) for subsequent analysis with the software PCLAMP10 (Axon Instruments).
Transmission electron microscopy

One mm³ of muscle tissue from surgical specimens were fixed in 4% paraformaldehyde and post-fixed in 2% osmium tetroxide [24]. After washing with 0.1 M phosphate buffer, the sample was dehydrated by a series of incubations in 30%, 50% and 70% ethanol. Dehydration was continued by incubation steps in 95% ethanol, absolute ethanol and propylene oxide, after which samples were embedded in Epon (Agar Scientific, Stansted, Essex CM24 8GF United Kingdom) [25]. Ultra-thin sections of 80 µm were mounted on copper grids and examined with a transmission electron microscope (Model JEM1400, JEOL).

Statistical analysis

For electrophysiological experiments, data are expressed as mean measurements ± SEM and n represents the number of slices analyzed. Data were compared with ANOVA and Tukey’s Multiple Comparison Test, and were considered significantly different if p<0.05. Excel 5.0 software was used for generation of graphs. For transmission electron microscopy, data expressed as mean ± SEM, were statistically analysed using the Student’s t test and Mann-Whitney test.

RESULTS

LTP analysis

Impairment of synaptic plasticity in CA1 hippocampal region of sedentary NPC1+/- mice. In a previous paper, we demonstrated that LTP recorded in the CA1 region of hippocampal slices from NPC1+/- mice was decreased respect to slices from WT mice [8]. In the present study, we analyzed whether in NPC1+/- mice an impairment of synaptic plasticity might occur. We elicited long term potentiation (LTP) at Schaffer collateral/commissural fiber-CA1 synapses in hippocampal slices by applying a train of high-frequency stimulation (HFS) (100 Hz for 1 s). In the NPC1+/- mouse slices an inhibition of the expression of both PTP and LTP was observed, while the LTP maintenance phase was not affected. In Figure 1 the trend of the potentiation both in the WT (n = 5) and in NPC1+/- (n = 6) mouse slices was described. The PS values at various times after tetanic stimulation were reported in Table 1, which indicates a significant statistical difference between the WT and NPC1+/- sedentary groups in the first 15 min. In fact, PTP and LTP values at 1, 5, 10 and 15 min after tetanic stimulation were, respectively, 359.5 ± 35.5 vs 212.9 ± 27.6 (**p<0.01), 330.1 ± 21.8 vs 187.1 ± 27.6 (**p<0.01), 298.5 ± 29.1 vs 193.9 ± 29.7 (*p<0.05), and 304.9 ± 39.5 vs 191.6 ± 30.3 (*p<0.05).

Effect of CU training protocol on synaptic plasticity in NPC1+/- and NPC1-/- mice. We demonstrated previously [21] that short term aerobic exercise exerts positive effects on synaptic plasticity in WT adult mice depending on the training modalities. These results prompt us to evaluate whether in the mouse genetic model of NPC1 disease, aerobic exercise might counteract synaptic deficits. First of all, we analyzed the slices from NPC1+/- trained mice and we observed that the CU protocol applied for three weeks was not able to improve synaptic plasticity in respect to the sedentary group, since an impairment of LTP induction phase was still present (Figure 1, n = 5). On the contrary, it was observed a robust training effect in the NPC1-/- trained mice slices, since the suppression of LTP, that was present in the sedentary group (n = 7) starting from the 10th min after tetanic stimulation, was completely counteracted (Figure 2, n = 4). Moreover, the increase in PS% observed in NPC1-/- trained mice slices was similar to the values recorded in the NPC1+/- sedentary mice slices (Figure 2). Regarding the WT trained mice no difference were appreciated respect to the sedentary group (data not shown).

Table 1

Percentage of PS amplitude values recorded in the CA1 region of hippocampal slices from WT, NPC1+/- and NPC1-/- mice at different times: Significance is reported between WT sedentary group, NPC1+/- sedentary and trained groups; between NPC1+/- sedentary, NPC1+/- trained and NPC1+/- sedentary groups. Note that a significant statistical difference is reported between WT and NPC1+/- sedentary and trained groups at 1, 5, 10 and 15 min (**p<0.01); between NPC1-/- sedentary and both NPC1-/- trained and NPC1+/- sedentary groups a significant statistical difference is also observed at 30, 40, 50 and 60 min (*p<0.05)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>WT Sedentary control (% PS amplitude)</th>
<th>NPC1 +/- Sedentary control (% PS amplitude)</th>
<th>NPC1 +/- Trained (% PS amplitude)</th>
<th>NPC1 -/- Sedentary control (% PS amplitude)</th>
<th>NPC1 -/- Trained (% PS amplitude)</th>
<th>Significance (WT sedentary vs NPC1+/- sedentary vs NPC1-/- trained)</th>
<th>Significance (NPC1 +/- sedentary vs NPC1 +/- trained vs NPC1+/- sedentary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>359.5 ± 35.5</td>
<td>212.9 ± 27.6</td>
<td>213.6 ± 33.6</td>
<td>221.8 ± 42.6</td>
<td>192.6 ± 66.6</td>
<td>0.01 (**)</td>
<td>0.89</td>
</tr>
<tr>
<td>5</td>
<td>330.1 ± 21.8</td>
<td>187.1 ± 27.6</td>
<td>210.8 ± 32.8</td>
<td>197.9 ± 35.9</td>
<td>193.0 ± 68.1</td>
<td>0.01 (**)</td>
<td>0.98</td>
</tr>
<tr>
<td>10</td>
<td>298.5 ± 29.1</td>
<td>193.9 ± 29.7</td>
<td>179.7 ± 20.8</td>
<td>137.4 ± 11.0</td>
<td>187.5 ± 49.2</td>
<td>0.02 (**)</td>
<td>0.23</td>
</tr>
<tr>
<td>15</td>
<td>304.9 ± 39.5</td>
<td>191.6 ± 30.3</td>
<td>193.9 ± 21.5</td>
<td>125.9 ± 13.9</td>
<td>177.8 ± 50.1</td>
<td>0.04 (**)</td>
<td>0.19</td>
</tr>
<tr>
<td>20</td>
<td>294.5 ± 43.7</td>
<td>182.2 ± 36.3</td>
<td>176.8 ± 13.9</td>
<td>117.8 ± 14.6</td>
<td>188.4 ± 49.8</td>
<td>0.06</td>
<td>0.19</td>
</tr>
<tr>
<td>30</td>
<td>245.2 ± 31.6</td>
<td>199.5 ± 25.7</td>
<td>161.5 ± 9.6</td>
<td>104.6 ± 19.4</td>
<td>191.2 ± 50.2</td>
<td>0.10</td>
<td>0.04 (*)</td>
</tr>
<tr>
<td>40</td>
<td>239.2 ± 36.8</td>
<td>200.4 ± 28.6</td>
<td>166.8 ± 10.2</td>
<td>101.8 ± 20.7</td>
<td>194.1 ± 35.4</td>
<td>0.24</td>
<td>0.02 (*)</td>
</tr>
<tr>
<td>50</td>
<td>235.3 ± 41.6</td>
<td>195.1 ± 21.3</td>
<td>156.1 ± 7.2</td>
<td>101.9 ± 21.3</td>
<td>181.8 ± 33.5</td>
<td>0.17</td>
<td>0.02 (*)</td>
</tr>
<tr>
<td>60</td>
<td>217.4 ± 41.3</td>
<td>206.5 ± 30.5</td>
<td>152.9 ± 9.7</td>
<td>103.4 ± 20.9</td>
<td>211.5 ± 46.5</td>
<td>0.35</td>
<td>0.03 (*)</td>
</tr>
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</table>

WT: Wild Type control; NPC1: Niemann Pick type C-1; % PS: percentage of population spikes amplitude.
The PS values recorded for each group of mice at various times after tetanic stimulation was reported in Table 1, where the values of significance was also showed: a significant statistical difference between the NPC1-/- sedentary and both NPC1+/+ trained and NPC1+/- sedentary groups at 30, 40, 50, 60 min was indicated. In fact, PTP and LTP values at 30, 40, 50 and 60 min after tetanic stimulation were, respectively, 104.6 ± 19.4 vs 191.2 ± 50.2 vs 199.5 ± 25.7 (*p<0.05), 101.8 ± 20.7 vs 194.1 ± 35.4 vs 200.4 ± 28.6 (*p<0.05), 101.9 ± 21.3 vs 181.8 ± 33.5 vs 195.1 ± 21.3 (*p<0.05), and 103.4 ± 20.9 vs 211.5 ± 46.5 vs 206.5 ± 30.5 (*p<0.05).

**Electron microscopy analysis**

Ultrastructural analysis of the hippocampus of WT, NPC1+/+ and NPC1-/- mice. To evaluate whether there were significant differences, from an ultrastructural point of view, between sedentary and trained mice, we performed an electron microscopy analysis, which is shown in Figure 3A. Ultrastructural analysis of the hippocampus of WT sedentary control mouse (a) shows a normal tissue organization with well-preserved neuronal and glial cells; nerve processes are well represented, rich in neurotubules and neurofilaments, and there is a slight vacuolization at the axonal level. WT trained mouse

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**Figure 1**

*Synaptic plasticity in CA1 hippocampal subfield of NPC1+/+ mice.* The % PS amplitude after tetanic stimulation (HFS) as a function of time is shown in WT sedentary control (grey bar, n = 5), in NPC1+/- sedentary control (red bar, n = 6), and in NPC1+/- trained (green bar, n = 5) mice slices at min 1, 5, 10, 15, 20, 30, 40, 50, 60 (*p<0.01, *p<0.05). Bars in the plot are means ± SEM of values obtained from different slices. The insert shows recordings obtained from slices of WT sedentary control (a), NPC1+/- sedentary control (b), and NPC1+/- trained (c) mice. The first curve of each group refers to the basal synaptic transmission (BST) and it was recorded before the application of the HFS, while the other curves refer to population spikes at times 1, 20 and 60 min after the HFS.

**Figure 2**

*Synaptic plasticity in CA1 hippocampal subfield of NPC1-/- mice.* The % PS amplitude after tetanic stimulation (HFS) as a function of time is shown in NPC1-/- sedentary control (pink bar, n = 7), in NPC1-/- trained (blue bar, n = 4), and in NPC1+/- sedentary control (red bar, n = 6) mice slices at min 1, 5, 10, 15, 20, 30, 40, 50, 60 (*p<0.05). Bars in the plot are means ± SEM of values obtained from different slices. The insert shows recordings obtained from slices of NPC1-/- sedentary control (a), NPC1-/- trained (b), and NPC1+/- sedentary control (c) mice. The first curve of each group refers to the BST and it was recorded before the application of the HFS, while the other curves refer to population spikes at times 1, 20 and 60 min after the HFS.
did not display any modification (data not shown). A recovery was observed in the hippocampus of NPC1+/- trained mice (c) as compared with NPC1+/- sedentary mice (b); specifically, we noted an higher number of axons structure in trained mouse (c). In NPC1-/- sedentary mice (d), we observed a significant vacuolation caused by axonal swelling with depletion of neurotubules and neurofilaments; synapses are well represented and morphologically well preserved. Trained NPC1-/- mice (e) register a significant decrease in axonal vacuolization and a better tissue organization respect to sedentary ones; specifically, both the number and size of axons structure is comparable with that observed in hippocampus of NPC1+/- sedentary mice (b).

Transmission electron microscopy analysis allows us to evaluate the number of axons for area in hippocampus of both NPC1+/- and NPC1-/- sedentary and trained mice (Figure 3B). In NPC1+/- sedentary mice, we observed a significant reduction of the axons density as compared with WT mice (4.16 ± 0.99 vs 8.11 ± 0.33, ***p<0.0001). Although axons density increased with training (6.52 ± 0.26, *p<0.05), the values were

![Figure 3](image_url)

**Figure 3**

Electron microscopy analysis of the hippocampus and axons evaluation. **A** shows the ultrastructural analysis of the hippocampus of WT, NPC1+/- and NPC1-/- mice. (a) Representative image of hippocampus area of a WT sedentary mouse (scale base represents 5 µm); numerous well conserved axons are shown (asterisk). (b) Hippocampus area of a NPC1+/- sedentary mouse displays a reduction of axons (asterisk; scale base represents 5 µm). (c) Representative image of hippocampus area of a NPC1+/- trained mouse showed several well conserved axons (asterisk; scale base represents 5 µm). (d) Image shows a degeneration of axons (asterisk) in a hippocampus area of a NPC1-/- sedentary mouse (scale base represents 5 µm). (e) Image displays some well conserved hippocampus axons (asterisk) in NPC1-/- trained mouse (scale base represents 5 µm). **B** shows the number of axons for area in hippocampus of WT, NPC1+/- and NPC1-/- mice. In NPC1+/- sedentary mice, a significant reduction of the axons density was observed respect to WT mice (***p<0.0001); axons density increased with training (*p<0.05), but the values were lower respect to the WT control (***p<0.0001). As concerns NPC1-/- mice, significant differences in the density of axons were observed both in sedentary and trained mice regarding to the WT control (***p<0.0001), even if training exerted a positive effect (***p<0.01).
lower respect to the WT control (**p<0.0001). As concerns NPC1-/- mice, a significant reduction of the axons density was observed both in sedentary and trained mice respect to the WT control (1.89 ± 0.15, 3.55 ± 0.56 vs 8.11 ± 0.33, ***p<0.0001), even if training exerted a positive effect (**p<0.01).

Ultrastructural analysis of the muscle tissues of WT, NPC1+/- and NPC1-/- mice. The adaptation of muscular plasticity after exposure to Continuous Uniform protocol was assessed through an ultrastructural analysis of muscle tissue of each experimental group, as shown in Figure 4A. Muscle tissue of WT sedentary control mice is characterized by normal sarcomeric network with well-preserved mitochondria (a). WT trained mice did not present any change (data not shown). In muscle tissue of NPC1+/- sedentary mice, we frequently observed slight degeneration areas (b), that were almost completely recovered in muscle tissues of NPC1+/- trained mice (c). The ultrastructural study of muscle tissue of NPC1-/- sedentary mice showed both a complete degeneration of sarcomeric network and the presence of several areas of vacuolization (d). The training protocol in NPC1-/- mice induced a reduction of degeneration area; in addition, we observed several

Figure 4
Electron microscopy analysis of the muscle tissues and atrophic fiber evaluation. A shows the ultrastructural analysis of the muscle tissue of WT, NPC1+/- and NPC1-/- mice. (a) Image shows normal sarcomeric network of a WT sedentary control mouse (scale base represents 50 µm). (b) Muscle tissue of a NPC1+/- sedentary mouse characterized by slight areas of degeneration (arrow; scale base represents 50 µm). (c) Muscle tissue of a NPC1+/- trained mouse shows normal sarcomeric structure (scale base represents 50 µm). (d) Image displays degenerated sarcomeric network of a NPC1-/- sedentary mouse (scale base represents 20 µm). (e) Muscle tissue of a NPC1-/- trained mouse characterized by altered sarcomeric structure, degenerated area (asterisk) and dark mitochondria (arrows; scale base represents 20 µm). B shows the percentage of atrophic fibers in WT, NPC1+/- and NPC1-/- mice. A significant increase of the percentage of atrophic fibers in NPC1-/- mice as compared to both NPC1+/- and WT mice was observed (**p<0.0001). Atrophic fibres both in NPC1+/- that NPC1+/- mice was reduced after training (**p<0.01), without reaching the values observed in WT sedentary control.
dark mitochondria whose appearance is probably linked to training (e).

We have also performed an atrophic fibers’ evaluation in sedentary and trained mice, as illustrated in Figure 4B. Morphometrical analysis of muscle fiber showed a significant increase of the percentage of atrophic fibers in NPC1-/- mice as compared to both NPC1+/+ - and WT mice; corresponding values were, respectively, 74.14 ± 2.25, 26.99 ± 1.56 and 8.12 ± 0.78 (***p<0.0001). The training protocol induced a slight reduction in atrophic fibres both in NPC1-/- that NPC1+/+ - mice; corresponding values were, respectively, 71.12 ± 3.52 and 22.01 ± 2.52 (**p<0.01).

DISCUSSION

In the present paper, we have evaluated the effect of a short term, aerobic uniform exercise training on synaptic and muscle plasticity in three different mice groups: WT controls, NPC1+/+ - and NPC1-/- animals. To this aim mouse hippocampal slices and anterior leg muscles were analyzed by employing extracellular recordings and electron microscopy analysis.

Synaptic changes, that underlie cognitive processes, depend on physiological mechanisms including LTP [26], a phenomenon especially represented in the hippocampus, an area which is critical for memory and learning [27, 28]. Recently, it was demonstrated both a dramatic reduction in the number of neurons and synapses in CA2 and CA3 hippocampal regions and a discontinuity of the connection pathways between the hippocampus and other regions of the central nervous system in NPC1-/- mice [29]. These observations are in agreement with our previous published data, that demonstrated an impairment of the LTP in NPC1-/- mice [8].

Since it has been reported that exercise mediates beneficial effects on brain functions [30-32] and that enhancement in synaptic plasticity depends on the training protocol proposed [21], in the present paper we tested if the aerobic uniform exercise protocol could influence synaptic plasticity and exert a positive effect on the LTP phenomena in the NPC1-/- mice. We included in the study WT as controls and NPC1+/+ - mice to evaluate if in the latter group the LTP was reduced too.

In vitro study the results indicate that synaptic plasticity was affected also in NPC1-/- untrained mice and that the aerobic uniform exercise protocol was able to rescue long term potentiation in NPC1+/+ - trained, but it was ineffective in NPC1+/+- trained mice slices; perhaps this type of protocol was too weak and not demanding for NPC1+/+ - mice.

Previous paper reported in NPCD patients a condition of dystonic and myoclonic movements, without any muscular morphologic study [33, 34]. Hence we performed an ultrastructural analysis of the mice anterior leg muscle to verify a degeneration of muscle organization, and to detect if significant differences could be appreciated in trained vs untrained mice. Moreover electron microscopy analysis was performed on the hippocampus. WT sedentary control mice show a normal muscle organization with well-preserved mitochondria, nerve processes well represented and a well-preserved neuronal and glial cells. A recovery was observed both in muscle and hippocampus of NPC1+/+ - trained mice as compared with NPC1+/+ - sedentary control: a reduction in atrophic fibres in trained mouse was observed as well as an higher number of axons structure. Then the protocol applied was able to induce morphological improvements probably not yet sufficient to produce functional changes like an LTP enhancement. Regarding NPC1+/+ - mice instead, the training protocol induced: i) a reduction of degeneration area in the muscle tissue; ii) a decrease of the percentage of the atrophic fibres in the hippocampus, that resemble a picture similar to that observed in the NPC1+/+ - sedentary mice, and iii) an increase of the number of axons.

CONCLUSION

The short term uniform protocol proposed in this study allows us to highlight the crucial role played by physical exercise as part of the therapeutic treatment. In fact, the physical exercise, even though it is not being able to reverse the disease itself, enhances LTP in the experimental murine model. Therefore, it could have a beneficial effect in improving quality of life of patients with Niemann Pick C disease, in addition to the current drug therapy that is currently only a symptomatic one.

Conflicts of interest statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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