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Electric-field induced sleep promotion and lifespan extension in Gaucher's disease model flies

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ABSTRACT

Gaucher's disease (GD) is a genetic disease characterized by a mutation in the metabolic enzyme glucocerebrosidase (GBA1), leading to the accumulation of glucosylceramide in tissues. We previously discovered that a *minos*-inserted mutation in the *GBA1* gene of fruit flies, *Drosophila melanogaster*, mimics human neuronopathic GD (nGD) characteristics, providing a promising model for studying the molecular mechanisms of the disease. We also reported that extremely low-frequency electric fields (ELF-EFs) promote sleep and extend the lifespan of wild-type flies.

In this study, we show that ELF-EFs have health-promoting effects on nGD model flies.

Firstly, the total sleep time and sleep episode duration of EF-exposed nGD model flies increased. EFs also extended the lifespans of nGD model flies. Additionally, the expression of the endoplasmic reticulum stress-related gene *PERK* and autophagy-related gene *p62* were elevated after EF exposure. The effects of EF exposure on nGD flies are associated with the change of these genes expression. Our findings suggest that EF exposure may be effective as an additional therapy for nGD.

1. Introduction

Gaucher's disease (GD) is caused by mutations in the glucocerebrosidase gene (*GBA1*), decreased enzymatic activity of glucocerebrosidase (GBA1), and accumulation of glucosylceramide as its substrate in the phagocyte system primarily in the spleen, liver, bone marrow, brain, and osteoclasts, leading to various symptoms in the bone, blood, internal organs, and nervous system. GD is classified into three subtypes: types I, II, and III. Type I GD is most common and known for non-neuronopathic symptoms such as osteopenia, anemia, and hepatosplenomegaly. Type II and III GD are acute and subacute neuronopathic forms, respectively, with symptoms such as seizures and convulsions. Type II GD has severer pathology than type III, for it is infantile or perinatal lethal [1,2,3,4]. Mutations in *GBA1* are known to increase the risk of Parkinson's disease (PD), suggesting a common molecular mechanism between GD and PD [5,6].

Because of abundant genetic tools and easy housing, pathological animal models of human neuronopathic GD (nGD) symptoms have been established in *GBA1* mutant fruit flies [7–9]. *GBA1* knockout mutants showed pathological phenotypes, such as neurodegeneration and reduced climbing ability, accompanied by autophagy disruption characterized by p62 protein accumulation [8,10]. These mutants had

Abbreviations: ELF, extremely low frequency; EF, electric field; GD, Gaucher's disease; PD, Parkinson's disease; GBA, glucocerebrosidase; ER, endoplasmic reticulum; UPR, unfolded protein response; XBP1, X-box binding protein 1; BiP, binding immunoglobulin protein; LD, light/dark; PERK, protein kinase R-like ER kinase; Ire1, inositol-requiring enzyme 1; Pink1, PTEN-induced kinase 1; cathD, cathepsin D; RpL32, ribosomal protein L32; Keap1, Kelch-like ECH-associated protein 1; Nrf2, NF-E2-related factor 2.

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shorter lifespans than the genetic background strains, which were rescued by rapamycin administration [8,10]. Lack of GBA1 also led to impairment in flies' cell growth, which manifested as reduced cell dimensions in the wings and fat body tissues [11]. Besides endogenous GBA1 mutants, flies expressing the human N370S or L444P mutant GBA1s also exhibit nGD phenotype accompanying endoplasmic reticulum (ER) stress and unfolded protein response (UPR) [12,13,14]. These phenotypes were, at least partially, rescued by molecular chaperons [12,14].

We previously reported that the expression of the human mutated glucocerebrosidase gene (*hGBA1*), which is associated with neuronopathy in patients with nGD, causes neurodevelopmental defects in *Drosophila* eyes. We showed that ER stress is elevated in *Drosophila* eyes carrying mutated *hGBA1* by using ER stress markers *dXBP1* and *dBiP*. We also found that ambroxol, a potential pharmacological chaperone for mutated hGBA1 protein, alleviated the neuronopathic phenotype by reducing ER stress.

In our previous study, we identified a *minos*-insertion mutant with a homologous GD gene (*CG31414*), which accumulated hydroxyglucosylceramide throughout the body of *Drosophila melanogaster*. Simultaneously, several genes were upregulated, namely the autophagy-related gene *p62*, ER-related gene *CG14715*, PD-related gene *Pink1*, and bone morphogenetic protein (BMP) signaling-related gene *tok* [7]. The *minos*-insertion mutant displayed abnormal phenotypes, including impaired climbing ability, disrupted sleep patterns, and a shortened lifespan. These abnormal phenotypes are similar to those observed in human nGD [7,15]. These nGD models exhibit autophagic changes, elevated ER stress, UPR, and inflammation, which represent cellular stress underlying nGD symptoms [7–9,15].

Compared to surgical or pharmaceutical approaches [2,16], physical therapy for GD is not well-documented. Recently, an extremely low-frequency electric field (ELF-EF) was reported to promote sleep and extend the lifespan of wild-type flies [17], suggesting that a physical approach may be an alternative to medication. In this study, *minos*-insertion mutant nGD model flies were exposed to ELF-EFs, and sleep parameters, longevity, and related gene expressions were evaluated to test their health-promoting effects.

2. Materials and methods

2.1. Study design

nGD flies were compared with normal control flies, each assigned to either the EF or sham group. The EF group received EF exposure, whereas the sham group did not. Male flies were used to reduce the variation caused by mating and egg-laying.

Sleep and longevity were monitored to test the acute and chronic effects of EF exposure on nGD. Gene expressions were evaluated to explore the molecular mechanisms underlying the effects of EF exposure.

2.2. Fly strains

The fruit fly *Drosophila melanogaster* was used in the experiments. Male flies with a homozygous *CG31414* gene mutation, established as a *GBA1* mutant, served as the nGD model [7]. Male w^{1118} flies, the same genetic background line of the nGD mutant, were used as a normal control. All flies were reared on standard cornmeal food in a temperature-controlled incubator at 25 °C under 12:12 h light/dark (LD) cycles. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Research Council.

2.3. EF exposure system

We used the same exposure system as used in a previous study [17]. Briefly, stainless steel parallel plate electrodes (upper electrode: 290 mm × 370 mm; lower electrode: 300 mm × 385 mm) were pasted on polyvinyl chloride plates. One electrode was applied with a 50 Hz alternating current high voltage and the other was grounded (0 V). Four Teflon pillars, each with a radius of 10 mm, were used as spacers between the parallel electrodes. A high-voltage device (Healthtron HEF-P3500; Hakuju Institute for Health Science, Tokyo, Japan) was used for voltage application [17]. EF, defined as the electric voltage per unit length (V/m), depends on the voltage and distance between the electrodes. In this experiment, an output voltage of 3.5 kV and a pillar length of 100 mm were employed to generate an EF of 35 kV/m. An identical pair of parallel electrodes was set for sham exposure, where both electrodes were connected and grounded to ensure a zero EF.

2.4. Sleep monitoring

The *Drosophila* Activity Monitoring (DAM) System (TriKinetics, Waltham, MA, USA) was used for sleep monitoring [7,17]. Activity count was recorded in 1-min bins, and an inactive period ≥5 min was defined as sleep.

Four-to- 8-day-old flies were collected and anesthetized with ether. 31–43 flies were used per group. Each fly was placed in a glass tube measuring 2 mm in diameter and 50 mm in length, with one end filled with 5 % sucrose and 2 % agar medium (Fig. 1; Supplementary Fig. 2). After resting overnight in an incubator, the glass tubes containing flies were placed between the electrodes on a desktop setup, where they were exposed to EFs for 9 h during daytime (Figs. 1 and 2a; Supplementary Fig. 2). The tubes were collected immediately after EF exposure and transferred to the DAM system. Subsequent nighttime sleep was monitored for 12 h (Fig. 2a; Supplementary Fig. 2) in the incubator. The parameters evaluated included sleep bout number, total sleep, and sleep episode duration.

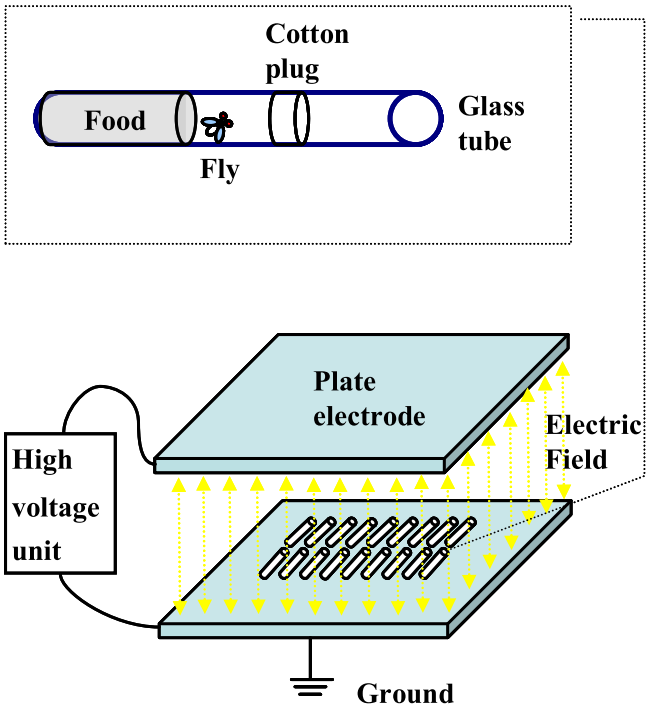

Fig. 1. Electric field (EF) exposure system. EFs were generated by applying a high voltage (3.5 kV, 50 Hz) between two parallel-plate electrodes (upper electrode: 290 mm × 370 mm; lower electrode: 300 mm × 385 mm) separated by a distance of 100 mm.

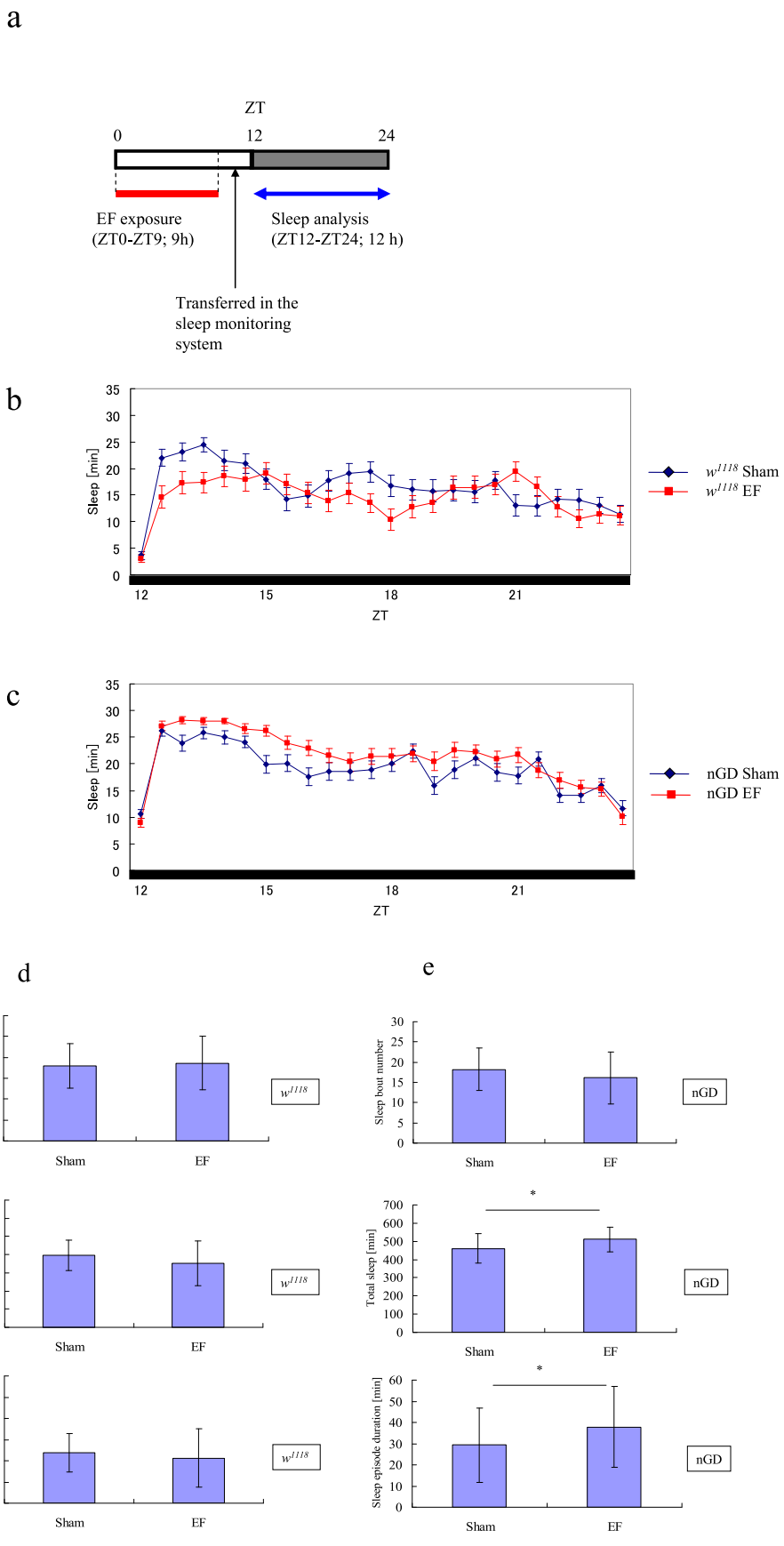

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Fig. 2. Sleep measurement schedule and effects of EF on sleep parameters in normal controls (w^{1118}) and nGD flies. (a) Flies were collected and placed in tubes during daytime on the day before exposure. EF exposure was conducted for 9 h; from ZT 0 to ZT 9 during the daytime. The tubes were then transferred to a sleep-monitoring device to measure the subsequent nighttime sleep. The time course of sleep time during night is shown in (b) and (c). The overall sleep amount was elevated in the EF-exposed nGD group (c). In w^{1118} flies (d), sleep bout number (top), total sleep (middle), and sleep episode duration (bottom) during nighttime were not changed by EF exposure. In nGD flies (e), total sleep time and sleep episode duration significantly increased in the EF group. Statistical data are expressed as the mean ± standard deviation (S.D.). *$p < 0.05$ using Student's *t*-test. N = 31 flies each for w^{1118}; n = 42–43 flies each for nGD.

2.5. Lifespan assay

Vials (φ22 × φ25 × H96 mm) containing a low-nutrition medium with 5 % glucose and 1 % agar were prepared. Newly eclosed male flies were collected under ether anesthesia within 48 h of eclosion and transferred into these vials for lifespan assays [7,17]. Fly longevity was assessed under continuous EF/sham exposure conditions in a temperature-controlled room at 25 °C under 12:12 h LD cycles. The medium was changed at least twice a week. Each vial contained 20 flies, and 79–80 flies were used per group.

2.6. qRT-PCR

Zero-to- 2-day-old flies treated with the same procedure as in the lifespan assay were collected after 7 days of EF/sham exposure (Fig. 4a) and homogenized in RNAiso reagent (Takara Bio, Shiga, Japan). Ten to twenty flies were homogenized per tube, and four to eight biological replicates were prepared for each group. RNA was extracted and stored under −20 °C. qRT-PCR was performed as previously described [7] and the data were analyzed by the delta delta Ct method. The genes evaluated included an autophagy-related gene *p62*; ER-related genes *BiP*, *Ire1*, *PERK*, *CG14715*; a PD-related gene *Pink1*; a lysosome-related gene *cathD* (cathepsin D); and a bone morphogenetic protein (BMP) signaling-related gene *tok*. Ribosomal protein L32 (*RpL32)* gene was used as a reference gene. The primer sequences used are listed in Table 1.

2.7. Statistical analysis

The significance of differences in sleep parameters between the sham and EF groups was estimated using Student's *t*-test. Significant differences in qRT-PCR data between the normal control and nGD groups or between the sham and EF groups were also estimated using Student's *t*-test. The longevity of the sham and EF groups was evaluated using the log-rank test. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using Excel (Microsoft, WA, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

3. Results

3.1. Changes in the sleep status following EF exposure

First of all, the EF exposure system was constructed to measure sleep of *Drosophila* (Fig. 1). The changes of sleep waveform after sham/EF exposure are shown in Fig. 2b and 2c. The sleep time after EF exposure was elevated in the EF-exposed nGD group (Fig. 2c). Total sleep time in the EF group significantly increased in nGD flies after EF exposure ($p = 0.0026$) (Fig. 2e). The mean value of total sleep in nGD flies was 460 min in the sham group and 511 min in the EF group. Sleep episode duration, a parameter defined as total sleep divided by sleep bout number, was significantly increased in nGD flies after EF exposure ($p = 0.034$), suggesting less fragmented sleep (Fig. 2e). The number of sleep bouts tended to decrease but did not significantly change in nGD flies after EF exposure ($p = 0.104$) (Fig. 2e). In contrast, these sleep parameters did not change significantly in the control w^{1118} flies (Fig. 2d).

3.2. Effects of EF exposure on the lifespan

To show effects of EF exposure to lifespan of w^{1118} and nGD flies, we exposed EF from day 0 to the end (Fig. 3).

The median survival time of nGD flies was approximately half that of the normal flies, suggesting severe effect of *GBA1* mutation to lifespan (Fig. 3a and 3b). The lifespans of normal flies were not significantly altered by EF exposure ($p = 0.548$) (Fig. 3a). In contrast, the lifespan of the EF-exposed nGD flies was significantly longer than that in the sham group ($p = 0.029$) (Fig. 3b). Thus, EF exposure prolonged the lifespan of the nGD flies. Furthermore, the survival curves of the nGD flies did not change during the first half of their lifetime, but a life-extension effect was observed 10–20 days after the initiation of EF exposure.

3.3. Changes in gene expression in nGD flies

To determine changes in gene expression in nGD flies compared with w^{1118} control, we carried out qRT-PCR analysis. Upregulation of the autophagy marker *p62* was observed in nGD flies compared to normal flies ($p = 0.0059$) (Supplementary Fig. 3a). The expression of other genes, such as the ER stress marker *PERK*, was not significantly altered

Table 1
Primer sequences used for qRT-PCR.

Name		Sequence	Exon No.	Accession No.
RpL32	Forward	5'-AGATCGTGAAGAAGCGCACCAAG-3'	E2	KJ746563
	Reverse	5'-CACCAGGAACTTCTTGAATCCGG-3'	E3	
p62	Forward	5'-TTACCCAAATGCGACGTAAG-3'	E1	AAF53824
	Reverse	5'-GTAGCGAACCCAATCAAG-3'	E2	
PERK	Forward	5'-GAAAACCCGTAATCAGTGGAC-3'	E2	AAF61200
	Reverse	5'-TGGATGCTGGACGAGATTAG-3'	E4	
Pink1	Forward	5'-TTGCGCAGCTATTGTAAACG-3'	E4	AAN09178
	Reverse	5'-AAGATTCCACTGCTGCTGGT-3'	E5	
Ire1	Forward	5'-AGCGAAATACGCTGGACAAT-3'	E2	ABW08704
	Reverse	5'-ACCAGTTGGGGAATGGTGTA-3'	E3	
BiP	Forward	5'-GCTATTGCCTACGGTCTGGA-3'	E5	AHN59609
	Reverse	5'-CATCACACGCTGATCGAAGT-3'	E5	
tok	Forward	5'-CATACCGCAGCAAATACACC-3'	E3	AY881278
	Reverse	5'-CAATGTCGCCCATGAAACC-3'	E4	
CG14715	Forward	5'-CATTAAGAAGCGGGTGGAGA-3'	E1	AAF54674
	Reverse	5'-GCTGCTCACCCTCACACATA-3'	E2	
cathD	Forward	5'-GGAAGAGGCCACCTCTATTAAC-3'	E1	AF220040
	Reverse	5'-ATGAGATCGCAAGAAACCAC-3'	E2	

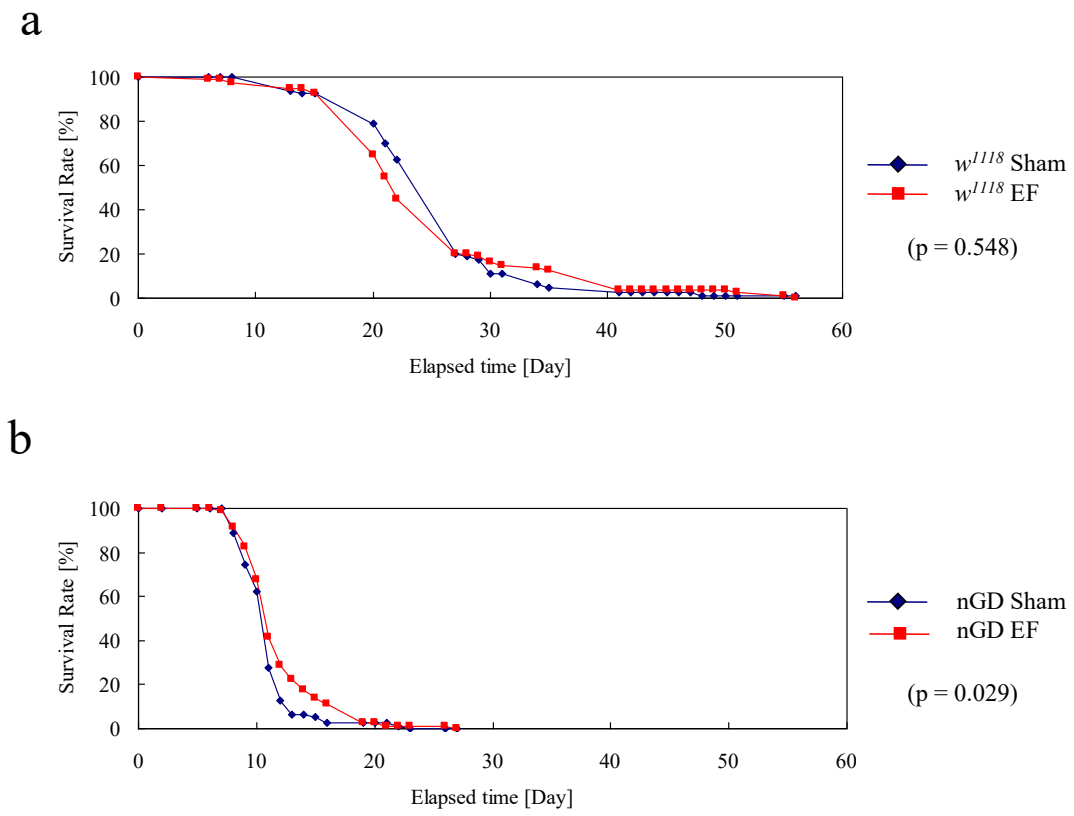

Fig. 3. Effects of the EF exposure on longevity. (a) The lifespan of w^{1118} flies (n = 80) did not change after exposure to EF ($p = 0.548$). (b) The lifespan of nGD flies (n = 79–80) was half that of normal flies (n = 80). EFs significantly extended the lifespan of nGD flies ($p = 0.029$). Statistical analyses were performed using log-rank tests.

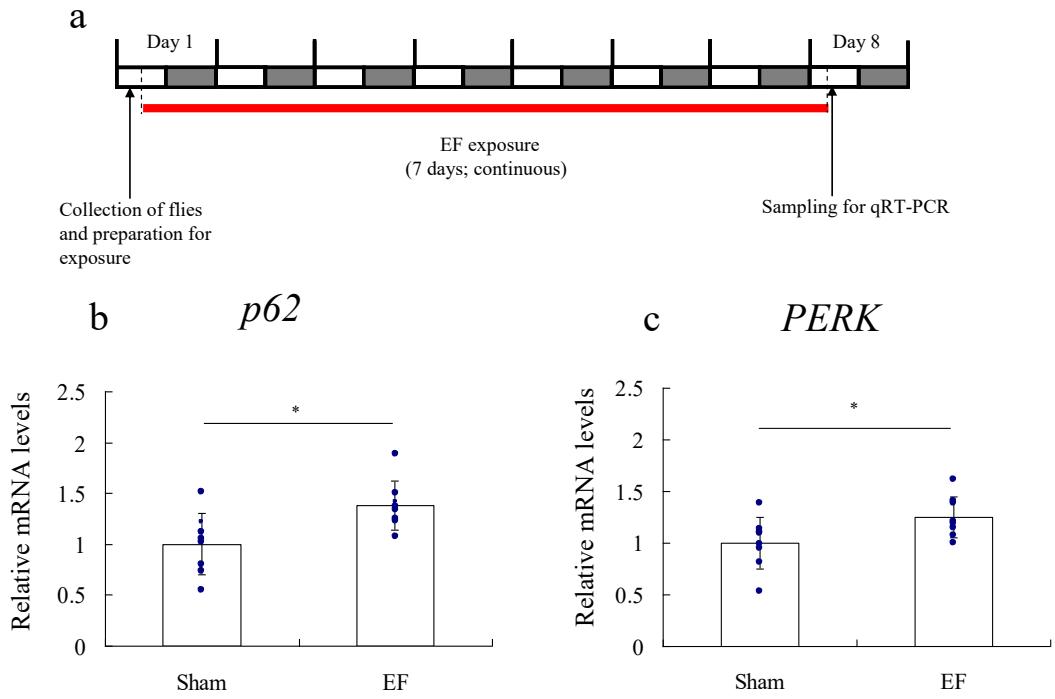

Fig. 4. Altered gene expression by the EF exposure in nGD flies. (a) EF exposure was conducted continuously for 7 days. The EF exposure significantly elevated the expression of *p62* (b) and *PERK* (c). Statistical data are expressed as the mean ± S.D. Statistical analysis was conducted using Student's *t*-test. *$p < 0.05$. N = 8 biological replicates per group. Gene expression of the other tested genes shown in Supplementary Fig. 1.

($p = 0.358$) (Supplementary Fig. 3b).

3.4. Changes in gene expression after 7-day EF exposure

To find out changes in gene expression after 7-day EF exposure, we carried out qRT-PCR analysis. Interestingly, *p62* and *PERK* were significantly upregulated in nGD flies after 7 days of EF exposure ($p = 0.015$ and $p = 0.047$, Fig. 4b and 4c, respectively). In contrast, the expression of other genes, such as *BiP*, *Ire1*, *CG14715*, *Pink1*, *cathD*, and *tok* did not differ significantly between the EF and sham groups (Supplementary Fig. 1).

4. Discussion

This study showed that in nGD model flies, EFs affected sleep improvement, lifespan extension, and caused upregulation of *p62* and *PERK* levels. The upregulation of *p62* in nGD compared to normal flies was consistent with previous findings [7]. Although we do not know the molecular mechanism for the upregulation of *p62*, one explanation is that the increased *p62* level may reflect autophagy [8,10] triggered by EF exposure. Previous study suggested that upregulation of *p62* prolongs lifespan by improving mitochondrial function and mitophagy in flies [18]. The relationship between *p62* and sleep remains controversial, as sleep deprivation has been reported to either increase [19] or decrease [20,21] p62 levels in some tissues.

PERK activates the *p62*-mediated non-canonical Kelch-like ECH-associated protein 1 (*Keap1*)–nuclear factor erythroid 2-related factor 2 (*Nrf2*) pathway and plays a protective role against lipotoxic stress [22]. Therefore, the co-upregulation of *p62* and *PERK* observed in the present study may represent a protective response against ER stress in nGD flies. Furthermore, *PERK* activation by ER stress promotes sleep in nematodes [23]. *PERK* overexpression in flies induces sleep, where the expression of wake-promoting neuropeptides is suppressed [24]. Thus, EFs may improve sleep, at least in part, by activating the *PERK* pathway. Further molecular evidence is required to explain the promotion of sleep and extension of lifespan in nGD flies by ELF-EFs.

As shown in our previous report on wild-type Oregon R flies [17], EF exposure had beneficial effects on sleep and longevity in nGD flies. However, these parameters did not significantly change in the control w^{1118} flies. Recently, w^{1118} flies have been reported to have a shorter lifespan and weakness in response to various stressors [25]. Thus, w^{1118} flies may differ from Oregon R flies, leading to unchanged sleep and lifespan following EF exposure.

The advantage of our study is that EFs can be applied using a commercially available simple device, and the electric power consumption is low during operation. However, EFs have certain limitations. ELF-EFs are largely shielded by the body surface, making it difficult to increase EF intensity inside the body, resulting in mild therapeutic efficacy. Therefore, EF therapy alone may not be sufficiently effective; however, it may be beneficial as a combination therapy for the treatment of nGD.

GBA1 gene deficiency promotes protein aggregation and is linked to neurodegenerative diseases, such as PD [12,26–29]. EFs prevent abnormal protein aggregation in solution [30]. If this occurs *in vivo*, EFs may alleviate GD symptoms by preventing protein aggregation. Our previous studies showed that EF treatment elevated N-palmitoyl serine levels in healthy humans [31], which extended the lifespan of PD model flies [32]. Further studies using *Drosophila* models of neurodegenerative diseases, such as PD and GD, should be conducted to clarify the effects of ELF-EFs.

CRediT authorship contribution statement

Takaki Nedachi: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Haruhisa Kawasaki:** Methodology, Investigation, Formal analysis, Conceptualization. **Eiji Inoue:** Methodology, Investigation, Formal analysis, Conceptualization. **Takahiro Suzuki:** Conceptualization. **Yuzo Nakagawa-Yagi:** Conceptualization. **Norio Ishida:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.101915.

Data availability

Data will be made available on request.

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