Experimental Animals
Exp. Anim. 69(2), 161–167, 2020

Original

Effects of environmental enrichment on autonomic nervous activity in NSY mice

Kentaro KANEKO[1–3], Akitoshi CHIKAMOTO[1], Julia Chu-Ning HSU[1], Ryota TOCHINAI[1], Shin-ichi SEKIZAWA[1], Machiko YAMAMOTO[2,3] and Masayoshi KUWAHARA[1]

[1]*Laboratory of Veterinary Pathophysiology and Animal Health, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*
[2]*School of Nursing, Ibaraki Christian University, 6-11-1 Omika, Hitachi, Ibaraki 319-1295, Japan*
[3]*Graduate School of Nursing, Ibaraki Christian University, 6-11-1 Omika, Hitachi, Ibaraki 319-1295, Japan*

Abstract: Environmental enrichment (EE) can reduce anxiety and stress in experimental animals, while little is known about the influence on autonomic nervous activity especially in disease animal models. Diabetes mellitus (DM) is associated with cardiovascular autonomic dysfunction, which can be characterized by a higher resting heart rate and a lower heart rate variability (HRV). We hypothesized that EE can enhance parasympathetic nervous activity while reducing disease progression in type 2 diabetic mice. A telemetry transmitter was implanted in NSY mice to continuously record electrocardiograms (ECG). Animals were kept in a cage with or without a nest box as EE. The autonomic nervous activity was evaluated using power spectral analysis of HRV. Four weeks of EE could increase high frequency (HF) power, but no change was observed in the absence of EE. Although animals showed impaired glucose tolerance at 48 weeks of age regardless of EE, a worsen case was observed in control. These results indicate that EE can be necessary for long-term housing of experimental animals and may reduce the risk of impaired glucose tolerance in NSY mice by enhancing parasympathetic nervous activity. In future, it is demanded whether increasing parasympathetic nervous activity, whatever the method is, can prevent diabetes from worsening.
Key words: diabetes mellitus, glucose tolerance, heart rate variability, power spectral analysis

Introduction

It is known that environmental enrichment (EE) promote the inherent behavior of laboratory animals, resulting in reduction of stress response as well as activation of natural killer cells [2, 3]. Especially for rodents such as mice, putting the material of nest box in the cage as EE made animals comfortable states [9, 10], while little is known about the influence on autonomic nervous function which might be closely related to the progression of diabetes mellitus (DM).

Autonomic nervous activity, which would have an influence on heart rate (HR) and thus has been frequently evaluated by a variability of the HR, is generally reduced in diabetic patients compared with non-diabetic patients of the same age [14]. Therefore, type 2 DM can lead to cardiac autonomic neuropathy [4, 5], which has been associated with elevated mortality in patients with DM [13, 23]. The earliest manifestation of cardiac autonomic neuropathy in diabetes has tended to be associated with parasympathetic denervation [15], but even healthy adults, who showed a decreased parasympathetic input and a higher resting HR, were known to be at an increased risk for developing type 2 DM [5]. In this context, it could be considered that increasing parasympathetic nervous activity and/or lowering resting HR decrease the incidence of diabetes onset.

Thus, we hypothesized that EE can enhance autonomic nervous function, especially resting parasympathetic components, while reducing the disease progres-

(Received 10 August 2019 / Accepted 24 October 2019 / Published online in J-STAGE 18 November 2019)
Corresponding author: M. Kuwahara. e-mail: akuwam@mail.ecc.u-tokyo.ac.jp

sion or decrease the incidence of worsen disease pathogenesis in type 2 diabetic mice. To prove this hypothesis, we used an NSY mouse as a model of developing DM. The advantages of using the NSY mouse are; developing type 2 DM spontaneously by 48 weeks of age and established evidences of the etiology and the pathology of the disease [21]. In addition, because a nest box has been used as common types of mouse enrichment [1], we selected it as an EE. It can provide narrow and dark places and behavioral opportunities for mice to climb and play. Moreover, using nest box made of paper might be suitable to record electrocardiogram (ECG) with telemetry system, because it does not interfere the radio wave from transmitter. Therefore, we performed this study to examine whether EE can enhance parasympathetic nervous system activity using power spectral analysis of heart rate variability (HRV) [11] and affect the progression of DM in NSY mice implanted with ECG telemetry.

Materials and Methods

Animals and housing

Total fourteen male NSY/Hos mice (8 weeks of age) were obtained from Japan SLC, Inc. for the experiment. All mice were kept individually in plastic cage with wood chips for bedding, and the cages were placed in an incubator (SANYO MIR-553, Osaka, Japan) maintained at constant temperature (24°C). Mice could freely access to water and normal food (MF, Oriental Yeast, Tokyo, Japan) and were kept on a 12-h light-dark cycle (lights on at 8:00 a.m.) according to previous studies [6, 12]. Experiments were performed in accordance with the Institute of Ethical Guidelines under the protocols approved by the Animal Experimental Expert Committee of the University of Tokyo.

Implanting the telemeter for ECG recording

A telemeter transmitter for ECG (ATE-02S, Softron, Tokyo, Japan) was surgically implanted at the cervical subcutaneous region under the isoflurane inhalation anesthesia. The electrodes were placed to the subcutaneous tissue so as to record marked R-wave of ECG.

Experimental protocol

This study was consisted of two parts of experiments. Firstly, effects of EE on autonomic nervous function in NSY mice were evaluated. Time course of the experiment is shown below. After implanted telemetry system by 14 weeks of age, eight mice were randomly divided into two groups: one, EE was provided in the cage after 16 weeks of age (EE group) and another, EE was never provided in the cage (control group). A nest box was used as EE (Shepherd Shack®:SS; Shepherd Specialty Papers, Watertown, TN, USA). SS was made of regenerated paper, and the shape had 146.0 × 89.0 × 64.0mm, with a front hole (approximately φ 40mm). The ECG recordings were started from 15 weeks until 20 weeks of age. SS was provided continuously during the recording period.

Secondly, long-term effects of EE on autonomic nervous function and type 2 DM condition were evaluated. As previously described, EE was provided to mice in the EE group at 16 weeks of age, and was continuously kept until 48 weeks of age, which was supposedly enough time for NSY mice to develop diabetes. Mice in the control group were kept without EE as usual. Three mice in each group were implanted with ECG telemetry transmitter at 45 weeks of age. After recovery, the ECG recordings were made at 48 weeks of age, and then oral glucose tolerance test (OGTT) described below was performed in all available mice which included animals not implanted with ECG telemetry transmitter. Blood samples were obtained from the tail vein, and blood glucose concentration was measured directly by glucose oxidase method at 0, 30, 60, 90 and 120 min after forced oral administration of 2g /kg weight glucose. Area under the glucose curve (AUC) was calculated according to the trapezoid rule from the glucose measurements at baseline (0 min), 30, 60, 90 and 120 min.

Data recording and analyses

A signal receiving board (ATR-1001, Softron) was placed underneath each mouse cage in the incubator. ECG signals were continuously recorded by an ECG processor (SBP2000, Softron). Power spectral analysis of HRV was performed as previously described [6, 12]. Briefly, using the recorded ECG data, an off-line analysis of HRV was performed on an ECG power spectral analysis software (SRV2W, Softron). To analyze the power spectrum obtained, we separated the frequency range into two classes according to previous studies; low frequency (LF) class between 0.1 and 1.0 Hz and high frequency (HF) class between 1.0 and 5.0 Hz [8, 20]. The ratio of LF and HF power (LF/HF) was also calculated. It is known that LF corresponds to both sympathetic and parasympathetic nervous activities, HF corresponds to parasympathetic nervous activities and LF/HF ratio corresponds to sympatho-vagal balance [11].

ECG data were analyzed at 16 to 20 weeks of age as well as at 48 weeks of age, each of which corresponded to historically pre-diabetes phase and complete diabetes phase, respectively [21, 22]. All values are expressed as mean ± SEM. To determine the diurnal/nocturnal varia-

tion in these parameters, the 24-h period was separated into light period (8:00–20:00; asleep) and dark period (20:00–8:00; awake). A repeated measures analysis of variance (ANOVA) was used to examine the time course effect within the group as well as EE effect between the groups. Post hoc multiple comparisons were performed with the use of the Least Significant Difference test only when interaction between the groups and the time course effects was significant. Student's *t*-tests were only used for OGTT to compare values between the control group and the EE group at 48 weeks of age. A value of $P<0.05$ was considered significant. Statistical analyses were performed using JMP® 14 (SAS Institute Inc., Cary, NC, USA).

Results

Autonomic nervous function

No abnormal features of ECG were observed in both control group and EE group. Figure 1A is an example of continuous HR data from a control mouse from 16 to 20 weeks of age, showing clear circadian oscillations. As seen in HR, LF and HF were also showing circadian oscillation which was not obvious in LF/HF ratio (Fig. 1B), and thus it was necessary to separate data in each photo period. Changes in body weights were no difference between the control group (40.9 ± 2.0 g to 43.1 ± 3.1 g) and the EE group (41.6 ± 2.2 g to 43.4 ± 4.3 g) during in this experimental period. Figure 2 shows time course changes in autonomic nervous function in the presence or the absence of EE. HR decreased from 16 to 20 weeks of age in both light and dark period (time: $P=0.004$ and $P=0.005$, respectively) but changes were not significantly different between the control group and the EE group (between groups: $P=0.112$ and $P=0.116$; interaction: $P=0.741$ and $P=0.766$, respectively). LF showed a significant increase from 16 to 20 weeks of age in light phase but not in dark phase (time: $P=0.027$ and $P=0.151$, respectively), but changes seen in light period were not significantly different between the control group and the EE group (between groups: $P=0.150$; interaction: $P=0.118$). HF in the EE group gradually increased from 16 to 20 weeks of age only in light period compared to that in the control group (between groups: $P=0.026$; time: $P=0.001$; interaction: $P=0.035$) and changes were significant at 19 and 20 weeks of age compared to that at 16 weeks of age and between groups (post hoc test; vs. 16 weeks: $P=0.004$ and $P<0.001$, respectively; between groups: $P=0.009$ and $P<0.001$, respectively) (Fig. 2). LF/HF ratio decreased similarly in both control and EE groups from 16 to 20 weeks of age only in light period (between groups: $P=0.303$; time: $P=0.023$; interaction: $P=0.285$) but not in dark period (between groups: $P=0.260$; time: $P=0.397$; interaction: $P=0.791$).

Figure 3A shows representative 24-h time-course data

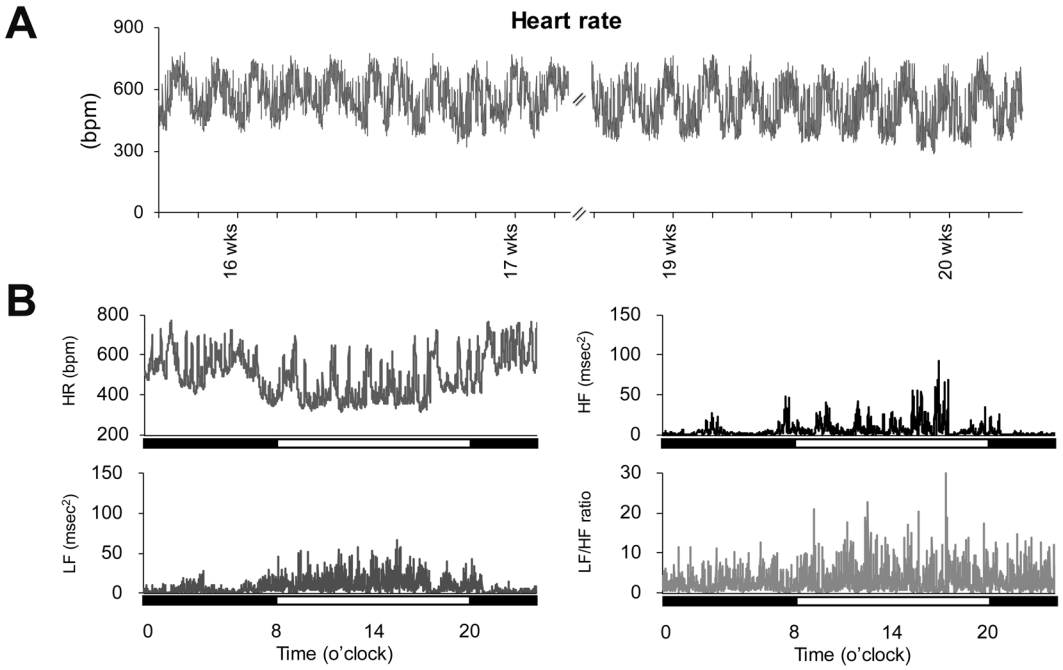

Fig. 1. Circadian oscillations in pre-diabetes mouse. A) Heart rate (HR) from a NSY mouse of control group. Data are plotted every 5 min. B) Time course changes of HR, Low frequency (LF), High frequency (HF) and LF/HF ratio from a 20-week-old mouse of control group. Clearly different activities are observed between light and dark periods in HR, LF and HF but not in LF/HF ratio. wks, weeks of age.

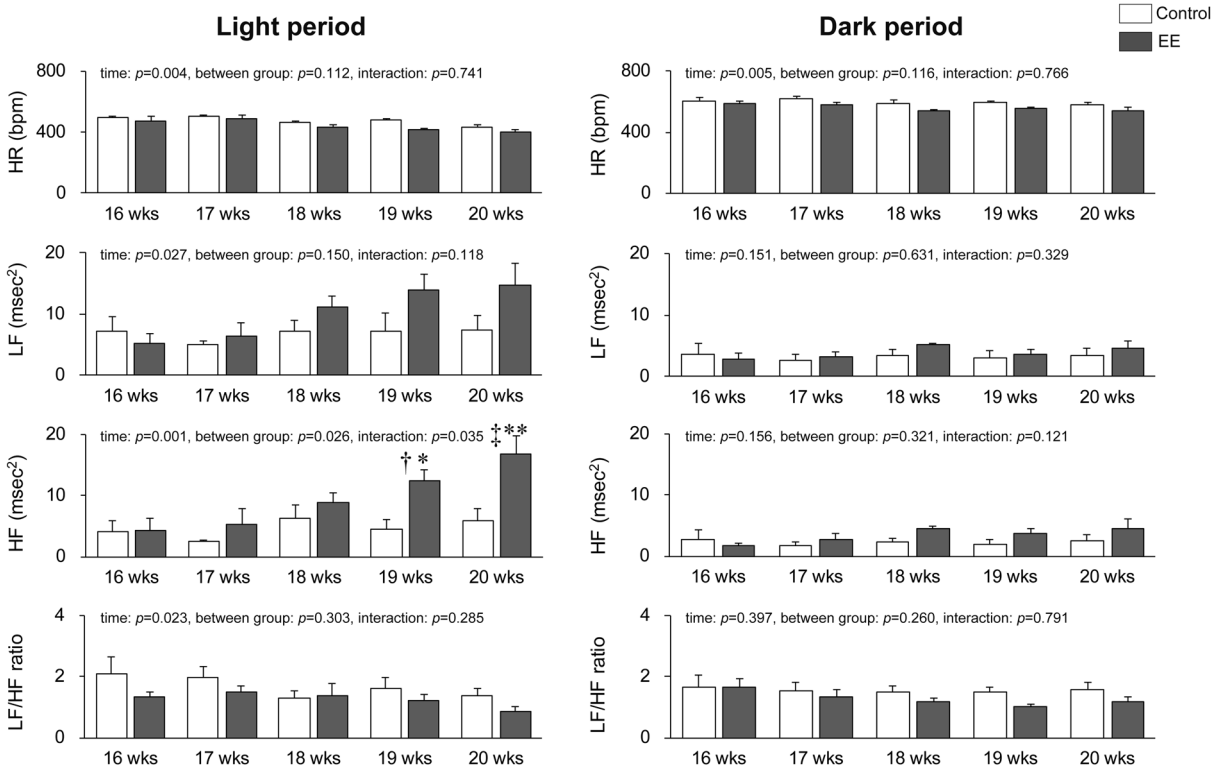

Fig. 2. Autonomic nervous functions in the control group (n=4, blank bar) and the environmental enrichment (EE) group (n=4, gray bar) from 16 to 20 weeks of age. Values are mean ± SEM and were analyzed using a repeated measures analysis of variance and multiple comparisons. **P*<0.01, ***P*<0.001 vs 16 weeks. †*P*<0.01, ‡*P*<0.001 vs control group at same phase. wks, weeks of age; HR, heart rate; LF, low frequency; HF, high frequency.

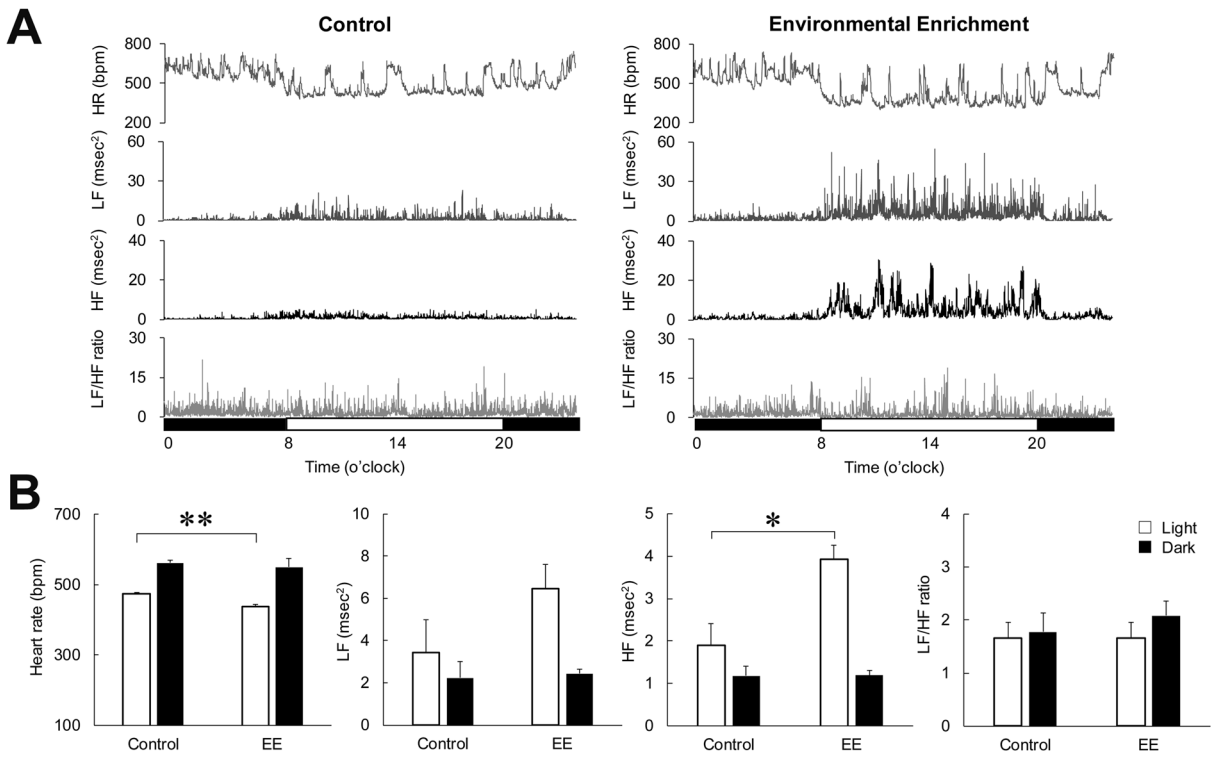

Fig. 3. Autonomic nervous function at 48 weeks of age in each light and dark period. A) Time course changes of heart rate (HR), Low frequency (LF), High frequency (HF) and LF/HF ratio from each mouse of control and environmental enrichment (EE) groups at 48 weeks of age. Much higher powers in both LF and HF are presented during light period in EE compared to control. B) In light period, EE group showed significantly lower HR and higher HF power than control group. Each group n=3. Values are mean ± SEM. **P*<0.05, ***P*<0.01.

 doi: 10.1538/expanim.19-0103

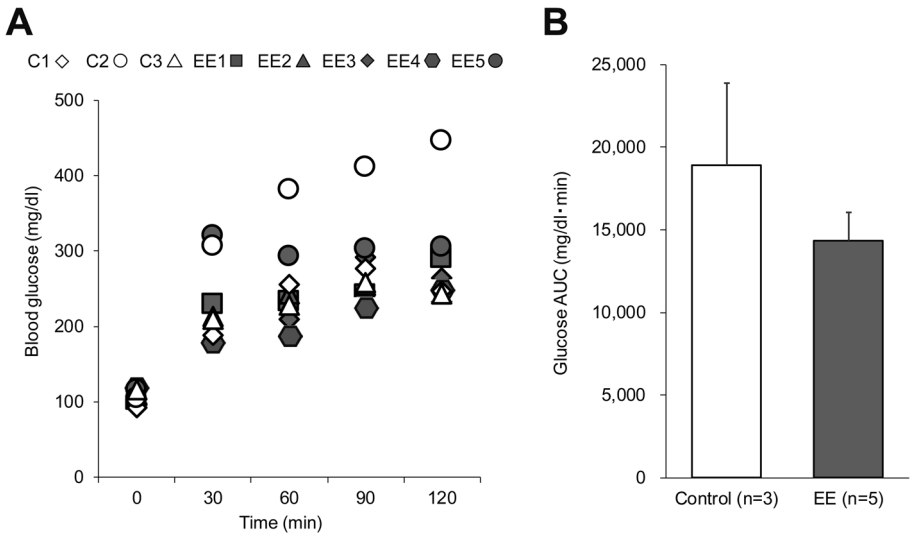

Fig. 4. Oral glucose tolerance test. A) Time course changes in blood glucose concentration are plotted in each individual mouse tested at 48 weeks of age. Open symbols are from control group and closed symbols are from environmental enrichment (EE) group. Note that open symbols of one control mouse (C2) show greater blood glucose concentration even at 120 min after 2 g/kg body weight glucose application. B) Glucose area under curve (AUC). Difference was not statistically significant (P=0.330). Values are mean ± SEM.

of autonomic nervous function in an NSY mouse each from control and EE groups at 48 weeks of age. Diurnal/nocturnal oscillation was remained to be seen in HR, LF and HF but not LF/HF ratio in both groups, while HR seemed to be lower and the power of LF and HF seemed to be greater in EE group compared to those in the control group (Fig. 3A). In fact, group data confirmed that HR and HF in the EE group, only in light period, were significantly lower (P=0.005) and greater (P=0.029) than those in the control group, respectively (Fig. 3B). Although LF in the EE group in light period tended to be greater than that in the control group, difference was not statistically significant (P=0.187). LF/HF ratio was not different between control and EE groups in light period. During dark period, autonomic nervous function was similar in both control and EE groups.

Blood glucose level and OGTT at 48 weeks of age

Fasting blood glucose levels were 106.4 ± 9.3 mg/dl in the control group and 108.2 ± 4.0 mg/dl in the EE group, and the difference was not statistically significant between both groups (P=0.863, n=5 each). Body weight were 46.5 ± 1.9 g in the control group and 45.2 ± 1.4 g in the EE group, and the difference was also not statistically significant between both groups (P=0.590, n=5 each). Figure 4A shows the results of the glucose tolerance test in both control and EE groups at 48 weeks of age. Although all mice were judged as a diabetic condition based on the glucose level of >200 mg/dl at 120 min after the oral glucose administration, AUC was not significantly different between both groups (Fig. 4B). However, as seen in Fig. 4A, one mouse in the control group showed abnormally high blood glucose values and was more than 2 standard deviation of the average of the rest values. Furthermore, this mouse died a week later even though other animals were survived.

Discussion

In this study, we investigated whether EE can enhance parasympathetic nervous system activity and affect the progression of DM in NSY mice implanted with ECG telemetry. The major findings of the present study were as follows. First, there was a clear nocturnal variation in HR and autonomic nervous system function in NSY mice. Second, the placement of the nest box as EE gradually increased the parasympathetic nervous activity in the light period as interpreted from an increased HF, and it became significant after 3 weeks of EE treatment. The effect of EE was not observed in the dark period. Third, mice in the EE group that were continuously received nest box in their cages showed high parasympathetic activity and low HR in light period (resting phase) even at 48 weeks of age. Although NSY mice showed impaired glucose tolerance in all cases at 48 weeks of age, there was an individual showing abnormally high blood glucose values in the group without EE and being dead soon after. This may indicate that EE have some positive effect on diabetes such as preventing severe progression.

Autonomic nervous activities of NSY mice

Among various experimental animal models in diabetes studies [18], we used NSY mice since this model seems to be more comparable to human type 2 diabetes in terms of disease progression compared to other mice models such as db/db mice which develop obesity and hyperglycemia very early and the severity increased progressively with age [16]. Since this study was also aimed to evaluate the long-term effects of EE, NSY mice were also appropriate due to the characteristics described below. The NSY mouse is a spontaneous disease model of type 2 DM with moderate obesity that was established by selective breeding for impaired glucose tolerance from a non-diabetic Jcl:ICR mouse colony [17]. Impaired glucose tolerance is specific to males and observed as early as at 12 weeks of age, and this impairment progressively worsens with growing. The cumulative incidence of impaired glucose tolerance is 98% for male at 48 weeks of age, accompanied by an increase in body fat and mild hyperinsulinemia [22]. Additionally, the hyperinsulinemia and high AUC of serum blood glucose at fasting condition were recognized from the age of 24 weeks [21, 22]. Therefore, the evaluation before 20 weeks of age in the present study seems to have been in a state without severity of impaired glucose tolerance.

This is the first report of autonomic nervous activities using HRV analysis in the NSY mouse. As seen in results, diurnal variation on HR and autonomic nervous function in the NSY mouse existed and synchronized to the light-dark cycle. Because it is important to consider both time of day/night and stress when evaluating HR and HRV in diabetes [19], this study preciously provides the basis for future investigation on autonomic nervous function in NSY mice.

Effects of environmental enrichment

EE in experimental rodents decreases anxiety and stress-responsive hormones and enhances natural killer cell activity [2, 3]. In the present study, we provided a nest box as an EE to NSY mice of young age and successfully enhance parasympathetic nervous activity as indicated by an increased HF power especially in the resting period. Whereas no change was observed in any parameters of autonomic nervous function immediately after EE was set, parasympathetic nervous activity during light period gradually increased afterward and became significant after 3 weeks. Since we used the nesting box as EE, which might be related to resting and/or sleep area for mice, effects in the present study may be observed only in resting phase (light period). If this is the case and EE were functioning as 'toys' for mice, effects on autonomic nervous activity may be seen in dark period which is an active phase for nocturnal animals. It is not known whether it is important to increase autonomic nervous function in active phase or resting phase, and this question would be remained for future studies.

In the present study, adding the nesting box into the cage did not have an immediate influence on any autonomic nervous function but took more than 3 weeks to increase parasympathetic nervous activity in resting phase. This suggests that changing steady state of autonomic nervous activity could not be achieved instantaneously such as one shot of medication but might rather result from continuous and/or long-term treatments. This effect was still remained and also confirmed by 48 weeks of age NSY mice as seen in Fig. 3B. In any case one should always consider how EE works for animals and thus what effects would be expected in a specific aspect.

In addition, continuous EE placement resulted in lower HR at 48 weeks of age when comparing to those in the control group, while HR effects were not observed at 20 weeks of age. This may indicate that EE treatment longer than 4 weeks can change steady state HR especially at resting phase, resulting from already increased parasympathetic activities. Since NSY mice at 48 weeks of age were all diabetic in the present study, the disease progression may have an influence on autonomic nervous function. In addition, there was no difference in body weight changes between the control group and the EE group. NSY mice were a model with mild obesity, and the changes were almost the same as the body weight changes shown in earlier studies [21]. However, even so, EE could increase parasympathetic activity and lower HR in diabetic mice, and therefore some kind of intervention capable to increase parasympathetic activity and/or decrease HR would be effective and beneficial for patients with DM as discussed below. Because low HRV and autonomic nervous imbalance are related to the progress of diabetes [4, 5, 14], continuing to increase the parasympathetic nerve activity might have a preventive effect on disease progression and severity. In the present study, the autonomic nervous functions, especially parasympathetic activity, was significantly greater, and the HR was significantly lower in the EE group at 48 weeks of age, while all mice in the EE group showed impaired glucose tolerance, indicating EE could not prevent onset of diabetes. However, those mice had been survived even at 52 weeks of age, one mouse in the control group showed severe impaired glucose tolerance and died after a few days after OGTT.

As described above, environmental enrichments had not immediate effects on autonomic function until >3 weeks placement of nesting box. This suggests that the short-term rearing in mice (or experimental rodents) may

not require environmental enrichment such as a nesting box in the cage, but the long-term may require it in terms of animal welfare, probably contributing refinement of experimental animals.

Future study

Except fasting blood glucose levels, there was no index related to the disease state at a given time point in the present study. Glycemic variability may be associated with autonomic imbalance in patients with DM [7], and thus investigating a relationship between glycemic variability and HRV may be a potential future study to detect disease conditions of diabetes. However, in the current study we did not try to test glycemic variability, because we considered important to avoid interruption of continuous recording of ECG with telemetry system as well as invasive stress by measurement of glycemic variability.

Acknowledgment

This work was supported by Japan Society for Promotion of Science KAKENHI Grant (grant numbers JP17H05107 and JP17K12091).

References

1. Bayne, K. 2018. Environmental enrichment and mouse models: Current perspectives. *Animal Model. Exp. Med.* 1: 82–90. [Medline] [CrossRef]
2. Belz, E.E., Kennell, J.S., Czambel, R.K., Rubin, R.T. and Rhodes, M.E. 2003. Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacol. Biochem. Behav.* 76: 481–486. [Medline] [CrossRef]
3. Benaroya-Milshtein, N., Hollander, N., Apter, A., Kukulansky, T., Raz, N., Wilf, A., Yaniv, I. and Pick, C.G. 2004. Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. *Eur. J. Neurosci.* 20: 1341–1347. [Medline] [CrossRef]
4. Benichou, T., Pereira, B., Mermillod, M., Tauveron, I., Pfabigan, D., Maqdasy, S. and Dutheil, F. 2018. Heart rate variability in type 2 diabetes mellitus: A systematic review and meta-analysis. *PLoS One* 13: e0195166. [Medline] [CrossRef]
5. Carnethon, M.R., Golden, S.H., Folsom, A.R., Haskell, W. and Liao, D. 2003. Prospective investigation of autonomic nervous system function and the development of type 2 diabetes: the Atherosclerosis Risk In Communities study, 1987-1998. *Circulation* 107: 2190–2195. [Medline] [CrossRef]
6. Chikamoto, A., Sekizawa, S.I., Tochinai, R. and Kuwahara, M. 2019. Early attenuation of autonomic nervous function in senescence accelerated mouse-prone 8 (SAMP8). *Exp. Anim.* 68: 511–517. [Medline] [CrossRef]
7. Fleischer, J. 2012. Diabetic autonomic imbalance and glycemic variability. *J. Diabetes Sci. Technol.* 6: 1207–1215. [Medline] [CrossRef]
8. Ishii, K., Kuwahara, M., Tsubone, H. and Sugano, S. 1996. Autonomic nervous function in mice and voles (Microtus arvalis): investigation by power spectral analysis of heart rate variability. *Lab. Anim.* 30: 359–364. [Medline] [CrossRef]
9. Kawakami, K., Shimosaki, S., Tongu, M., Kobayashi, Y., Nabika, T., Nomura, M. and Yamada, T. 2007. Evaluation of bedding and nesting materials for laboratory mice by preference tests. *Exp. Anim.* 56: 363–368. [Medline] [CrossRef]
10. Kawakami, K., Xiao, B., Ohno, R., Ferdaus, M.Z., Tongu, M., Yamada, K., Yamada, T., Nomura, M., Kobayashi, Y. and Nabika, T. 2012. Color preferences of laboratory mice for bedding materials: evaluation using radiotelemetry. *Exp. Anim.* 61: 109–117. [Medline] [CrossRef]
11. Kuwahara, M., Yayou, K., Ishii, K., Hashimoto, S., Tsubone, H. and Sugano, S. 1994. Power spectral analysis of heart rate variability as a new method for assessing autonomic activity in the rat. *J. Electrocardiol.* 27: 333–337. [Medline] [CrossRef]
12. Kuwahara, M., Ito, K., Hayakawa, K., Yagi, S. and Shiota, K. 2015. N-Acetylmannosamine improves sleep-wake quality in middle-aged mice: relevance to autonomic nervous function. *Auton. Neurosci.* 187: 56–62. [Medline] [CrossRef]
13. Maser, R.E., Mitchell, B.D., Vinik, A.I. and Freeman, R. 2003. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. *Diabetes Care* 26: 1895–1901. [Medline] [CrossRef]
14. Perciaccante, A., Fiorentini, A., Paris, A., Serra, P. and Tubani, L. 2006. Circadian rhythm of the autonomic nervous system in insulin resistant subjects with normoglycemia, impaired fasting glycemia, impaired glucose tolerance, type 2 diabetes mellitus. *BMC Cardiovasc. Disord.* 6: 19. [Medline] [CrossRef]
15. Pop-Busui, R. 2010. Cardiac autonomic neuropathy in diabetes: a clinical perspective. *Diabetes Care* 33: 434–441. [Medline] [CrossRef]
16. Senador, D., Kanakamedala, K., Irigoyen, M.C., Morris, M. and Elased, K.M. 2009. Cardiovascular and autonomic phenotype of db/db diabetic mice. *Exp. Physiol.* 94: 648–658. [Medline] [CrossRef]
17. Shibata, M., and Yasuda, B. 1980. New experimental congenital diabetic mice (N.S.Y. mice). *Tohoku J. Exp. Med.* 130: 139–142. [Medline] [CrossRef]
18. Stables, C.L., Glasser, R.L. and Feldman, E.L. 2013. Diabetic cardiac autonomic neuropathy: insights from animal models. *Auton. Neurosci.* 177: 74–80. [Medline] [CrossRef]
19. Stables, C.L., Auerbach, D.S., Whitesall, S.E., D'Alecy, L.G. and Feldman, E.L. 2016. Differential impact of type-1 and type-2 diabetes on control of heart rate in mice. *Auton. Neurosci.* 194: 17–25. [Medline] [CrossRef]
20. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93: 1043–1065. [Medline] [CrossRef]
21. Ueda, H., Ikegami, H., Yamato, E., Fu, J., Fukuda, M., Shen, G., Kawaguchi, Y., Takekawa, K., Fujioka, Y., Fujisawa, T., Nakagawa, Y., Hamada, M., Shibata, M. and Ogihara, T. 1995. The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. *Diabetologia* 38: 503–508. [Medline] [CrossRef]
22. Ueda, H., Ikegami, H., Kawaguchi, Y., Fujisawa, T., Nojima, K., Babaya, N., Yamada, K., Shibata, M., Yamato, E. and Ogihara, T. 2000. Age-dependent changes in phenotypes and candidate gene analysis in a polygenic animal model of Type II diabetes mellitus; NSY mouse. *Diabetologia* 43: 932–938. [Medline] [CrossRef]
23. Vinik, A.I., Maser, R.E., Mitchell, B.D. and Freeman, R. 2003. Diabetic autonomic neuropathy. *Diabetes Care* 26: 1553–1579. [Medline] [CrossRef]