Studies on the Autonomic Nervous Function in Tsukuba Emotional Rats—with Special Reference to Their Cardiac Function

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ABSTRACT. Autonomic nervous function in Tsukuba high (THE) and low (TLE) emotional rats as well as Wistar rats (control) was studied by means of electrocardiographical examination in addition to the runway test. In the runway test, locomotive activity differed significantly among the three strains, the greatest activity in TLE and the smallest in THE. Electrocardiogram (ECG) and locomotive activity were successively recorded for 24 hr using a small size of telemeter system. Though the locomotive activity showed a clear nocturnal rhythm, no significant difference was observed among the three strains. The mean value of R-R intervals in THE tended to be smaller than those in other two strains; showing a significant difference between THE and TLE. The mean value of standard deviation (SD) of R-R intervals in TLE tended to be larger than those in other two strains; showing a significant difference between TLE and Wistar in the light and dark periods, and also between TLE and THE in the light period. As regards coefficients of variation of R-R intervals (CV), there was no significant difference among the three strains. Autonomic nervous tone in THE and TLE was evaluated by means of autonomic blockades using atropine and/or propranolol. The sympathetic nervous tone represented 21.6% in THE and 16.8% in TLE, while the parasympathetic nervous tone −9.0% and −12.3% in THE and TLE, respectively. There was a significant difference in the net autonomic nervous tone between THE (10.7%) and TLE (−6.6%). These results demonstrated that the sympathetic nervous activity was higher in THE than in TLE, while conversely, the parasympathetic nervous activity was higher in TLE than in THE.—KEY WORDS: autonomic nervous function, ECG, runway test, Tsukuba emotional strain of rat

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When animals are faced to a life-or-death crisis, they have two strategies to cope with it. One is active behavioral response such as fight-or-flight response accompanied by predominance of sympathetic nervous activity. The other is passive behavioral response represented by freezing or sham-death response, accompanied by predominance of parasympathetic nervous activity [7, 20, 21].

It has been reported that stimulation or destruction of some brain regions can induce changes in emotional states of animals [17]. For example, stimulation to the mesencephalic central gray matter [3] or ventromedial amygdaloid nucleus [24] can induce attacking behavior, whereas removal of septal area resulted in hyperemotionality [5], and corpus amygdaloideum lack of fear and rage [4].

These emotional changes would be accompanied by changes in the autonomic nervous function, as suggested by the reports of horses [15, 16] in which heart rate level in response to novel environment was found to be different between normal and nervous individuals.

However, it is not clear whether the autonomic nervous function may alter in animals having variant emotionality during their normal life without any marked stimulation to affect their behavior.

Tsukuba low emotional rats (TLE) and Tsukuba high emotional rats (THE) have been well-known as the congenitally breeding animals for studies on the emotionality [8, 9]. They were obtained by bidirectional inbreeding of the Wistar strain for high (TLE) and low (THE) activity in the runway test which consisted of a dark starting box with a small exit and a bright straight runway of which length was divided into 5 sections (25 cm each) for recording the locomotive activity [8]. This criterion is based on the supposition that the suppression of locomotive activity, represented by 'freezing' to a novel situation reflects a certain emotional reaction, which has been thought as an expression of fear or anxiety of rats. Therefore, it is of great interest to investigate the differences in the autonomic nervous function between these animal models having different emotionality each other.

The heart beat rhythm has been extensively recognized as an excellent parameter to show the alteration of the autonomic nervous function. In the present study, the electrocardiogram (ECG) of freely moving rats was recorded for 24 hr by using a small size of biotelemetry system implanted into the body of rats, which would not restrict their natural behavior. Based on the ECG recorded, the autonomic nervous function in THE and TLE was investigated by analysis for heart rate (HR), variation of R-R intervals, and also effects of autonomic blockades on HR.

MATERIALS AND METHODS

Locomotive activity and defecation scores in runway test: In order to ascertain the background emotional difference among THE, TLE and Wistar rats, the runway test was conducted according to the report by Fujita [8] using 4 male THE, 5 male TLE rats (originated from Institute of Psychology, University of Tsukuba) and 5 male Wistar rats (from SLC Co., Ltd., Shizuoka); all aged 8 to 9 weeks and weighing 250 to 300 g. Rats were tested for 3 consecutive days and total number of sections traversed on the bright straight runway and total number of fecal boli were recorded.

Locomotive activity and autonomic nervous function during 24 hr in the home cages: Four male THE, 4 male TLE and 4 male Wistar rats were used in this experiment. They were accustomed to 12 hr light-dark cycle (L=8:00–20:00; D=20:00–8:00). The rats remained in their home cages throughout the experiment. Food and water were available *ad libitum*. The rats were housed individually after the surgery described below and kept in a temperature-controlled room (23 ± 2°C).

A small size of telemeter system (TA10EA-F2, Data Science Co., Ltd., Minnesota, U.S.A.) for transmitting ECG was implanted chronically into the notal subcutanea under pentobarbital sodium anesthesia (40 mg/kg, i.p.). For recording the ECG, paired wire electrodes connected to the body of the telemeter were placed under the skin of dosal and ventral thorax, and the Apex-Base (A-B) lead ECG was introduced. After 3 days of recovery period of the surgery, each rat with the home cage was placed on a signal-receiving board (RA 1610, Data Science Co., Ltd., Minnesota, U.S.A.), and ECG signals were recorded for 24 hr from 8:00. In addition, paired infrared photo-sensors were set at both sides across the home cage to record locomotive activity (photically-monitored activity, PA). The PA was continuously measured for 24 hr, and the total amount of it during each 5 min was memorized on a personal computer (PC-9801 VM) successively. ECG was subsequently stored on a laser disk by means of an ECG processor (Softron, Tokyo). All the data of R-R intervals during each 5 min-term were assigned for analysis on their mean value, standard deviation (SD) and coefficient of variance (CV). On these data, statistical analysis using Student's *t*-test was performed for the comparison between light and dark periods and among the three strain groups. Statistical significant difference was considered if P value was less than 0.05.

Autonomic nervous tone in THE and TLE: Five male THE and 5 male TLE rats were used in this study. The same telemeter system as described above was implanted into the rats and the A-B lead ECG was monitored through the receiver.

In order to block the autonomic nervous activity, atropine (2 mg/kg), propranolol (4 mg/kg) and atropine (2 mg/kg) + propranolol (4 mg/kg) were intraperitoneally injected in this order at 24-h intervals to both the THE and TLE. The doses of these drugs used were determined according to the previous studies [1, 23, 26]. As a control, 1 m*l* of saline was intraperitoneally injected to both the THE and TLE rats. After the injection of these drugs or saline, ECG was continuously recorded for 10 min or more in each rat.

Jose [10] termed the HR under the total autonomic nervous blockade induced by a combined atropine and propranolol injection the intrinsic HR (IHR). In order to determine the sympathetic and parasympathetic tone in the autonomic nervous system, the following equations adapted from Walsh [26] and Lin and Horvath [13] were used.

Sympathetic tone (S-tone) = 100 (HRatr. - IHR) / IHR

Parasympathetic tone (P-tone) = 100 (HRprop. - IHR) / IHR

Net autonomic tone (N-tone) = 100 (HRcont. - IHR) / IHR

In these equations, HRatr., HRprop., HRcont. and IHR show the mean HR for 3 min at a quiet state of the animal after the atropine, propranolol, saline and atropine + propranolol injections, respectively. Although the handling effect related to the injection was observed, the animals got quiet within 5 min in most cases. Therefore, the data for calculating the mean HR were obtained 5 and more min after the injection when animal was in a quiet condition.

Statistical analysis based on Student's *t*-test was performed for the comparison between the THE and TLE, and the difference was considered to be significant if P value was less than 0.05.

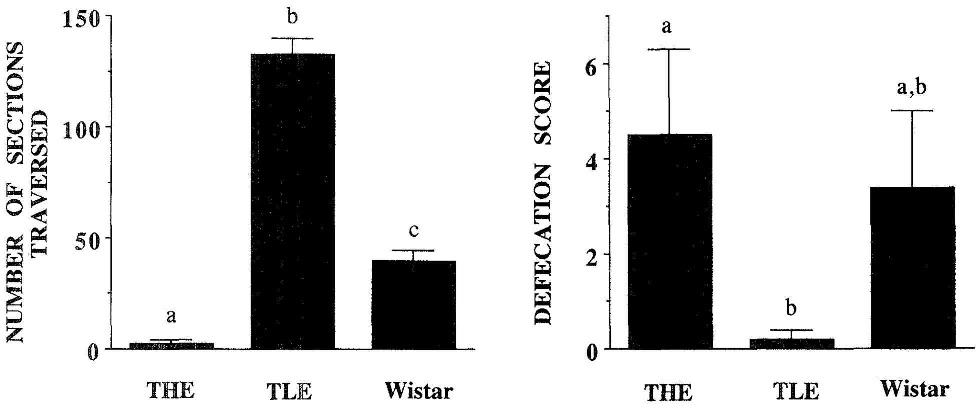

Fig. 1. The number of sections traversed and defecation score in THE (n=4), TLE (n=5) and Wistar (n=5) strains in the runway test. Each column and vertical bar represents a mean ± S.E. A significant difference ($p<0.05$) is shown among values with different alphabets in each graph.

RESULTS

Locomotive activity and defecation scores in runway test: As shown in Fig. 1, the number of sections traversed on the runway differed significantly among three strains ($p<0.05$); the largest number in TLE and the smallest number in THE. The locomotive activity in THE was pronouncedly inhibited, conversely, that in TLE was considerably high level. The locomotive activity in Wistar rats was positioned between the levels of THE and TLE. Moreover, the mean number of fecal boli was 4.5, 0.2 and 3.4 for THE, TLE and Wistar, respectively: a statistically significant difference was shown between THE and TLE.

Locomotive activity and autonomic nervous function during 24 hr in the home cages : The diurnal change of mean PA in each strain is shown in Fig. 2. All the strains showed a clear nocturnal activity rhythm. Mean values of PA in light and dark periods of the three strains are summarized in Fig. 3. Though PA in dark period of Wistar strain rats tended to be less than that of other two strains, no significant difference was observed among the three strains in either light or dark period.

The diurnal change of mean R-R intervals in each strain is shown in Fig. 4. In all the strains, mean R-R intervals were maximum before and after 8:00 related to the light-on, whereas they were minimum after 20:00 related to the light-off. However, these R-R intervals gradually increased or decreased toward the light-on or light-off, respectively. As shown in Fig. 5, the mean R-R interval had a tendency being longer in the light period than in the dark period in each strain; especially, a significant difference ($p<0.05$) in TLE. Moreover, both in light and dark periods, the mean R-R interval in TLE was significantly longer than that in THE ($p<0.05$).

Changes in mean standard deviations (SD) of R-R intervals and mean values of SD in light and dark periods of each strain are shown in Figs. 6 and 7, respectively. The mean SD in TLE in the light period was significantly larger than that in THE ($p<0.05$), while in the dark period TLE showed a larger, but not statistically significant, value than THE (Fig. 7). Moreover, both in the light and dark periods, the mean SD in TLE was significantly larger than that in Wistar ($p<0.05$).

The mean value of CV in each strain indicated 4.8 (light period), 4.4 (dark period) in THE, 5.5 (light period), 5.1 (dark period) in TLE and 4.6 (light period), 4.4 (dark period) in Wistar, respectively. Although those values in TLE at both the light and dark periods were slightly higher than those in other two strains, there was no significant difference among them ($0.2>p>0.05$).

Autonomic nervous tone in THE and TLE: As shown in Fig. 8, the control HR (HRcont.) in THE was significantly higher than that in TLE ($p<0.05$). However, the HR in both strains was changed to almost the same level by atropine (HRatr.) or propranolol (HRprop.) injection. The IHR in TLE tended to be higher than that in THE. The S-tone, P-tone and N-tone were compared between THE and TLE (Fig. 9). The S-tone represented 21.6% in

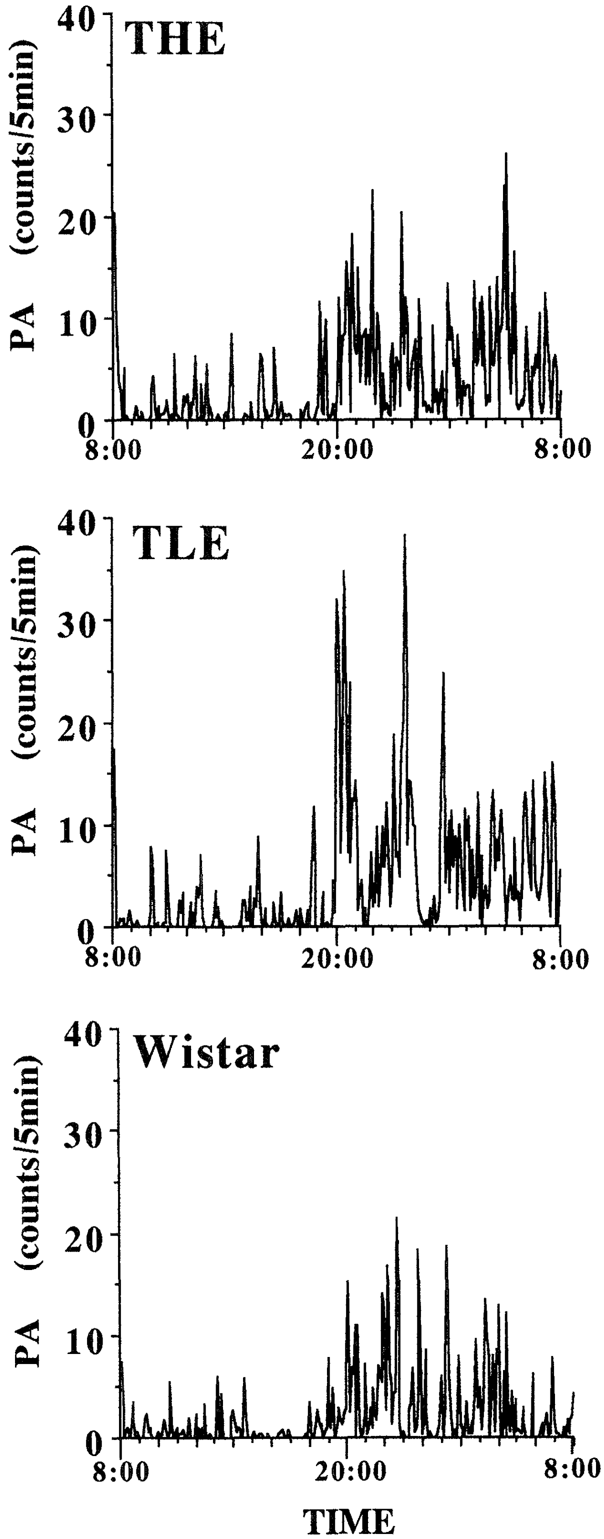

Fig. 2. Mean changes in photically-monitored locomotive activity (PA) in THE (n=5), TLE (n=5) and Wistar (n=4) strains during 24 hr. The number of sections traversed was counted every 5 min in all rats. Light period; 8:00–20:00, dark period; 20:00–8:00.

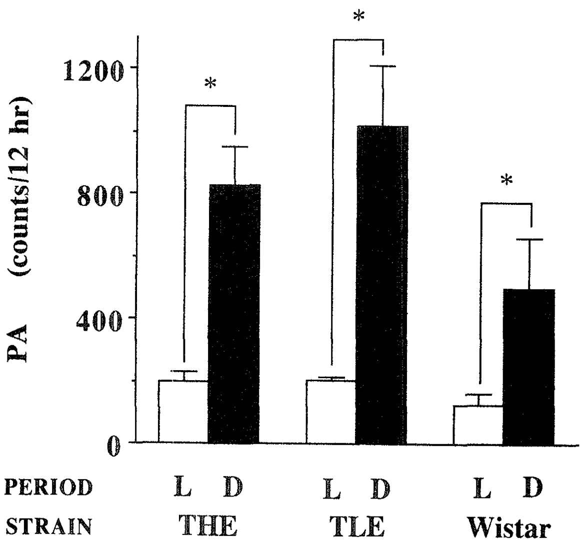

Fig. 3. Summary of photically-monitored locomtive activity (PA) in THE (n=5), TLE (n=5) and Wistar (n=4) strains during light (L) and dark (D) periods. L; 8:00–20:00, D; 20:00–8:00. Each column and vertical bar represents a mean ± S.E. *: Significantly different from each other (p<0.05).

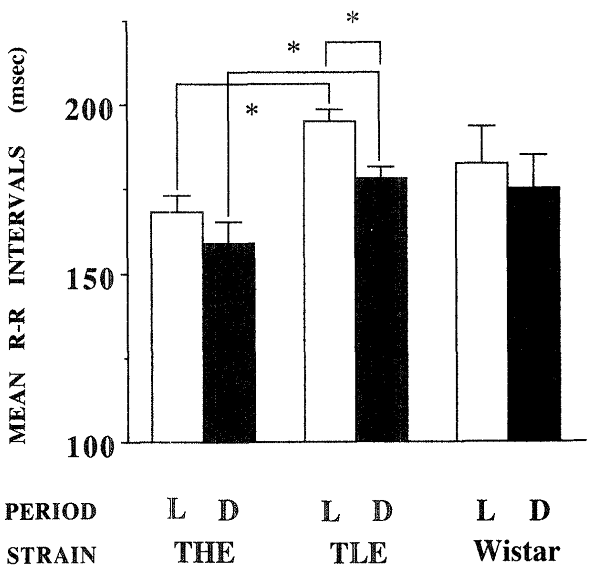

Fig. 5. Summary of R-R intervals in THE (n=5), TLE (n=5) and Wistar (n=4) strains during light (L) and dark (D) periods. L; 8:00–20:00, D; 20:00–8:00. Each column and vertical bar represents a mean ± S.E. *: Significantly different from each other (p<0.05).

THE and 16.8% in TLE, while the P-tone −9.0% and −12.3% in THE and TLE, respectively. The N-tone resulted in a predominant sympathetic tone of 10.7% in THE but a predominant parasympathetic tone of −6.6% in TLE.

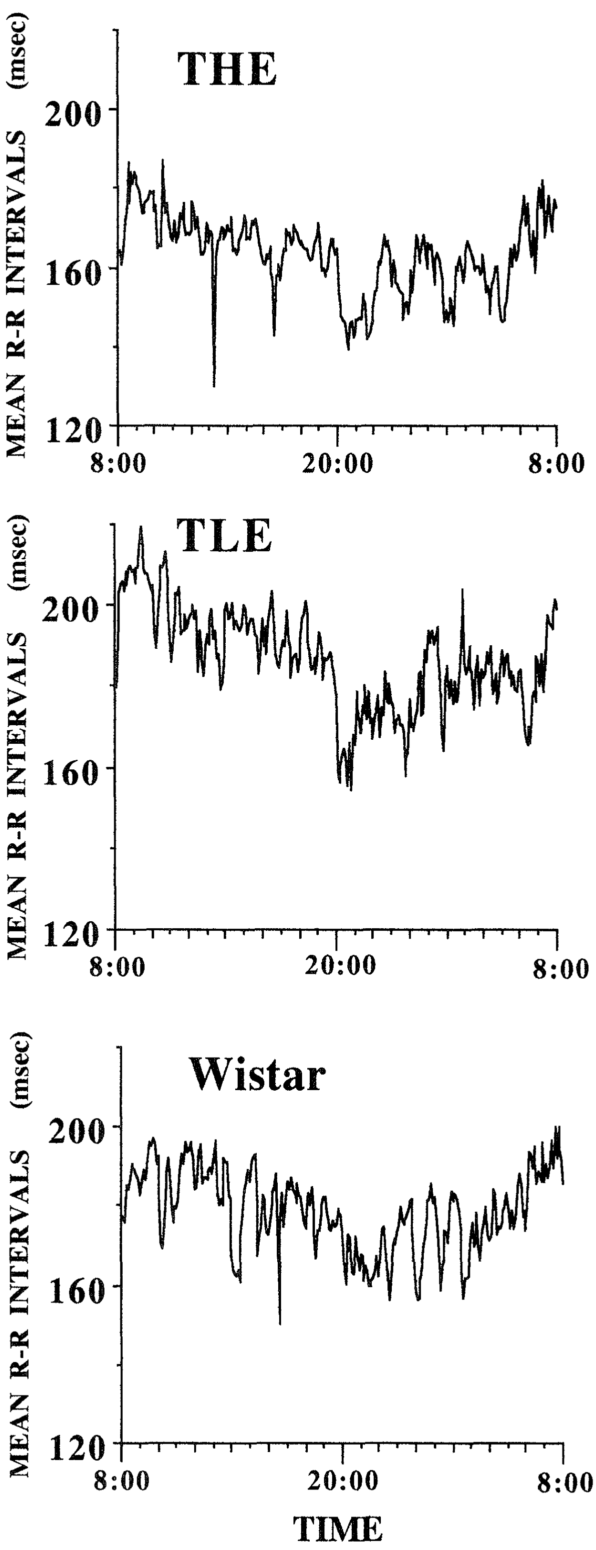

Fig. 4. Mean changes in R-R intervals in THE (n=5), TLE (n=5) and Wistar (n=4) strains during 24 hr. The analysis was performed for all R-R intervals during each 5 min. Light period; 8:00–20:00, dark period; 20:00–8:00.

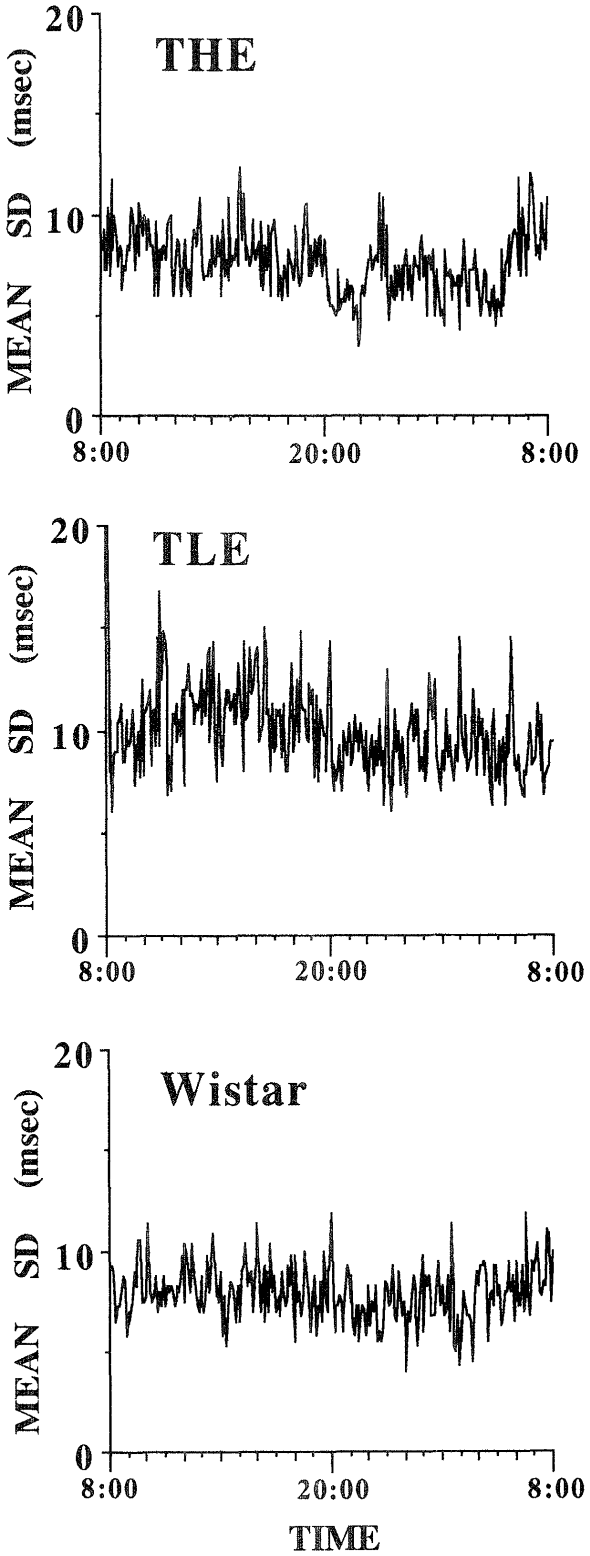

Fig. 6. Mean changes in standard deviation (SD) of R-R intervals in THE (n=5), TLE (n=5) and Wistar (n=4) strains during 24 hr. The analysis was performed for all R-R intervals during each 5 min in all rats. Light period; 8:00–20:00, dark period; 20:00–8:00.

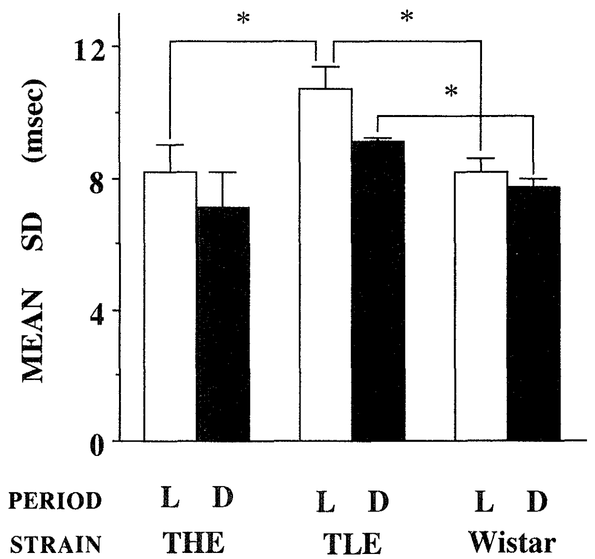

Fig. 7. Summary of standard deviation (SD) in THE (n=5), TLE (n=5) and Wistar (n=4) strains during light (L) and dark (D) periods. L; 8:00–20:00, D; 20:00–8:00. Each column and vertical bar represents a mean ± S.E. *: Significantly different from each other ($p<0.05$).

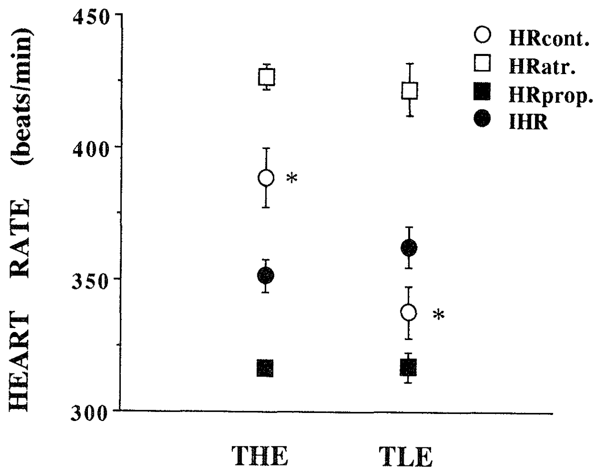

Fig. 8. The effect of autonomic blockades on HR in THE (n=5) and TLE (n=5). HRcont., HRatr., HRprop. and IHR represent the HR after the saline, atropine, propranolol and atropine + propranolol injections, respectively. Each symbol and vertical bar represents a mean ± S.E. The asterisk shows a significant difference between the same symbols ($p<0.05$).

DISCUSSION

The emotion in animals and human could be defined as various mental changes revealed by neurological responses in the central nervous system associated with various environmental changes. It appears a complex reaction of autonomic nervous, endocrine, humoral, and behavioral systems and in human has four standard

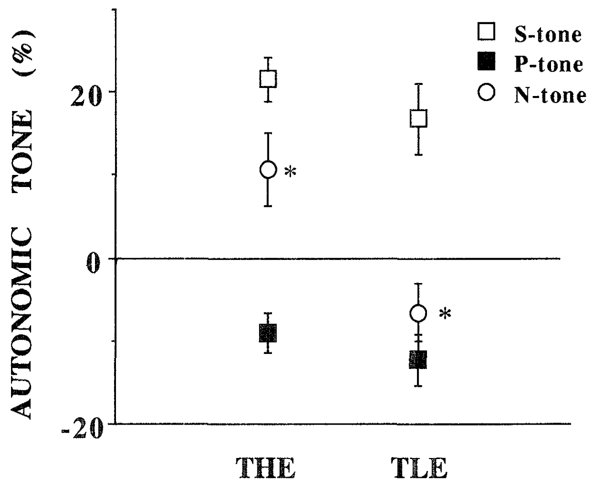

Fig. 9. Autonomic nervous tone calculated from the HR with and without autonomic blockades. The positive direction applies to sympathetic nervous activity, while negative direction parasympathetic activity. S-tone: sympathetic nervous tone, P-tone: parasympathetic nervous tone, N-tone: net autonomic nervous tone. Each symbol and vertical bar represents a mean ± S.E. The asterisk shows a significant difference between the same symbols ($p<0.05$).

aspects, i.e., delight, sorrow, rage and fear [12]. Freezing behavior shown by rats is a typical manifestation of highly augmented emotional states recognized as a certain expression of fear or anxiety [6]. THE and TLE, as a result of bidirectional inbreeding, possess different emotionality evaluated by amounts of locomotive activity depending on their freezing potency in the runway test [8]. In the present study, as compared with Wistar rats, a significantly lower and higher locomotive activity was observed in THE and TLE, respectively (Fig. 1). Considering that the photically monitored locomotive activities in their home cages during 24 hr did not differed significantly among the three strains, the clear difference in locomotive activity between THE and TLE observed in the runway test might reflect the difference in emotional reactivity of these animals in face to novel environment. In fact, the number of fecal boli in the runway test was the largest in THE and the smallest in TLE, indicating a clear relationship between the emotionality and locomotive activity in such circumstances. In accordance, these animals are considered to be valuable animal models for investigating various physiological mechanisms related to emotionality.

As to assessment for the autonomic nervous function, the R-R interval of ECG is widely used in mainly human studies [18, 22]. Recently, the analysis by standard deviation (SD) of R-R intervals and coefficient of variation (CV: 100×SD/mean R-R) has been developed for evaluation of the autonomic nervous activity, especially, the parasympathetic nervous activity [11, 19]. Therefore, it is of great interest to apply these methods for the emotional animal models. In the present study, the short R-R interval (high HR) in THE suggests a greater influence of sympathetic nervous activity (S-tone) and/or a minor influence of parasympathetic nervous activity (P-tone) in this strain than in TLE. In general, the HR is largely influenced by the activity of locomotion: the higher HR is associated with an increased locomotive activity. In the present study, however, such effect due to locomotion seems to be minimum, since the locomotive activity in THE was almost the same level as that in TLE. In the studies on human, an increased CV value indicates a large influence of P-tone [11]. However, in our study, this could not be applicable to the animal models such as THE and TLE, though only a minor increase of CV value was observed in TLE.

The significant difference in the mean R-R interval between THE and TLE (Fig. 5) suggested the presence of the difference in the autonomic nervous tone during their natural life. In fact, pharmacological denervation conducted for THE and TLE demonstrated the clear difference in the autonomic nervous tone between them; the higher S-tone (lower P-tone) in THE and the higher P-tone (lower S-tone) in TLE.

Masui [14] investigated the quantitative differences of monoamines and their metabolites in the brain between THE and TLE. He reported that there were quantitative differences in noradrenergic, adrenergic, serotonergic and dopaminergic neurons between THE and TLE. Considering that the degree of emotional behavior can be changed by stimulation or destruction of various monoaminergic neurons [3–5, 24], or infusion of agonists or antagonists of various monoamines [2, 25], these quantitative difference of monoamines in the brain might have important roles on the strain difference of emotional changes revealed by THE and TLE. However, it is still not clear whether or how such variation in the central nervous system can affect the autonomic nervous activity.

This study demonstrated the presence of different autonomic nervous activity in genetically-controlled emotional animals, which could be estimated by analysis on the cardiac activity.

REFERENCES

1. Adolph, E. F. 1967. Ranges of heart rates and their regulations at various ages (rat). *Am. J. Physiol.* 212: 595–602.
2. Bandler, R. J. 1970. Cholinergic synapses in the lateral hypothalamus for the control of predatory aggression in the rat. *Brain Res.* 20: 409–424.
3. Bandler, R. J. 1982. Induction of rage by glutamate microinjection of midbrain but not hypothalamus of cats. *Neurosci. Lett.* 30: 183–188.
4. Blanchard, D. C. and Blanchard, R. J. 1972. Innate and conditioned reactions to threat in rats with amygdaloid lesions. *J. Comp. Physiol. Psychol.* 81: 281–290.
5. Brady, J. V. and Nauta, W. J. H. 1953. Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. *J. Comp. Physiol. Psychol.* 46: 339–346.
6. Brush, F. R. 1971. Aversive Conditioning and Learning,

American Press, New York.
7. Cannon, W. B. 1929. Bodily Changes in Pain, Hunger, Fear and Rage, 2nd ed., Appleton, New York.
8. Fujita, O. 1975. Behavior-genetic analysis of responses in runway tests as measures of emotional reactivity in rats. 1. Phenotype variation and heritability estimates based on offspring-parents regression. *Jpn. J. Psychol.* 46: 281–292 (in Japanese).
9. Fujita, O. 1984. "Tsukuba Emotionality": New selected rats. *Rats News Lett.* 13: 31.
10. Jose, A. D. 1966. Effects of combined sympathetic and parasympathetic blockade on heart rate and cardiac function in man. *Am. J. Cardiol.* 18: 476–478.
11. Kageyama, S. 1984. Autonomic function test using R-R interval variation in ECG. *Brain and Nerve (Tokyo)* 36: 433–439 (in Japanese).
12. LeDoux, J. E. 1987. Higher Function. pp. 419–459. *In*: Handbook of Physiology, Nervous System (Flum, F. and Mountcastle, V. B. eds.), Am. Physiol. Society, Washington D. C.
13. Lin, Y. C. and Horvath, S. M. 1972. Autonomic nervous control of cardiac frequency in the exercise-trained rat. *J. Appl. Physiol.* 33: 796–799.
14. Masui, S. 1987. Differences in the levels of brain monoamines and their metabolites between Tsukuba high and low emotionality strains of rats selectively bred for differences in behavioral responses to novelty. *Jpn. J. Psychopharmacol.* 7: 207–208 (in Japanese).
15. McCann, J. S., Heird, J. C., Bell, R. W., and Lutherer, L. O. 1988. Normal and more highly reactive horses. 1. Heart rate, respiration rate and behavioral observation. *Appl. Anim. Behav. Sci.* 19: 201–214.
16. McCann, J. S., Heird, J. C., Bell, R. W., and Lutherer, L. O. 1988. Normal and more highly reactive horses. 2. The effects of handling and reserpine on the cardiac response to stimuli. *Appl. Anim. Behav. Sci.* 19: 215–226.
17. McGeer, P. L., Eccles, J. C., and McGeer, E. G. 1978. Molecular Neurobiology of the Mammalian Brain. Plenum Press, New York.
18. Musha, H., Murayama, M., Ito, H., Ono, S., Itai, T., and Kawabata, T. 1986. Estimation of autonomic nervous tone by evaluating minimal hourly heart rate. *Respiration & Circulation* 34: 1003–1006 (in Japanese with English abstract).
19. Musha, H., Murayama, M., Ito, H., Ono, S., and Itai, T. 1987. Evaluation of autonomic nervous system by R-R interval variation in sinus bradycardia. *Jpn. J. Electrocardiol.* 7: 643–646 (in Japanese).
20. Pascoe, J. and Kapp, B. S. 1985. Electrophysiological characteristics of amygdaloid central nucleus neurons during Pavlovian fear conditioning in the rabbit. *Behav. Brain Res.* 16: 117–133.
21. Robertshaw, D. and William, J. T. Jr. 1984. Autonomic nervous system — Emergency reaction. pp. 684–685. *In*: Duke's Physiology of Domestic Animals, 10th ed. (Melvin, J. S. ed.), Comstock Publishing Associates, a division of Cornell University Press, Ithaca and London.
22. Robinson, B. F. 1966. Control of heart rate by autonomic nervous system. *Circ. Res.* 19: 400–411.
23. Tucker, D. C. and Johnson, A. K. 1984. Development of autonomic control of heart rate in genetically hypertensive and normotensive rats. *Am. J. Phsyiol.* 246: R570–R577.
24. Ursin, H. and Kaada, B. R. 1960. Functional localization within the amygdaloid complex in the cat. *EEG Clin. Neurophysiol.* 12: 1–20.
25. Waldbilling, R. T. 1979. The role of the dosal and median raphe in the inhibition of muricide. *Brain Res.* 160: 341–346.
26. Walsh, R. R. 1969. Heart rate and its neural regulation with rising body temperature in anesthetized rats. *Am. J. Physiol.* 217: 1139–1143.