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EFFECTS OF TRANSIENT PAINFUL HEAT STIMULATION ON OPEN-FIELD BEHAVIOR IN SHR

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The present study aimed at investigating effects of heat stimulation on locomotor activity of the spontaneously hypertensive rat (SHR) which was insensitive to environmental stimulation. The locomotor activity in an open-field situation were observed for 15 min under two testing environment: (1) a test without any heat stimulation, followed by (2) a test just after a transient painful heat stimulation on a hot-plate method. For these tests, we used 9 male SHRs and 9 male Wistar-Kyoto strain rats (WKYs), which were all 19-week-old at the beginning of the experiment. The results showed that in the test after heat stimulation as well as without any stimulation, the SHRs maintained a significantly higher level of locomotor activity than the WKYs. In addition, the stimulation had a significant effect on the activity of each strain group to produce an increase in number of crossings at the outer area of open-field apparatus. We concluded that the heat stimulation could be useful for activating the SHR's locomotor activities even more.

Key words: SHR, open-field behavior, heat pain stimulation.

Introduction

A spontaneously hypertensive rat (SHR; Okamoto & Aoki, 1963) often showed a higher level of the locomotor activity than a normotensive Wistar-Kyoto strain (WKY) rat in an open-field apparatus (Danysz, Plaznik, Pucilowski, Plewako, Obersztyn, & Kostowski, 1983; Knardahl & Sagvolden, 1979). Such hyperactivity might be related to hypertensive symptoms because our longitudinal observation (Sato, Miyazaki, Shimizu, & Hatayama, 1993) as well as earlier studies have proved that SHRs would reach to systolic blood pressure of 200 mmHg or more until about 12 weeks old after birth.

Sato, Shimizu, and Hatayama (1995) pointed out that with novel objects put into an open field rose the activity in SHRs to a higher level; This suggests that some cognitive function would play a role in regulating their activity as well. An important system of controlling the SHR's behavior seems to be a pain regulatory system associated with activity in enkephalinergic cells in the periaqueductal gray matter of the brain stem because the SHR's reaction to painful stimulation is generally insensitive compared with normotensive rats (Sato &

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Hatayama, 1995). The regulatory system in SHRs might be usually much activated by way of the nucleus of the solitary tract with continuous visceral afferents of vagus generated by baroreceptors which are sensitive to blood pressure in the carotid artery and in the ascending aorta. Then we assume that a way of how the regulatory system works is likely to be one of key factors to control the hyperactive behavior of SHRs. Suarz and Gallup (1981), who attempted to examine an effect of behavior activation in normotensive rats with painful stimuli, suggested that the painful stimulation was useful for modifying the rats' total behavior.

Then in the present study, an attempt was made to investigate behavioral changes in SHRs when the regulatory system were much more activated. We took measurements of the changes of locomotor behavior in SHRs using an open field just after a painful stimulation was presented to rats through a hot-plate method.

METHOD

Subjects: Nine male SHRs and 9 male WKYs were used. These rats were kept in individual cages at a room temperature of 23 ± 3 ℃. Food and water were available ad libitum.

Systolic blood pressure measurement[3]: This measurement was taken 2 weeks before the open-field test. Systolic blood pressure (SBP) was measured in a non-invasive way, using a tail-cuff plethysmography (Natsume, manometer-tachometer system KN-210) which made it possible to record heart rate (HR) simultaneously. Age of all *Ss* in week at the time of measurement was 19-week-old.

Apparatus: We used a plastic open field (90 × 90 × 37 cm high) under a dimly-lit condition (about 55 lx on the surface of the floor) illuminated by four 20-W bulbs. The inside of this apparatus was painted flat gray with black lines dividing the floor of the field into a grid of 100 squares, each 9 × 9 cm. Whole area of this floor was covered with two transparent plastic panels (90 × 45 cm), and these were cleansed by using of an antiseptic solution (Takeda chemical corp., Osban R) just after every experimental trial was finished.

Procedure: Each rat was at first observed over a control period of 15 min at a OPEN-FIELD condition. Following this test, we performed the open-field test with heat stimulation loaded using the same animals. In this test, a stimulation-cum-start box (20 × 20 × 23 cm high) was attached to the outside of open-field apparatus. On the floor of this box, electrically-conductive hot plate (20 × 30 × 1 cm, Nihon Kohden, ST101J) was built in for heating limbs of animals. The surface temperature of this hot plate was monitored by electric thermometer (Yokogawa, LR 4110). A diagram of the apparatus was depicted in Fig. 1. An opening 12 cm square to the open field enabled the subject to move on from the start box. At the beginning of this test, each subject was put in the start box with the opening closed

3. The SHRs showed the mean SBP of 214.89 and their *SD* of 17.30, the mean HR of 322.22 and the *SD* of 12.02, while the WKY did the SBP of 129.17 and 5.01, the HR of 272.78 and 19.22 at 19-week-old. *T*-test by the Cochran and Cox approximation revealed that the SBP and HR in the SHRs were higher than in the WKYs [SBP: $t = 14.275$, $df = 8.0$, $p < 0.0001$; HR: $t = 6.5434$, $df = 8.0$, $p < 0.0002$). This results confirmed that the SHRs used in this study were in the severe hypertensive state.

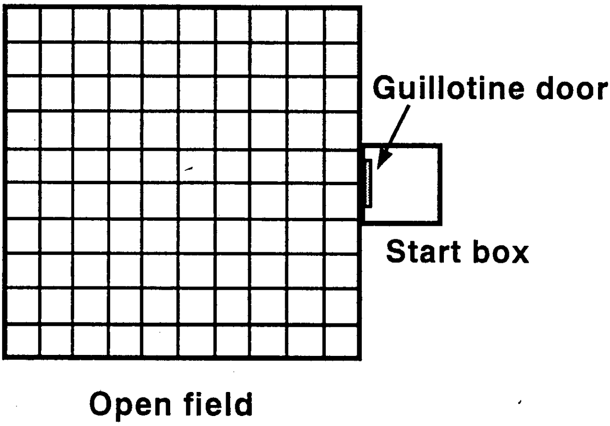

Fig. 1 A diagram of the open-field apparatus and the start box.

with a guillotine door, and the surface temperature of hot plate was regulated at 34 ± 1℃. After placement of the rat in the box, a heat stimulus was applied from the hot plate with an electrical alternating current of 120 V and 2.5 A to the feet of a rat. If the rats jumped during presenting heat stimuli, we opened the guillotine door for the rats to move out the open field and the value of the temperature at jumping was recorded as pain reaction index. At the time when subjects go out to the open field, we closed the door, and conducted an open-field test for 15 min (AFTER HOT-PLATE condition).

During two kinds of the open-field test, we recorded the rats' behavior by using a video camera (Sony, Handycam CCD-F330). Based on the analysis of the video recordings, we counted two indices of ambulatory behavior: (a) the crossings observed in central 36 section as crossings in inner area, and (b) the ones observed in peripheral 64 sections, which formed two columns along the wall of the apparatus, as crossings in the outer area.

Results and Discussion

Temperatures at pain reaction: Surface temperatures of the hot plate at the time when the jumping reaction occurred was shown in Table 1. The results of t-test suggested that the mean temperature at jumping in the SHRs was significantly higher than in the WKYs ($t = 2.8597$, $df = 16.0$, $p < 0.05$). This was in agreement with our previous study (Sato & Hatayama, 1995) and with the others (Maixner, Touw, Brody, Gebhart, & Long, 1982; Saavedra, 1981); The hypoalgesia the SHRs showed was likely to result from activation of a pain regulatory system with afferents generated by stimulating strongly baroreceptors because of the high blood pressure.

Table 1 Temperatures at jumping on hot-plate apparatus in SHRs and WKYs†

Strain	N	Temperature(℃)
SHR	9	56.68(4.76)
WKY	9	51.26(3.12)

† The values listed are means (*SD*) for each strain group.

Open-field test: The number of crossings at the inner area was illustrated in Fig. 2. A two-way ANOVA with repeated measure [strain (2) × condition (2)] indicated that the main effect of strain was significant [$F(1, 16) = 34.94$, $p < 0.0001$].

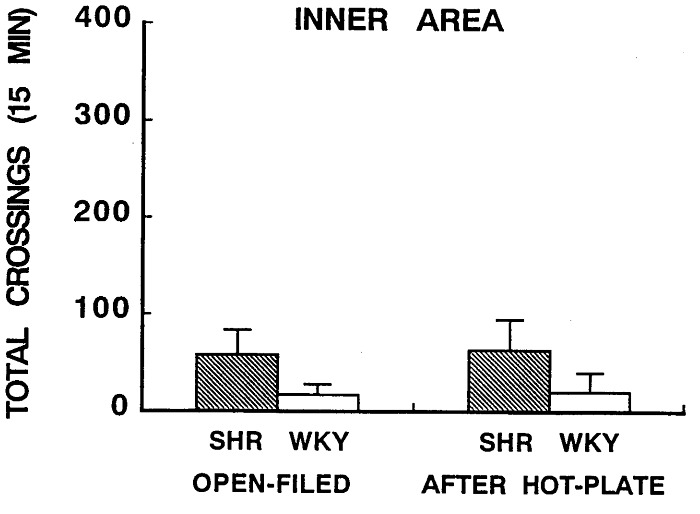

Fig. 2 Mean number of crossings at the inner area of the open-field apparatus with two conditions in a 15-min period. Vertical bars indicate *SD*s. Nine SHRs and 9 WKYs were used.

Figure 3 illustrated the number of crossings at the outer area. The ANOVA with repeated measure showed that two main effects were significant [strain: $F(1, 16) = 8.77$, $p < 0.0092$; condition: $F(1, 16) = 9.77$, $p < 0.0065$]. The results revealed that the effect of heat pain stimulation could be produced in such a way that locomotor activities in both groups of strain would be increased in the amount of total crossings at the outer area. Then, we examined the extent of which each of both groups enhanced the activities after the OPEN-FIELD condition was exchanged with AFTER-HOT-PLATE; In percent-change rates there was no significant difference between the two groups. These results suggested that although the amount of locomotor activities in the outer area at the SHRs were significantly larger than the WKYs, the effect of the heat stimulation would be produced in much the same way even at the WKYs.

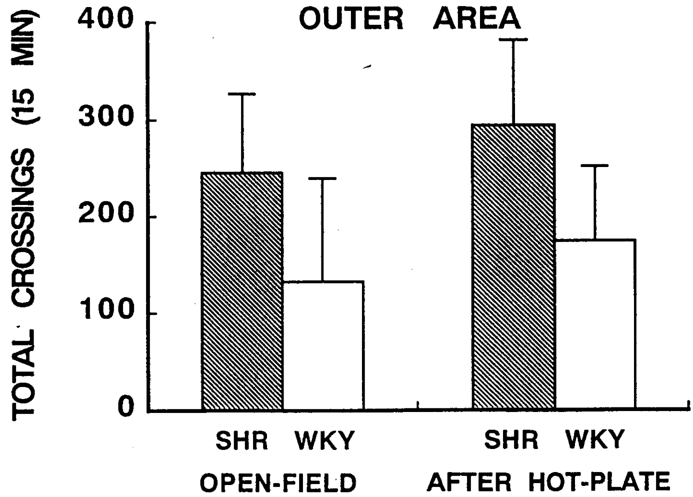

Fig. 3 Mean number of crossings at the outer area of the open-field apparatus with two conditions in a 15-min period.

With the comparison of the behavior in the outer area with the inner, we found that the crossings in each strain were significantly increased only in the outer area; it is simply at the outer area which the level of ambulatory activity was activated with the heat pain stimulation. Because this stimulation was expected to cause intense activity of sympathetic nervous system in the rats, it probably elicits their emotional response; The effect of emotional arousal would be reflected in an enhanced ambulatory behavior along the wall just after the stimulation. Besides, Sato, Shimizu, and Hatayama (1995) suggested that the rats exposed to environmental enrichment were made more active even in the inner area. Locomotor behavior in rats could be changed in the direction depending upon the nature of given stimuli. This difference in directional behavior changes between two types of stimulation might be based on the degree of activation of pain regulatory system, particularly in SHRs.

The increased locomotor activity along the wall just after heat stimulation probably reflected the occurrence of emotional behavior as characterized by escape. Such a behavioral change should have had evoked much higher level of excitation in rats' cardiovascular activity, particularly in SHRs, which would stimulate baroreceptors within aorta much stronger than usual: This means that potentiated afferentation originating in baroreceptors would promote activation for the pain regulatory system. This internal stimulation is likely to play an important role in elevating SHRs' activity level.

Although it remains still unclear whether the hyperactivity in SHRs may result from the pain regulatory system or not, the study of SHRs' behavior has a clinical importance because SHRs might be one of animal models of attention-deficit hyperactivity disorder (Sagvolden, Pettersen, & Larsen, 1993), characterized by persistent patterns of inattention and/or hyperactivity-impulsivity (Diagnostic and statistical manual of mental disorders, fourth edition; American Psychiatric Association, 1994).

Conclusion

The results of this study indicated that the painful heat stimulation, elicited jumping behavior in the SHRs at the higher temperature on the hot plate than in the WKYs, had a significant effect on the SHRs' activity to produce an increase in the number of crossings at the outer area of the open-field apparatus. This increase in the crossings reflected a kind of escape behavior associated with the activation of the pain regulatory system, particularly in the SHRs.

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