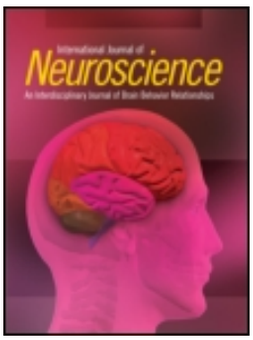

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IMMUNOLOGICAL AND BEHAVIORAL EFFECTS OF FRAGRANCE IN MICE

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The aim of this study was to determine the effects of olfactory stimulation on immunological and behavioral states in mice. Anti-SRBC (IgM) plaque forming cell (PFC) count and spontaneous running activity (SRA) were measured to demonstrate the effects of exposure to a given fragrance. The decreased PFC count and thymic involution induced by high pressure stress in mice were recovered after exposing the stressed mice to the fragrance continuously for 4 days after the stress was given. The PFC and SRA also appeared to be maintained at normal levels by oflactory stimulation with the fragrance for 24 hrs after the given stress. The immunological suppression induced by high pressure stress was considered to be caused by the induction and activation of suppressor cells. However, exposure to the fragrance after the stress did not enhance suppressor activity. The restoration of the stress–induced immune suppression by olfactory stimulation was blocked by procain administration onto the oflactory cells.

Keywords: stress, olfactory, fragrance, plaque forming cell

Aggressive behavior can be experimentally induced by the removal of the olfactory bulb in animals (Yoshimura, Gomita & Ueki, 1974), and this phenomenon suggests that emotional behavior may be regulated by the olfactory bulb. The foreign molecules which are perceived as odors vary widely in structure but they are readily detected and identified. However, the mechanism by which these different odors are identified by the olfacactory system is not clear. The olfactory epithelium, where the odor receptor neurons are located, has a dozen separate types of receptors (Kashiwayanagi & Kurihara, 1984), each of which shows a different degree of reactivity towards different odors (Kashiwayanagi & Kurihara, 1985). This olfactory epithelium may be responsible for odor detection.

We have previously demonstrated that various forms of stress applied to mice influenced humoral and cellular immune responses (Fujiwara, Tanaka & Orita 1985; Fujiwara & Orita, 1987; Fujiwara Orita & Yokoyama, 1989). This study attempted to determine the influence of odors on the restoration of emotional, behavioral and biological responses which are altered by stress. Mice showing immunological and behavioral changes induced by high pressure stress. Mice were exposed to an aromatic fragrance, and the recovery from these stress–induced changes was investigated.

MATERIALS AND METHODS

BALB/c male mice, 8–10 wks old, were used throughout the experiments and were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan.

Spleen cell preparation: The spleen was removed aseptically and the cells were finely minced in RPMI 1640 medium, and were passed through a No. 150 wire mesh. The cells obtained were washed three times in phosphate–buffered saline (PBS; pH 7.4), and then were hemolyzed by adding 0.75% Tris–ammonium chloride solution (ph 7.65). The cells were washed again three times and were suspended in RPMI 1640 at a concentration of 1×10^7 cells/ml.

Plaque forming cells (PFC) for sheep red blood cells (SPBC) were measured in vivo and in vitro tests. In the PFC test in vivo, SRBC (2×10^8) were injected into the tail vein of the mouse immediately after exposing it to high pressure stress. Five days after the immunization, the spleen was removed and assessed for anti-SRBC IgM PFC according to the method of Cunningham and Azenberg (1968). In the PFC test in vitro, the spleen of the mouse was removed at 24 hrs after the stress and the spleen cells were used for further experiments. Spleen cells were isolated and the cell suspension was divided into two groups. One of the two groups of the cell suspension was used as regulator cells. These cells were treated with 25 μg/ml mitomycin C(MMC) at 37° C for 30 min. The MMC–treated cell suspension was mixed with a spleen cell suspension from non–stressed mice and a SRBC suspension. The resulting cell suspensions were adjusted to a concentration of 5×10^6/well. The second cell suspension group was used as effector cells and was mixed with a suspension of SRBC. The mixture was then cultured for 5 days according to the method of Mishell and Dutton (1967) and assayed for PFC. The cell mixture was cultured in RPMI 1640 supplemented with 25 mM HEPES, 300 μg/ml of L-glutamine, 100 μg/ml of L-glutamine, 100 μg/ml of streptomycin, 5×10^{-5} M 2-mercaptoethanol and 10% fatal bovine serum (FBS).

A chamber (diameter 125 mm, length 180 mm, volume 2210 cm^3) was used in applying high pressure to mice. Three mice were put into one chamber and were exposed to 2.2 kg/cm^3 pressure of compressed air for 60 min, once a day, for 2 days. Control mice were placed in this chamber without exposure to pressure.

The mice exposed to an aromatic fragrance (Aroma Mini derived from various woods, Japan Fitness Co., Osaka) in an environmentally controlled cage with a clean air system.

A 2% procain solution was sprayed into the nasal cavity after the given stress but before the exposure of fragrance.

Behavioral change induced by stress was also evaluated by measuring the spontaneous running activity of mice in a photocell counter (300 × 300 × 150 mm).

RESULTS

The mice injected with SRBC immediately after exposure to high pressure stress showed a significant reduction of PFC in the spleen cells on day 5 (Figure 1). However, PFC was restored by exposing the mice to the fragrance before or during the stress. When the mice were exposed to the fragrance after the stress, PFC was markedly enhanced.

Mice immunized with SRBC immediately after the given stress were exposed to the fragrance for 24 hrs and the PFC test in vivo was completely restored to the control level on day 5 (Figure 2). Thymic involution of the mice after the stress disappeared after exposure to the fragrance.

The effect of the fragrance on the suppressed PFC production induced by high pressure stress was studied in vitro. Spleen cells of mice with suppressor activity for PFC production appeared at 24 hrs after the high pressure stress. However, the

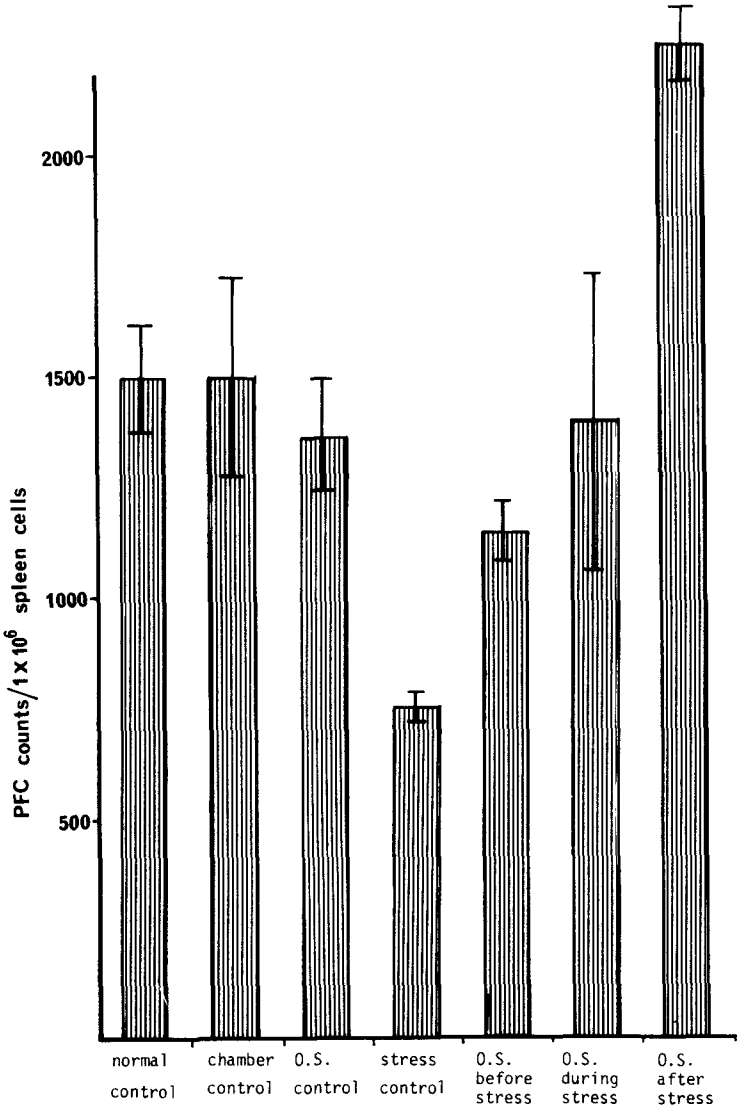

FIGURE 1 Effects of fragrance (O.S.) on PFC counts suppressed by high pressure stress in mice on day 5. PFC counts was enhanced by the exposure of the mice to the fragrance before, during and after the stress.

suppressor activity in the spleen cells and thymic involution disappeared after the mice were exposed to the fragrance for 24 hrs after the given stress (Figure 3).

In order to demonstrate the effect of fragrance on immune suppression via the reduction of PFC induced by high pressure stress, the duration of the exposure to the fragrance was experimentally varied. The exposure to the fragrance was carried out for either a 4 hr or 24 hr period, after the high pressure stress. Immune suppression with a reduction of PFC had been observed at 24 hrs after the stress in a previous experiment, and therefore the PFC was examined after exposure to the fragrance for

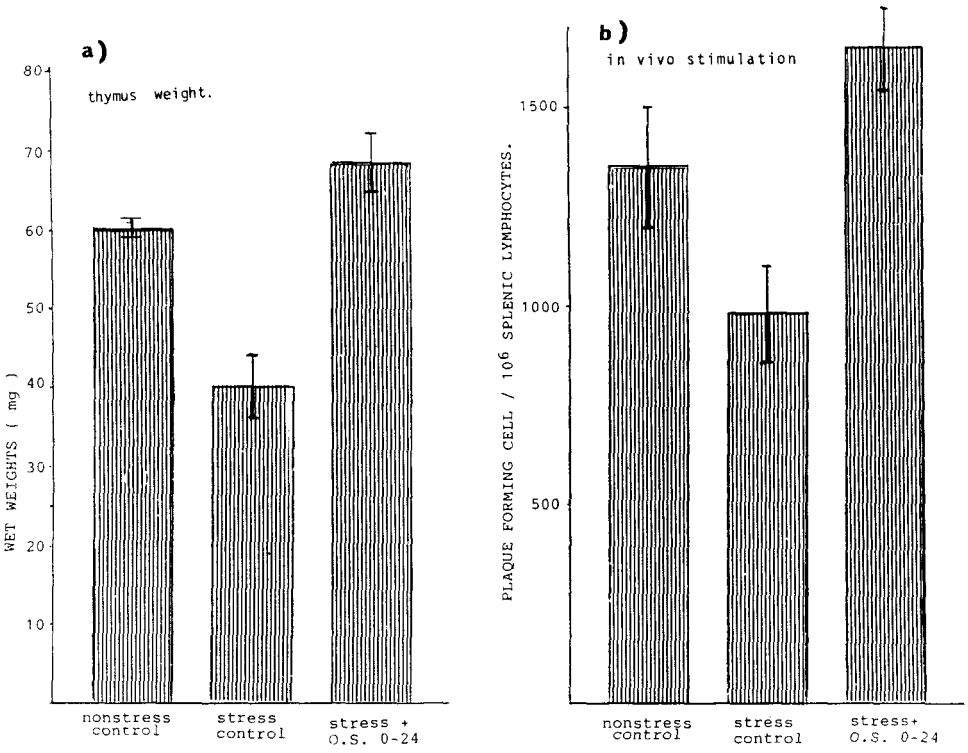

FIGURE 2 Effect of fragrance (O.S.) applied for 24 hrs immediately after the stress was given. The thymic involution (a) and the suppression of PFC production (b) induced by high pressure stress were observed on day 5. The thymic involution and in vivo PFC production were reconstituted by the exposure of fragrance.

a 4 hr period during the first 24 hrs after the stress. Three different groups of mice which had been immunized with SRBC immediately after the stress were exposed to the fragrance for 4 hrs during the periods 4th–8th, 10th–14th and 16th–20th hr, respectively, after the stress. The results revealed that the group exposed to the fragrance at the 4th–8th and 10th–14th hr were found to have the PFC restored to the normal level, but no recovery was seen in the 16th–20th hr exposure group (Figure 4). The data suggests that the suppressed immune response induced by high pressure stress was restored to normal levels by exposure fragrance during to the period between the 4th and 14th hrs after the stress.

Thereafter, an additional study was carried out to find whether the effect of fragrance on the suppressed immune response can be blocked by pretreatment of the olfactory cells in the nasal cavity of mice with procain. As shown in Figure 5, the suppression of PFC induced by stress was blocked by the exposure of fragrance during the 4th, 8th, 10th and 14th hrs after the stress, but the effect was absent when mice were pretreated with 2% procain sprayed onto the olfactory cells.

In order to evaluate the behavioral change induced by stress, spontaneous running activity was measured by a photocell counter. Running activity in mice was enhanced by high pressure stress, however, the enhancement of running activity was reduced by exposure to the fragrance (Figure 6).

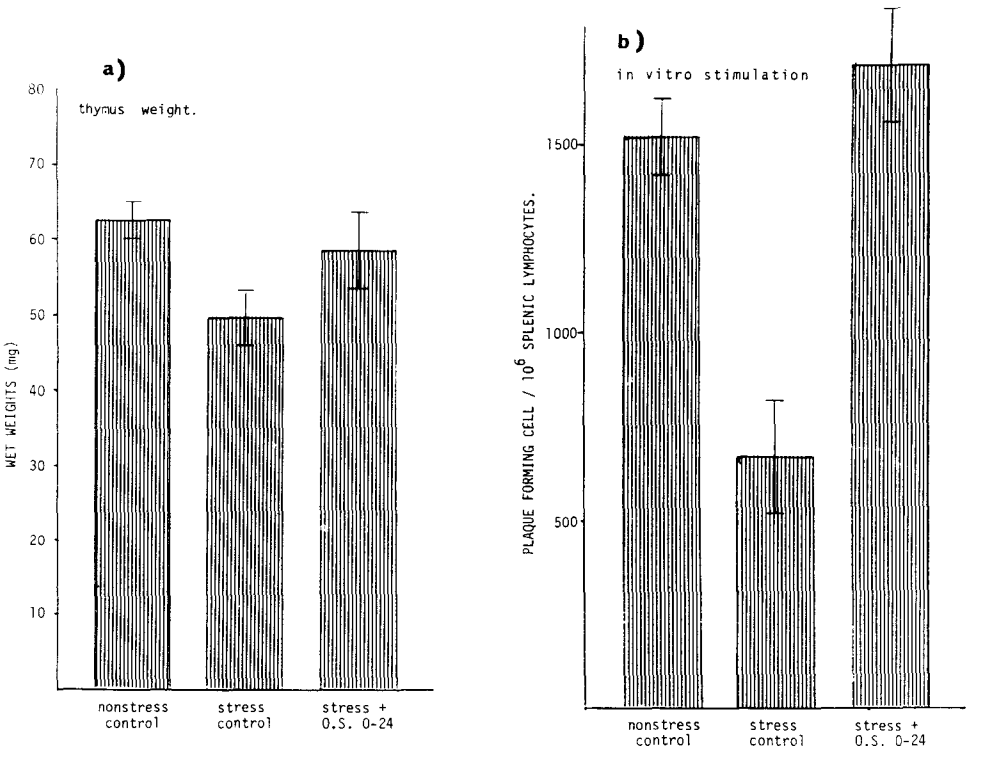

FIGURE 3 Effects of fragrance (O.S.) on thymic involution (a) and suppressed PFC production induced by high pressure stress in mice. The thymic involution was observed 24 hrs after the stress and the thymus weight was recovered some extent by the exposure of fragrance for 24 hrs after the stress. In the citro PFC test, the PFC count of spleen cells (responder cells) of non-stressed mice for 4 days stress and the count tested on day 5 was recovered by the exposure of fragrance for 24 hrs after the stress.

DISCUSSION

The results indicate that the exposure to an aromatic fragrance may block the suppression of the immune response induced by stress by causing the disappearance of supressor cells, and that the behavioral alteration induced by stress was blocked by the fragrance as well.

Many legends have been handed down regarding aromatherapies using various aromatic fragrances. However, very little information has been provided to scientifically prove the efficacy of these legends. For example, Rovesti and Colombo (1973) reported that the sniffing of some fragrances, e.g. mint, lavender, lemon or jasmin etc., improved the symptoms of patients in anxious or depressive states. They then suggested that the fragrance served as a tranquilizing drug in neuropsychosis. Arora, Taneja and Sharma (1973), reported that smelling musk oil inhibited inflammation, and it is well known that inflammatory reactions and the products from inflammation enhance the immune responses (Yoshinaga, Nakamura & Hayashi, 1975). In the case of pain stimulation, PFC production was enhanced, but with exposure to fragrance the immune response returned to normal (Shibata, Fujiwara, Shichijo & Yokoyama,

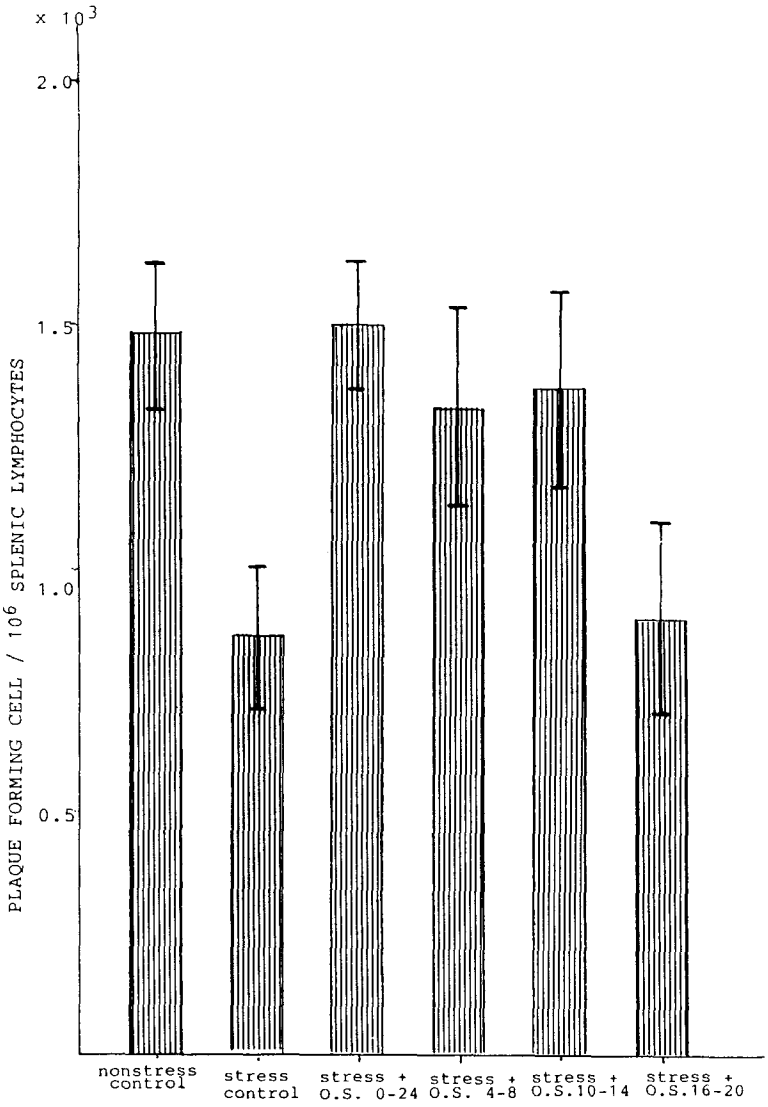

FIGURE 4 Effect of fragrance (O.S.) on suppressed PFC production induced by high pressure stress in mice. The exposure of fragrance was carried out for 24 hrs and 4 to 8, 10 to 14 and 16 to 20 hrs periods after stress was given.

1989). Various stressful events, e.g. inflammation, pain, and neurosychosis were therefore found to possibly be inhibited by exposure to fragrance. The present results revealed that exposure to the fragrance was effective against some forms of physical and emotional stress. We have previously studied (Fujiwara, et al. 1985; Fujiwara & Orita 1987; Fujiwara, et al. 1989;) the influence of the neuroendocrine system on immune responses in mice given pain stimulation, and the results have demonstrated that the stimulation of the neuroendocrine system alters the immune resposes. The alteration was considered to be due to the activation of regulatory helper or suppres-

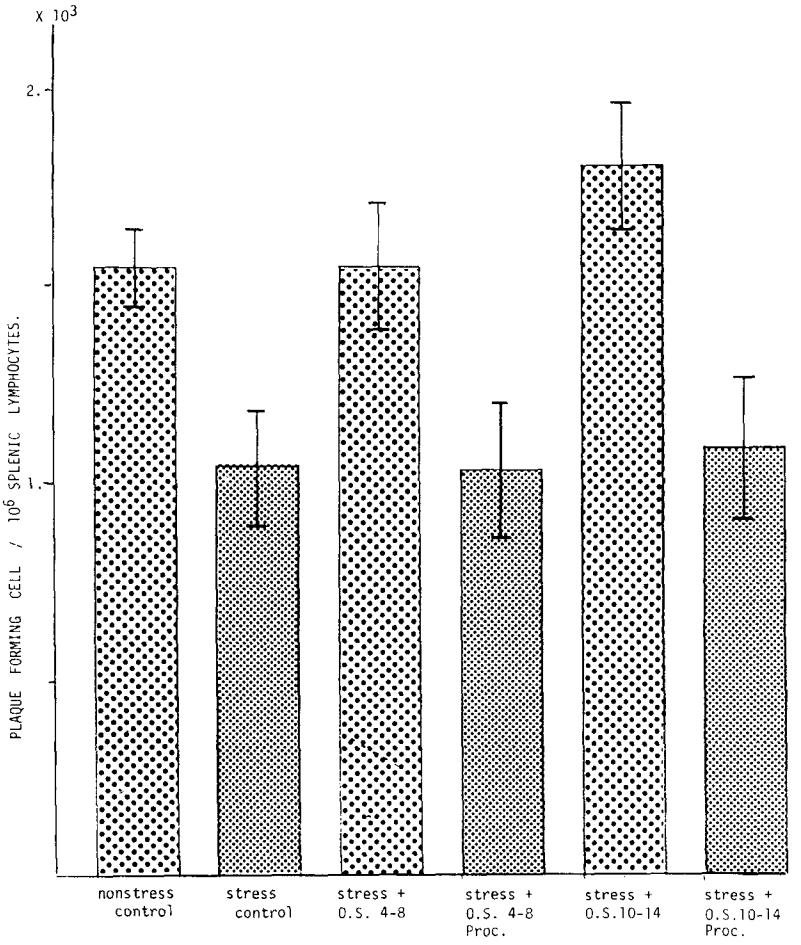

FIGURE 5 Effects of procain on suppressed PFC production restored by exposure of fragrance in mice. The exposure of fragrance during 4 to 8 and 10 to 14 hrs periods after the stress was not effected in PFC production after the stress was not effected in PFC production after procain was administrated.

sor T lymphocytes, via mediators from the neuroendocrine system e.g., adrenaline or glucocorticoid etc. Therefore, responses against stress were divided into two types, those involving thymic involution and those that did not. It is known that a stress involving thymic involution suppresses the immune response due to the activation of suppressor T cells via glucocorticoid released from corticoid of the adrenal gland (Munster, 1976). It is also known that a stress not involving thymic involution enhances immune response due to activation of helper T cells via the effect of adrenaline released from medulla of the adrenal gland (Fujiwara, et al., 1985; Fujiwara & Orita, 1987). Thus, we suggest that the alteration of immune response induced by various stress was due to the activation of either suppressor or helper T cells via chemical mediators which are released from the neuroendocrine system. In this study, the stress induced by exposure to high pressure was found to involve thymic involution and reduce PFC production via the activation of suppressor T cells in mice. The results showed that this response to stress involving thymic involution was

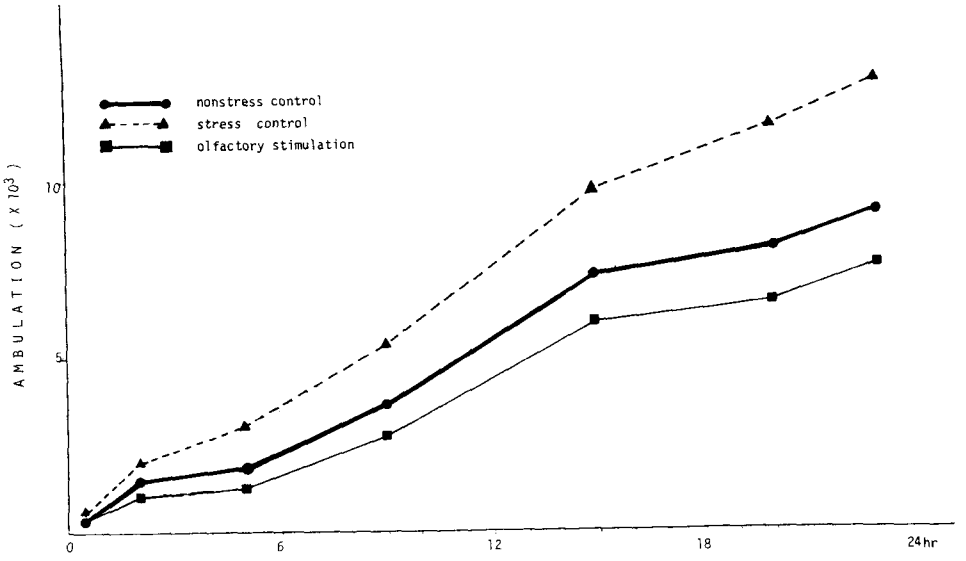

FIGURE 6 Effects of fragrance (O.S.) on enhancement of spontaneous runing activity induced by high pressure stress in mice.

blocked by the exposure to fragrance. We have previously observed (Fujiwara & Orita, 1987) that the enhancement of PFC production induced by stress not involving thymic involution, e.g. pain stimulation, was also blocked by fragrance (Shibata, et al. 1989). These facts indicate that fragrances may exhibited an antistress effect. It is also known that various tranquilizers block immun suppression induced by stress (Pericic, Manev, Boranic, Poljak-Blazi & Cakic 1987) and the effect of tranquilizers alter the biological rhythem of the body, is found to be due to the inhibition of emotional disorders induced by stress in limbic system. This accords with chemical meditators which were not released from the neuro-endocrine system (Lister & Nutt, 1986). Similarly, it is suggested that disorders induced by stress were blocked by the exposure to a fragrance which was modulated by the central nervous system. It could also be assumed from these results, that the limbic system is innervated by nerve fibres (Johnson, 1959) from the olfactory bulb, and that the activation of the limbic system induced by stress was therefore blocked by the olfactory stimulation with an aromatic fragrance.

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