



“Green odor” inhalation by stressed rat dams reduces behavioral and neuroendocrine signs of prenatal stress in the offspring

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ABSTRACT

Chronic maternal stress during pregnancy results in the “prenatally stressed” offspring displaying behavioral and neuroendocrine alterations that persist into adulthood. We investigated how inhalation of green odor (a mixture of equal amounts of trans-2-hexenal and cis-3-hexenol) by stressed dams might alter certain indices of prenatal stress in their offspring. These indices were depression-like behavior (increased immobility time in the forced-swim test) and acute restraint stress-induced changes in hypothalamo–pituitary–adrenocortical (HPA) axis activity [plasma corticosterone (CORT) and ACTH levels and the number of Fos-immunoreactive cells in the hypothalamic paraventricular nucleus (an index of neuronal activity)]. Pregnant rats were exposed to restraint stress for 60 min/day for 10 days (gestational days 10–19). The prenatally stressed offspring exhibited significant increases in depression-like behavior and in restraint stress-induced ACTH, CORT, and Fos responses, unless their dam had been exposed to green odor. The behavioral effect of the odor was also seen in offspring that were fostered by unstressed dams. The results obtained in the dams themselves were as follows. In vehicle-exposed stressed dams, but not in green odor-exposed ones, total body and adrenal weights were significantly decreased or increased, respectively. Depression-like behavior was not observed in the vehicle-exposed stressed dams themselves. Green odor inhalation prevented the impairment of maternal behavior induced by restraint stress. Thus, exposure of dams to stress may affect both the fetal brain and fetal HPA axis, and also maternal behavior, leading to altered behavioral and neuroendocrine responses in the offspring. Such effects may be prevented by the stressed dams inhaling green odor.

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Introduction

Events that are stressful to pregnant mothers also induce “stress” in the fetus (prenatal stress) and can lead to long-term physiological/behavioral alterations in the offspring (Darnaudéry and Maccari, 2008; Lupien et al., 2009; Mastorci et al., 2009; Weinstock, 2008). For example: (a) prenatally stressed animals exhibit increases in anxiety- and depression-related behaviors together with basal and/or stress-induced activation of the hypothalamo–pituitary–adrenocortical (HPA) axis and (b) chronic treatment of such prenatally stressed rats (but not of the controls) with an antidepressant can prevent both depression-like behavior in the forced-swim test and HPA axis dysregulation in adulthood (Darnaudéry and Maccari, 2008; Weinstock, 2008). Further, it has been pointed out that excess maternal glucocorticoid may be responsible for the alterations in the offspring’s behavior and HPA activity (Darnaudéry and Maccari, 2008; Lupien et al., 2009; Mastorci et al., 2009; Weinstock, 2008). The idea that maternal glucocorticoids may pass through the placenta to increase fetal HPA axis activity and modify fetal brain development is supported by the finding that

controlling glucocorticoid levels in stressed dams, by adrenalectomy and hormone replacement, prevent prenatal stress having these effects on the offspring (Barbazanges et al., 1996).

Recently, Nakashima et al. (2004) found that in rats, inhalation of “green odor” (extracted from green leaves) attenuated stress-induced elevations in plasma ACTH. A few years later, Ito et al. (2009) found, also in rats, that green odor inhalation reduced the stress-induced increases in plasma corticosterone (CORT) as well as the stress-induced activation of neurons located within the hypothalamic paraventricular nucleus (PVN). These results indicate that so-called green odor may inhibit the activation of the HPA axis that occurs during stress. On the basis of the above evidence, we hypothesized that green odor inhalation by a stressed dam might limit or prevent the stress-induced increase in glucocorticoid levels in the maternal plasma, and thereby limit or prevent any future alterations in the HPA axis and/or behavior of the offspring.

Here, we tested this hypothesis, in rats, by examining the effects of green odor inhalation by stressed dams (a) on certain prenatal stress-induced changes in the offspring, namely, increased HPA axis activity and depression-like behavior (increase in immobility time in the forced-swim test), (b) on maternal behavior and depression-like behavior in the dams, and (c) on the stress-induced decrease in total body weight and increase in adrenal weight in the dams. Smith et al. (2004) showed

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that rat dams exposed to restraint stress (1 h/day, on days 10–20 of gestation) displayed increased depression-like behavior in the forced-swim test as well as decreased maternal behavior. Conceivably, such reduced maternal behavior might be responsible for the neuroendocrine and behavioral changes observed in the prenatally stressed offspring. Therefore, in additional experiments, neonate offspring were fostered by dams that had not been stressed during pregnancy to exclude the possible influence of changes in the maternal behavior of the stressed dams on the observed variables.

Materials and methods

Green odor

The green odor used here was a mixture of equal amounts of trans-2-hexenal and cis-3-hexenol, diluted with triethyl citrate to 0.03% (w/w). This concentration of green odor has been reported to be effective in reducing the increase in the plasma concentration of ACTH induced by acute immobilization stress (Nakashima et al., 2004). Furthermore, a mixture of the above two compounds is more potent than either compound alone (Sano et al., 2002).

Experimental animals and prenatal treatment

Wistar rats were housed in a room maintained at 22 ± 1 °C with a photoperiod of 12-h light:12-h dark (lights on at 7:00 a.m.). All had ad libitum access to drink and standard laboratory rat chow. Three females (5–6 weeks old) were placed overnight with a single male (7 weeks old) for mating, and a vaginal smear from each female was examined the following morning. The day on which the smear was sperm positive was taken as day 0 of pregnancy. Each pregnant rat was then housed separately in a plastic home cage (40×25×20 cm; length×width×depth) with wood-chip bedding for later experimentation. The protocols were reviewed by the Committee on the Ethics of Animal Experiments in Tottori University Faculty of Medicine, and the experiments were carried out in accordance both with the Guidelines for Animal Experiments at Tottori University Faculty of Medicine and with the Federal Law (no. 221) and Notification (no. 6) issued by the Japanese Government.

On gestational days 10–19, pregnant rats were restrained as required in a small cylindrical restrainer made from steel wire [7 cm×22 cm (diameter×length)]. This was done for 60 min (1200 to 1300 h) each day. Green odor or its vehicle (i.e., triethyl citrate) was administered throughout the stress period (i.e., for 60 min). To this end, a cotton bowl (diameter = 15 mm) was impregnated with 0.2 ml of green odor or its vehicle then held 3 cm from the nose of the pregnant rat. When green odor was to be administered to freely moving rats, the same type of bowl impregnated with 0.2 ml of green odor was held in the restrainer mentioned above, and this was placed in one corner of the rat's "home cage". Control pregnant dams were left undisturbed in their home cages and a restrainer was not placed in the control rat's "home cage". Since the restrained rats were unable to reach the food and water, all pregnant rats were deprived of food and water from 1200 to 1300 h each day during the stress-exposure period.

After delivery, a dam and its own litter (of both sexes) were housed together in a single cage (40×25×20 cm; length×width×depth) with wood-chip bedding. In some cases, fostering was carried out. In brief, pups were fostered by other (non-stressed) dams in order to examine whether postnatal factors, such as stress-induced changes in maternal behavior in the pre-weaning period, might be involved in the observed effects seen in the offspring. At birth, six male pups plus 4–6 female pups per dam were randomly selected to be raised by foster dams that had not been stressed during pregnancy. The pups were transferred to the cage of the foster dam within the first 3–6 h after birth. During this procedure, the foster dams were briefly (for less than 1 min) removed from their cages.

Male pups, whether raised by their biological mother or by a foster mother, were weaned on postnatal day 25 and housed in a cage (40×25×20 cm; length×width×depth) in groups of five. For subsequent experimentation, at 8–9 weeks of age (250–300 g), the male offspring were divided into groups of 5–22 (obtained from at least three dams).

Offspring were divided into the following four groups. In the vehicle + stress and green + stress groups, vehicle or green odor was administered to their dams during stress-exposure. In the green group, green odor was administered to dams without imposing stress, while in the control group, nothing was administered at all to their dams, nor were the dams subjected to restraint.

Experimental protocols for biological dams

Forced-swim test (FST) and measurement of total body weight and adrenal weight

On postnatal days 3 and 4, randomly selected biological dams were temporarily removed from their litters to assess stress-induced depression-like behavior. This was done using a forced-swim test (Porsolt test) (Porsolt et al., 1978; Smith et al., 2004). Dams were placed in a 50-cm-diameter cylinder containing water 40 cm deep (25 ± 1 °C). After remaining in the water for 15 min, they were returned to their home cages. Then, 24 h later, they were put back in the same cylinder and subjected to a 5-min swim trial. We monitored the time spent immobile (a minimal amount of effort being used to stay float; Smith et al., 2004) during a 15-min trial on day 3 and a 5-min trial on day 4. This procedure was carried out between 0930 and 1500 h. The initial exposure on day 3 is designed to evoke a "helpless" response on the subsequent test day, and therefore data analyses were performed on the results obtained on day 4.

After the FST, the animals were given a lethal dose of sodium pentobarbitone. The rat's body weight and the combined weight of its two adrenal glands were measured.

Assessment of maternal behavior

In a separate experiment, maternal behavior was studied between 0830 and 1130 h on gestational days 2, 4, and 6. The experiment was begun by removing the pups from the nest and putting them at the opposite corner of the cage. This was done without changing anything in the cage; in particular, the nest was not destroyed. Then, for a 30-min period the maternal behavior was recorded using a video recorder. Our screening of maternal behavior (nursing, licking pups, nest building, sniffing, retrieving) was based on the screening described by Patin et al. (2002) and Akuta (1979).

The pups of the stressed dams used in the above experiments were excluded from the studies described below.

Experimental protocols for pups

Forced-swim test (FST)

Prenatal stress-induced depression-like behavior in the offspring was assessed using a forced-swim test (Porsolt test; see above). For this, we used male offspring (8–9 weeks old) raised either by their "biological" mother or by a "foster" mother.

Intravenous-cannulation surgery and assessment of plasma ACTH and CORT levels

To permit blood sampling, separate groups of male offspring (8–9 weeks old) were anesthetized with pentobarbital sodium (50 mg/kg i.p.), and a polyvinyl tube was inserted into the jugular vein so that its tip lay in the superior caval vein near the right atrium (Harms and Ojeda, 1974). The free end of the catheter was passed subcutaneously to the midscapular region, where it was exteriorized dorsally behind the neck. It was kept patent by flushing it every day with heparinized 0.9% saline (50 U/ml). This

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