Original Article

# Stress-reactive rats (high-avoidance female rats) have a shorter lifespan than stress-nonreactive rats (low-avoidance female rats)

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**Abstract:** Although Hatano high-avoidance and low-avoidance rats (HAA and LAA, respectively) have been selectively bred for good versus poor avoidance learning, HAA rats are known to be more reactive to stress than LAA rats. In this study, HAA and LAA female rats were compared during reproductive aging by observing estrous cycles from 8 to 11 months of age. Furthermore, these rats were allowed to live out their natural lifespans, that is, until 24 months of age, in order to compare their survival and to clarify the relationship between reproductive aging and tumor development. At eight months of age, 2 of 35 HAA rats and 20 of 35 LAA rats had abnormal estrous cycles. The median lifespan of the HAA rats (673 days) was shorter than that of the LAA rats (733 days). The incidence of pituitary neoplasia was higher in the HAA rats than in the LAA rats. These results suggest that HAA female rats (i.e., stress-reactive rats) have a shorter lifespan than LAA female rats (i.e., stress-nonreactive rats) and develop pituitary neoplasia, which was one of the causal factors in their accelerated mortality. However, the onset of an age-matched abnormal cycle did not correspond with their lifespan. (DOI: 10.1293/tox.2015-0045; J Toxicol Pathol 2016; 29: 77–84)

**Key words:** aging, estrous cycle, neoplastic and non-neoplastic lesions, incidence, inbred strain, Sprague-Dawley rat

## Introduction

Cavigelli and McClintock[1] found that male Sprague-Dawley rats identified as fearful of novelty, with a greater glucocorticoid response to novelty during infancy, died sooner than their less fearful brothers. Furthermore, Cavigelli *et al.*[2] demonstrated that the least exploratory (neophobic) female Sprague-Dawley rats as infants died about 6 months earlier than their most exploratory (neophilic) sisters did and that the ovarian function of the neophobic females aged more rapidly than that of their neophilic sisters did. These results suggest that, in the lifespan of rats, there are individual differences related to stress reaction and ovarian steroids.

Although Sprague-Dawley rats are generally used in toxicological studies, it is known to be easy to disturb their estrous cycles during middle age[3, 4] and that they develop spontaneous mammary and pituitary tumors[5–9]. Therefore, Sprague-Dawley rats are considered a powerful model for understanding the relationship between reproductive aging

and tumor development in female rats[2]. It is also expected that the cause of death can be determined more easily in female rats than in male rats. This strain is maintained as an outbred closed colony without selection. They are not uniformly homozygous; that is, they are not inbred.

Hatano rats have been bred from Sprague-Dawley rats on the basis of their active avoidance learning in a shuttle-box task[10]. High-avoidance (HAA) rats show a high rate of avoidance response, and low-avoidance (LAA) rats show a low rate of avoidance response. The HAA and LAA strains are the result of more than twenty consecutive generations of "sister × brother" mating. As a result, all individuals in these two inbred strains are homozygous, or genetically identical. Phenome data on these two strains are available from the National BioResource Project for the Rat (http://www.anim.med.kyoto-u.ac.jp/NBR/).

Although HAA and LAA rats have been selectively bred for good versus poor avoidance learning, HAA rats are known to have larger adrenal glands and to be more responsive to stress than LAA rats[11]. For instance, gastric erosion under restraint stress in water occurs at a higher rate in HAA rats than in LAA rats[12]. In addition, the effect of acute restraint stress on plasma ACTH levels is higher in HAA rats than in LAA rats[13]. Furthermore, it is known that prolactin plays an important role in the regulation of corticosterone release during stress in LAA rats, but not in HAA rats[14]. A recent study revealed that HAA rats showed a higher anxiety-like behavior in an elevated plus maze test than LAA

rats[15]. However, all of the HAA rats showed a regular 4-day estrous cycle, whereas most of the LAA rats (70.8%) exhibited prolonged diestrus and a regular 5-day cycle[16]. Regarding reproductive endocrinology during the estrous cycle, the LAA rats showed a smaller LH surge and a larger prolactin surge on the day of proestrus, accompanied by lower estradiol-17β secretion, compared with the HAA rats[16]. A subsequent *in vitro* study suggested that the difference in ovarian function between the two Hatano strains was due to a difference in regulation of the hypothalamic-pituitary axis for gonadotropin secretion[17]. There are, however, some differences between HAA rats and LAA rats in behavioral and endocrinological characteristics that are within the normal range of variation in the original Sprague-Dawley rats.

It is possible that a difference in lifespan between the two Hatano strains may be observed if HAA and LAA rats are observed over a long period. In order to evaluate this possibility, we examined reproductive aging and lifespan using HAA (as stress-reactive rats) and LAA (as stress-nonreactive rats) female rats, and we were able to verify the hypothesis suggested by Cavigelli *et al.*[2]

## Materials and Methods

HAA rats of the 60th generation and LAA rats of the 58th generation, which were maintained by sib-mating at the Hatano Research Institute, were used in the present study. Thirty-five healthy adult female rats of each strain (between 25 and 30 weeks old) were kept individually in metal cages with a metal meshed floor (220 mm (w) × 270 mm (d) × 190 mm (h)) in an animal room maintained with a room temperature of 22–25°C, a relative humidity of 50–65%, and 12-h lighting (lighting period: 7:00–19:00). Feed (CE-2 pellet feed, CLEA Japan) and tap water were available *ad libitum*. The animal protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the Food and Drug Safety Center (FDSC) and were carried out in compliance with the Guidelines for Animal Experiments at Hatano Research Institute, FDSC.

The general condition of all of the rats was observed once a day. In particular, visible or palpable masses were checked for carefully, and the dates on which masses were observed and their sites and sizes were noted. Vaginal smears were collected from all of the rats on each day during every other two-week period from 8 months to 11 months of age, as it was expected based on a previous study[18] that an age-matched abnormal cycle would appear in Sprague-Dawley rats at this time. Based on the cell types observed in the vaginal smears, the estrous cycles were categorized as normal cycles (regular 4- or 5-day cycles) or age-matched abnormal cycles, which included long cycles (cycles of 6 days or more), persistent estrus (a minimum of 3 consecutive days of estrus or proestrus), and constant diestrus (noncycling and anestrus).

To determine survival rate, all of the rats were allowed to live out their natural lifespans, that is, until 24 months of age. Those rats still alive at 24 months of age were ex-

**Table 1.** Summary of the Clinical Signs Observed in the HAA and LAA Female Rats

| Clinical signs | LAA | HAA |
|---|---|---|
| Subcutaneous mass | 22 | 10 |
|     Thoracic | 4 | 1 |
|     Abdominal | 8# | 3 |
|     Armpit | 6 | 1 |
|     Other | 4 | 5 |
| Decreased feces | 8 | 5 |
| Cataract | 2 | 0 |
| Malocclusion | 2 | 0 |
| Reddish urine | 0 | 5 |
| Soiled fur | 0 | 3 |
| Loss of fur | 0 | 1 |

Numerals represent the number of animals. #Including three animals that showed two or more masses.

sanguinated under sodium pentobarbital anesthesia and subjected to necropsy. Moribund rats and rats that died before 24 months of age were also subjected to necropsy. Histopathological examination was performed in a routine manner on the following organs and tissues of all the rats: skin and subcutaneous tissues, mammary glands, brain, spinal cord, pituitary gland, submandibular glands, Harderian glands, tongue, Zymbal's glands, thyroid glands, parathyroid glands, thymus and mediastinal lymph nodes, aorta, trachea, lungs and bronchi, heart, liver, spleen, pancreas, kidneys, adrenal glands, esophagus, stomach, intestines, urinary bladder, ovaries, uterus, vagina, clitoral gland, sciatic nerves, skeletal muscles, eyes, optic nerves, femurs, bone marrow, submandibular and mesenteric lymph nodes, and other organs or tissues with macroscopically abnormal lesions. All organs and tissues were fixed in 10% neutral buffered formalin. Excised specimens were processed as paraffin blocks, and 3 to 5 μm sections of every specimen were obtained. Sections were stained with hematoxylin and eosin in the routine manner and subjected to histopathological examination.

## Results

### General conditions

A summary of the clinical signs observed in both the HAA and LAA rats is shown in Table 1. Although subcutaneous masses were found in 22 of the 35 LAA rats, such masses were found in fewer than half that many HAA rats (10 of 35 rats). The times of earliest occurrence of visible masses were 57 weeks and 67 weeks of age in the LAA and HAA rats, respectively. Regarding clinical signs, besides masses, decreased feces were observed prior to death in both strains. At necropsy, a pituitary tumor was often found in the animals that showed decreased feces. In addition, cataracts and malocclusion (two rats each) were observed in the LAA rats, and reddish urine and soiled fur (five and three rats, respectively) were observed in the HAA rats.
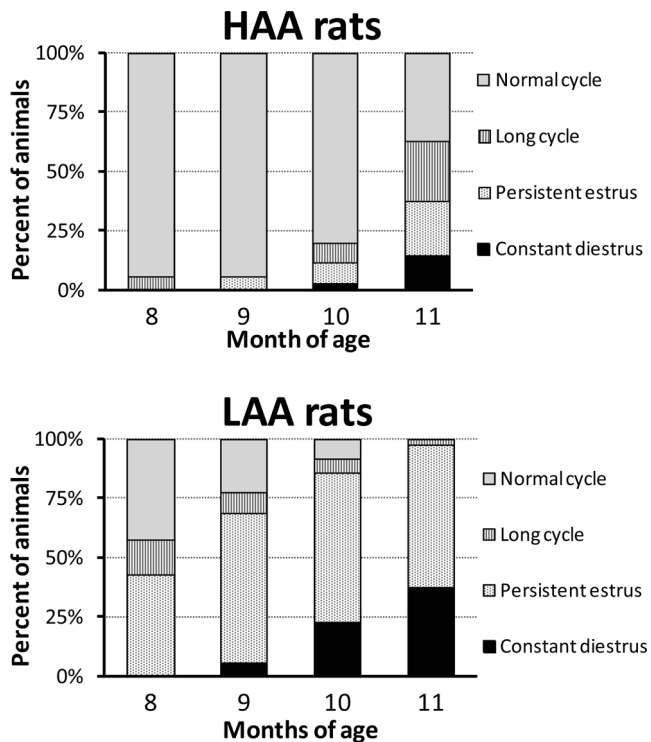
**Fig. 2.** Survival curves for the HAA and LAA female rats.

**Fig. 1.** Types of estrous cycles observed from 8 to 11 months of age in the HAA and LAA female rats.

was undetermined (6 cases).

*Histopathological findings*

A summary of the neoplastic and preneoplastic lesions in both the HAA and LAA rats is shown in Table 2. A neoplastic lesion was found in the pituitary gland in 23 of the 33 HAA rats examined and in 16 of the 35 LAA rats examined. The pituitary tumors were classified as either carcinomas or adenomas, and the incidence of adenomas was higher in the HAA rats. A neoplastic lesion was found in the mammary glands in 16 of the 34 HAA rats examined and in 23 of the 35 LAA rats examined. Regarding the types of mammary tumors, the incidence of the adenocarcinomas was higher in the HAA rats; however, the incidence of fibroadenomas was higher in the LAA rats. In the other organs, the incidences of thyroid C-cell adenomas and uterine endometrial stromal polyps were higher in the HAA rats than in the LAA rats. Hepatic foci of cellular alterations were frequently observed in the HAA rats. Clear cell foci and basophilic cell foci were observed in 5 and 4 of the HAA rats, respectively. However, these findings might be not related to stress reaction or sex hormones.

A summary of the non-neoplastic lesions in both the HAA and LAA rats is shown in Table 3. In the kidney, chronic progressive nephropathy (CPN) was observed in all of the LAA rats; however, it was observed in only three of the 35 HAA rats. Dilatation of the renal pelvis was observed in all of the HAA rats but in none of the LAA rats. In the thymus, cystic dilatation in the epithelial tubules and cords was observed in nearly all of the live HAA rats. In the thyroid gland, diffuse C-cell proliferation was frequently observed in the HAA rats. In the ovary, sex cord-stromal hyperplasia and Sertoli cells were observed in nearly all of the live HAA rats. In the uterus, the incidences of severe atrophy with fibrosis were similar in the live HAA and live LAA rats. In the adrenal glands, hypertrophy of the zona fasciculata was frequently observed in the HAA rats, suggesting a stress-related reaction. However, hypertrophy of the zona glomerulosa was frequently observed in most of

*Estrous cycles*

The types of estrous cycles observed from 8 to 11 months of age in both the HAA and LAA rats are shown in Fig. 1. Although 2 of the 35 (6%) HAA rats showed abnormal estrous cycles from 8 to 9 months of age, the number increased to 22 of the 35 rats (63%) at 11 months of age. On the other hand, 20 of the 35 (57%) LAA rats showed abnormal estrous cycles at 8 months of age, and all of the LAA rats showed abnormal estrous cycles by 11 months of age. In the HAA rats, long cycles (26%) and persistent estrus (23%) were frequently observed at 11 months of age. In the LAA rats, long cycles (14%) and persistent estrus (43%) were frequently observed at 8 months of age, and then persistent estrus (60%) and constant diestrus (37%) were frequently observed at 11 months of age.

*Survival curve*

The survival curves for both the HAA and LAA rats are shown in Fig. 2. The survival rate at 105 weeks of age was lower in the HAA rats (40%) than in the LAA rats (54%). The median survival time of the HAA rats (673 days) was about 2 months shorter than that of the LAA rats (733 days). The cause of death or moribund condition in 17 cases of the LAA rats was identified as renal lesion (7 cases), exhaustion causing large mammary tumor (5 cases), compression deformity of the cerebral base by pituitary tumor (4 cases), and nasal fracture (1 case). That in 21 cases of the HAA rats was identified as compression deformity of the cerebral base by pituitary tumor (12 cases) or malignant tumor (3 cases) or
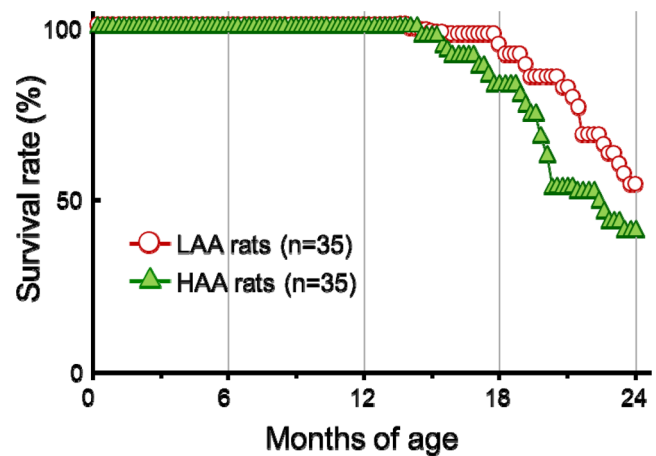
**Table 2.** Summary of the Neoplastic Lesions in the HAA and LAA Female Rats

| Neoplastic lesions | Live LAA | | Live HAA | | | Dead/sacrificed LAA | | Dead/sacrificed HAA | | | Total LAA | | Total HAA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pituitary gland | <18> | | <14> | | | <17> | | <19> | | | <35> | | <33> | | |
|   Carcinoma | 5 | (27.8) | 1 | (7.1) | | 2 | (11.8) | 5 | (26.3) | | 7 | (20.0) | 5 | (15.2) | |
|   Adenoma | 6 | (33.3) | 10 | (71.4) | * | 3 | (17.6) | 8 | (42.1) | | 9 | (25.7) | 18 | (54.5) | * |
|   Hyperplasia | 3 | (16.7) | 3 | (21.4) | | 5 | (29.4) | 2 | (10.5) | | 8 | (22.9) | 5 | (15.2) | |
| Mammary gland | <18> | | <14> | | | <17> | | <20> | | | <35> | | <34> | | |
|   Adenocarcinoma | 0 | (0.0) | 4 | (28.6) | | 1 | (5.9) | 4 | (20.0) | | 1 | (2.9) | 8 | (23.5) | * |
|   Adenocarcinoma arising in fibroadenoma | 1 | (5.6) | 1 | (7.1) | | 0 | (0.0) | 0 | (0.0) | | 1 | (2.9) | 1 | (2.9) | |
|   Adenoma | 0 | (0.0) | 0 | (0.0) | | 0 | (0.0) | 1 | (5.0) | | 0 | (0.0) | 1 | (2.9) | |
|   Fibroadenoma | 10 | (55.6) | 5 | (35.7) | | 11 | (64.7) | 1 | (5.0) | ** | 21 | (60.0) | 6 | (17.6) | ** |
|   Rhabdomyosarcoma | 1 | (5.6) | 0 | (0.0) | | 0 | (0.0) | 0 | (0.0) | | 1 | (2.9) | 0 | (0.0) | |
|   Hyperplasia | 4 | (22.2) | 1 | (7.1) | | 3 | (17.6) | 0 | (0.0) | | 7 | (20.0) | 1 | (2.9) | * |
| Thyroid gland | <18> | | <14> | | | <11> | | <14> | | | <29> | | <28> | | |
|   Adenoma, C-cell | 1 | (5.6) | 6 | (42.9) | * | 0 | (0.0) | 4 | (28.6) | | 1 | (3.4) | 10 | (35.7) | ** |
|   Carcinoma, follicular cell | 0 | (0.0) | 0 | (0.0) | | 1 | (9.1) | 0 | (0.0) | | 1 | (3.4) | 0 | (0.0) | |
|   Hyperplasia, C-cell | 3 | (16.7) | 4 | (28.6) | | 2 | (18.2) | 4 | (28.6) | | 5 | (17.2) | 8 | (28.6) | |
|   Hyperplasia, follicular cell, focal | 1 | (5.6) | 1 | (7.1) | | 0 | (0.0) | 0 | (0.0) | | 1 | (3.4) | 1 | (3.6) | |
| Parathyroid gland | <18> | | <13> | | | <14> | | <20> | | | <32> | | <33> | | |
|   Adenoma | 0 | (0.0) | 1 | (7.7) | | 0 | (0.0) | 0 | (0.0) | | 0 | (0.0) | 1 | (3.0) | |
| Liver | <18> | | <14> | | | <17> | | <21> | | | <35> | | <35> | | |
|   Adenoma, hepatocellular | 0 | (0.0) | 1 | (7.1) | | 0 | (0.0) | 0 | (0.0) | | 0 | (0.0) | 1 | (2.9) | |
|   Foci/area, cellular alteration | 2 | (11.1) | 8 | (57.1) | ** | 0 | (0.0) | 1 | (4.8) | | 2 | (5.7) | 9 | (25.7) | * |
| Kidney | <18> | | <14> | | | <17> | | <21> | | | <35> | | <35> | | |
|   Carcinoma, transitional cell | 0 | (0.0) | 0 | (0.0) | | 0 | (0.0) | 1 | (4.8) | | 0 | (0.0) | 1 | (2.9) | |
| Adrenal gland | <18> | | <14> | | | <17> | | <19> | | | <35> | | <33> | | |
|   Adenocarcinoma, cortical cell | 0 | (0.0) | 0 | (0.0) | | 0 | (0.0) | 1 | (5.3) | | 0 | (0.0) | 1 | (3.0) | |
|   Adenoma, cortical cell | 1 | (5.6) | 0 | (0.0) | | 0 | (0.0) | 1 | (5.3) | | 1 | (2.9) | 1 | (3.0) | |
|   Hyperplasia, medulla | 0 | (0.0) | 0 | (0.0) | | 1 | (5.9) | 0 | (0.0) | | 1 | (2.9) | 0 | (0.0) | |
|   Pheochromocytoma, malignant | 1 | (5.6) | 0 | (0.0) | | 0 | (0.0) | 0 | (0.0) | | 1 | (2.9) | 0 | (0.0) | |
| Uterus | <18> | | <13> | | | <17> | | <19> | | | <35> | | <32> | | |
|   Adenocarcinoma, endometrial | 1 | (5.6) | 0 | (0.0) | | 0 | (0.0) | 0 | (0.0) | | 1 | (2.9) | 0 | (0.0) | |
|   Adenoma, endometrial | 0 | (0.0) | 0 | (0.0) | | 1 | (5.9) | 0 | (0.0) | | 1 | (2.9) | 0 | (0.0) | |
|   Fibrosarcoma | 0 | (0.0) | 0 | (0.0) | | 0 | (0.0) | 1 | (5.3) | | 0 | (0.0) | 1 | (3.1) | |
|   Hyperplasia, glandular, focal/cystic | 2 | (11.1) | 0 | (0.0) | | 1 | (5.9) | 1 | (5.3) | | 3 | (8.6) | 1 | (3.1) | |
|   Polyp, endometrial stromal | 0 | (0.0) | 4 | (30.8) | | 0 | (0.0) | 4 | (21.1) | | 0 | (0.0) | 8 | (25.0) | ** |
|   Schwannoma, malignant | 0 | (0.0) | 0 | (0.0) | | 0 | (0.0) | 1 | (5.3) | | 0 | (0.0) | 1 | (3.1) | |

< >, number of animals examined. Values are expressed as number of animals with a neoplastic or preneoplastic lesion. The percentages of animals with the indicated neoplastic or preneoplastic lesion are shown in parentheses. Asterisks indicate significant differences from the incidences of LAA rats (*$p<0.05$ by Fisher's exact test; **$p<0.01$ by Fisher's exact test).

the LAA rats with CPN. In the circulatory organ system, periarteritis nodosa was observed in 16 of the 35 LAA rats but in only three of the 35 HAA rats.

## Discussion

Based on the hypothesis suggested by Cavigelli et al.[2], we compared the lifespans of HAA (stress-reactive) and LAA (stress-nonreactive) female rats. As a result, the lifespan of the HAA female rats was found to be shorter than that of the LAA female rats. This result agreed with the hypothesis suggested by Cavigelli et al.[2] that nervous female rats die sooner than their less nervous sisters.

Cavigelli et al.[2, 19] revealed that one of the causes of early death in nervous females is the occurrence of pituitary tumors. Similarly, the HAA (stress-reactive) rats showed compression deformity of the cerebral base by markedly enlarged pituitary gland, which was one of the causal factors in their accelerated mortality. It is known that pituitary tumors develop spontaneously in Sprague-Dawley rats[5–9, 20], which is a lethal lesion in rats because tumors of the pituitary can compress important brain structures as they enlarge[21]. HAA rats were selected from Sprague-Dawley rats for high avoidance learning. In this process, animals prone to development of pituitary tumors may have also been selected. Because the breeding program for HAA and LAA rats did not discard animals with a pituitary tumor, we might have included individuals that easily develop a spontaneous tumor. However, the timing of occurrence of pituitary tumors in rats and mice can also be altered by postnatal exposure to low doses of estrogenic compounds[18, 22]. Therefore, the increased rate of pituitary tumors in the HAA rats may have been induced by their endocrine characteristics. For example, the HAA rats show higher estrogen secretion during the estrous cycle. Chronic administration of estrogen to rats is known to result in the development of pituitary tumors[23, 24], even though Sprague-Dawley rats appear to be insensitive to estrogen induction of pituitary tumors[25]. An elevated es-

**Table 3.** Summary of the Non-neoplastic Lesions in the HAA and LAA Female Rats

| Non-neoplastic lesions | Total (including dead/sacrificed animals) | | | | | | | | | | | Live (excluding dead/sacrificed animals) | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | LAA | | | | | HAA | | | | | | LAA | | | | | HAA | | | | | |
| | − | ± | + | 2+ | 3+ | − | ± | + | 2+ | 3+ | | − | ± | + | 2+ | 3+ | − | ± | + | 2+ | 3+ | |
| Kidney | | | | | | | | | | | | | | | | | | | | | | |
|   Chronic progressive nephropathy | 0 | 6 | 11 | 8 | 10 | 32 | 3 | 0 | 0 | 0 | ** | 0 | 3 | 4 | 6 | 5 | 11 | 3 | 0 | 0 | 0 | ** |
|   Dilatation, pelvis | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 22 | 9 | ** | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 8 | 5 | ** |
| Thymus | | | | | | | | | | | | | | | | | | | | | | |
|   Involution | 0 | 0 | 0 | 11 | 22 | 0 | 0 | 5 | 20 | 9 | ** | 0 | 0 | 0 | 10 | 7 | 0 | 0 | 3 | 9 | 2 | |
|   Dilatation, epithelial tubules and cords | 33 | 0 | 0 | 0 | 0 | 9 | 3 | 9 | 12 | 1 | ** | 17 | 0 | 0 | 0 | 0 | 1 | 1 | 5 | 6 | 1 | ** |
|   Hyperplasia, epithelial tubules and cords | 22 | 9 | 2 | 0 | 0 | 1 | 13 | 13 | 6 | 1 | ** | 8 | 7 | 2 | 0 | 0 | 0 | 1 | 7 | 5 | 1 | |
| Thyroid gland | | | | | | | | | | | | | | | | | | | | | | |
|   Proliferation, C-cell, diffuse | 26 | 1 | 1 | 0 | 0 | 6 | 16 | 6 | 0 | 0 | ** | 17 | 0 | 1 | 0 | 0 | 3 | 8 | 3 | 0 | 0 | ** |
| Ovary | | | | | | | | | | | | | | | | | | | | | | |
|   Atrophy | 0 | 5 | 9 | 18 | 3 | 0 | 3 | 17 | 9 | 2 | | 0 | 3 | 6 | 7 | 2 | 0 | 2 | 5 | 6 | 0 | |
|   Hyperplasia, sex cord stromal, mixed | 35 | 0 | 0 | 0 | 0 | 1 | 22 | 6 | 2 | 0 | ** | 18 | 0 | 0 | 0 | 0 | 0 | 9 | 2 | 2 | 0 | ** |
|   Appearance, Sertoli cell | 33 | 1 | 1 | 0 | 0 | 2 | 24 | 5 | 0 | 0 | ** | 16 | 1 | 1 | 0 | 0 | 1 | 10 | 2 | 0 | 0 | ** |
|   Cyst, follicular/luteum | 29 | 4 | 2 | 0 | 0 | 13 | 14 | 4 | 0 | 0 | ** | 15 | 3 | 0 | 0 | 0 | 2 | 8 | 3 | 0 | 0 | |
|   Appearance, atretic follicle | 6 | 19 | 9 | 1 | 0 | 1 | 16 | 11 | 3 | 0 | | 1 | 9 | 7 | 1 | 0 | 1 | 7 | 5 | 0 | 0 | |
|   Appearance, corpus luteum | 3 | 9 | 11 | 11 | 1 | 11 | 8 | 4 | 7 | 1 | ** | 3 | 4 | 4 | 6 | 1 | 4 | 3 | 3 | 2 | 1 | |
| Uterus | | | | | | | | | | | | | | | | | | | | | | |
|   Atrophy | 0 | 3 | 13 | 17 | 2 | 11 | 5 | 12 | 3 | 1 | ** | 0 | 3 | 9 | 5 | 1 | 4 | 2 | 7 | 0 | 0 | |
| Vagina | | | | | | | | | | | | | | | | | | | | | | |
|   Cornification, epithelium | 34 | 1 | 0 | 0 | 0 | 25 | 2 | 1 | 2 | 2 | * | 17 | 1 | 0 | 0 | 0 | 12 | 1 | 0 | 0 | 0 | |
|   Mucification, epithelium | 25 | 4 | 1 | 4 | 1 | 10 | 2 | 2 | 10 | 8 | ** | 14 | 0 | 1 | 3 | 0 | 3 | 1 | 1 | 6 | 2 | ** |
| Adrenal gland | | | | | | | | | | | | | | | | | | | | | | |
|   Hypertrophy, zona fasciculata | 27 | 6 | 2 | 0 | 0 | 5 | 12 | 13 | 3 | 0 | ** | 11 | 5 | 2 | 0 | 0 | 1 | 4 | 8 | 1 | 0 | ** |
|   Hypertrophy, zona glomerulosa | 5 | 28 | 2 | 0 | 0 | 26 | 7 | 0 | 0 | 0 | ** | 0 | 17 | 1 | 0 | 0 | 12 | 2 | 0 | 0 | 0 | ** |
|   Necrosis, focal | 17 | 10 | 2 | 5 | 1 | 25 | 5 | 2 | 1 | 0 | * | 6 | 7 | 2 | 3 | 0 | 10 | 4 | 0 | 0 | 0 | * |
| Liver | | | | | | | | | | | | | | | | | | | | | | |
|   Necrosis, focal | 28 | 3 | 3 | 1 | 0 | 34 | 0 | 0 | 1 | 0 | * | 17 | 1 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | |
| Circulatory organ system | | | | | | | | | | | | | | | | | | | | | | |
|   Periarteritis nodosa | 19 | 0 | 4 | 6 | 6 | 32 | 2 | 1 | 0 | 0 | ** | 9 | 0 | 3 | 2 | 4 | 13 | 1 | 0 | 0 | 0 | * |

'Symbols: −, no abnormal changes; ±, very slight; +, slight; 2+, moderate; 3+, marked. Numerals represent the number of animals. Asterisks indicate significant differences from the incidences of the LAA rats (*p<0.05 by Mann–Whitney U test; **p<0.01 by Mann–Whitney U test).

tradiol/progesterone ratio during persistent estrus results from reduced estradiol and elevated prolactin, often as a result of a pituitary tumor[26]. However, persistent estrus was observed earlier in the LAA rats than in the HAA rats in this study. Therefore, the increased rate of pituitary tumors in the HAA rats may have been caused by other factors, such as dopamine receptors. It is known that hypothalamic dopamine inhibits proliferation of prolactin-producing lactotroph cells by activating lactotroph dopamine D2 receptors[27]. Specifically, apomorphine-susceptible (APO-SUS) rats show more resistance to tumor angiogenesis than apomorphine-unsusceptible (APO-USUS) rats, and APO-SUS rats also show more reaction to stressors than APO-USUS rats[28]. Further study is needed regarding the dopaminergic reaction in HAA and LAA rats.

Although Cavigelli *et al.*[2] reported that ovarian dysfunction occurred earlier in nervous female rats than in their less nervous sisters, ovarian dysfunction occurred earlier in the LAA (stress-nonreactive) rats than in the HAA (stress-reactive) rats in this study. This discrepancy may have been caused by the strain differences in their reproductive endocrinology during the estrous cycle[16]. For instance, most LAA rats exhibit prolonged diestrus and a regular 5-day estrous cycle, whereas all HAA rats show a regular 4-day estrous cycle[16]. Additionally, LAA rats show a smaller LH surge and a larger prolactin surge on the day of proestrus, accompanied by lower estradiol-17β secretion, compared with HAA rats[16]. It is thought that the onset of an age-matched abnormal cycle in HAA and LAA rats is dependent on their reproductive endocrinology during the estrous cycle rather than on ovarian aging.

In the present study, tumors of the mammary glands were observed more frequently in the LAA rats, in which spontaneous ovarian dysfunction occurred earlier, as stated above. Furthermore, visible masses in the LAA rats developed at a younger age than in the HAA rats. In LAA rats, prolactin, rather than ACTH, is secreted from the anterior pituitary under stressful conditions[13, 14]. These results suggest that these factors made LAA rats stress resistant. Stress-nonreactive (stress-resistant) rats (i.e., LAA rats) secreted more prolactin when they faced stressful situations, such as door opening, changing cage, etc., compared with HAA rats. It is known that hyperprolactinemia induces abnormal estrous cycles, prolactin promotes the development of mammary glands, and hyperprolactinemia is related to the risk of mammary tumors[29]. This information suggests that the high

incidence of mammary tumors in the LAA rats is related to the high levels of plasma prolactin. In the HAA rats, hyperplasia of the mammary gland was observed in only one animal, though pituitary gland tumors were frequently observed. Therefore, it was thought that most of the pituitary tumors observed in the HAA rats were nonfunctional adenomas. Unfortunately, measurements of body weight and food consumption were not carried out in the present study. Even so, it has been confirmed that there is no difference between LAA and HAA female rats in body weight gain during the aging period (unpublished data). Body weight and food consumption should have been measured in order to determine the profile of the pituitary tumor.

In the thyroid, the proliferation of C-cells observed in the HAA rats was considered to be a non-neoplastic lesion. On the other hand, there was no difference between the HAA and LAA rats in the incidence of C-cell hyperplasia, which is considered to be a preneoplastic lesion, though the incidence of C-cell adenomas was higher in the HAA rats than in the LAA rats. Moreover, calcitonin is known to increase pituitary gland tumors[30]. It is known that calcitonin secretion is increased by estrogens or dopamine. It should also be remembered that the estrogen level is higher in HAA rats than in LAA rats[16]. Therefore, higher calcitonin levels induced by estrogen in HAA rats may be associated with more pronounced proliferation of C-cells.

Unexpectedly, CPN, a non-neoplastic lesion, was observed in all of the LAA rats but in only a few of the HAA rats. CPN is known to be an age-related renal disease commonly observed in various rat strains, including Sprague-Dawley rats[31, 32]. It can be induced by high-protein or high-calorie diets, and the incidence of CPN can, therefore, depend on the breeder[33]. Furthermore, circulating sex steroids or prolactin levels are associated with development of CPN[34]. Use of female HAA rats makes it possible to avoid interaction between CPN and the effects of chemicals in a long-term toxicity study. The dilatation of the renal pelvis observed in the older HAA rats may be a congenital anomaly because this phenomenon can be observed in all HAA rats at a young age (unpublished data). The presence of reddish urine in five HAA rats may be involved in the renal pelvic dilation. Therefore, this finding might not be related to stress reaction.

Hyperplasia in the epithelial tubules and cords in the thymus, which is stimulated by estrogens[35], occurred more often in the HAA rats than in the LAA rats. Hyperplasia of the sex cord-stromal or Sertoli cells in the ovary, which is thought to be associated with a deficiency in gonadotropins[36], was also observed in the HAA rats. Moreover, uterine polyps occurred more often in the HAA rats than in the LAA rats. Uterine polyps are common in Sprague-Dawley rats. Our observations and previous studies suggest the LAA rats may be resistant to age-related lesions in the thymus, ovary, and uterus even though they showed earlier metaphase.

Periarteritis nodosa was observed more in the LAA rats than in the HAA rats on histopathological examination.

Periarteritis nodosa is observed in the mesenteric arteries, testicular arteries, and renal arteries in spontaneously hypertensive rats[37]. It is suggested that the renin-angiotensin system can play a primary role in the development of periarteritis nodosa in rats[38]. In general, LAA rats seem to have a low blood pressure rather than a high blood pressure (http://www.anim.med.kyoto-u.ac.jp/NBR/). Therefore, further study of blood pressure is needed in older LAA rats, since age-related CPN was observed in all of the LAA rats. While polyarteritis nodosa also involves vasculitis associated with immune complex deposition that can lead to renal failure, the occurrence of polyarteritis nodosa might also be related to the fact that antibody production is higher in LAA rats than in HAA rats[39].

The present study demonstrated that older female HAA and LAA rats showed differences in the incidence of neoplastic and non-neoplastic lesions, and some of these differences may be related to the difference in stress reaction or ovarian function between HAA and LAA rats, and resulted in their different lifespans, with the lifespans of the HAA rats being shorter than the lifespans of the LAA rats. However, the LAA rats had accelerated ovarian aging compared with the HAA rats. Therefore, ovarian aging did not necessarily correspond to their lifespan. Since there were clear differences between the HAA and LAA female rats in age-related histopathological findings other than behavioral and endocrine characteristics, Hatano rats may be a good experimental models for understanding the relationships among hormones, oncogenesis, and stress reactions. Because the number of animals in this study was less than that normally used in a carcinogenicity study, further study is necessary. In addition, further study should be carried out using male rats to clarify the relationship between stress reactivity and lifespan.

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