Available online at www.sciencedirect.com
ScienceDirect
Hormones and Behavior 50 (2006) 454–462
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Infant temperament predicts life span in female rats that develop spontaneous tumors

Sonia A. Cavigelli [a,*], Jason R. Yee [b,c], Martha K. McClintock [b,c,d]

[a] *Department of Biobehavioral Health, The Pennsylvania State University, 315 E. Health and Human Development Building-East, University Park, PA 16802, USA*
[b] *Department of Comparative Human Development, University of Chicago, 5730 S. Woodlawn Avenue, Chicago, IL 60637, USA*
[c] *Institute for Mind and Biology, University of Chicago, 940 E. 57th Street, Chicago, IL 60637, USA*
[d] *Department of Psychology, University of Chicago, 5730 S. Woodlawn Avenue, Chicago, IL 60637, USA*

Received 3 February 2006; revised 21 April 2006; accepted 1 June 2006
Available online 11 July 2006

Abstract

In a recent study, we found that male rats that minimally explored a novel environment as infants died significantly faster than their more exploratory brothers. At death, these males had various complex pathologies, precluding identification of specific hormonal mechanisms underlying adult disease progression and mortality. To minimize the variance of disease processes at the end of life, we conducted a longitudinal study with female Sprague–Dawley rats prone to high rates of spontaneous mammary and pituitary tumors. For females that developed either mammary or pituitary tumors, those that had been neophobic (least exploratory) as infants died approximately 6 months earlier than their neophilic (most exploratory) sisters. In the case of mammary tumors, both benign and malignant, neophobic females developed palpable tumors earlier than neophilic females, whereas the interval between first palpation and death was the same for all females, indicating psychosocial regulation of early rather than later stages of the disease. Neophobic females' ovarian function aged more rapidly than their neophilic sisters. Concomitantly, they had lower corticosterone responses to restraint in late adulthood, ruling out high estrogen or corticosterone levels during senescence as causal factors in their accelerated mortality. During puberty, when mammary tissue is proliferating and differentiating, neophobic females experienced more irregular cycles with prolonged "luteal" phases, suggesting a role for prolactin, prolonged progesterone and fewer estrogen surges during this sensitive period for mammary tumor risk. Thus, we identified prolactin, estrogen, progesterone and possibly corticosterone dynamics as candidates for neuroendocrine mechanisms linking infant temperament with onset of adult neoplastic disease.

Keywords: Temperament; Personality; Mammary tumors; Pituitary tumors; Reproductive cycles; Glucocorticoid dynamics; Longevity; Sibling differences

Stable behavioral traits (e.g. temperament, personalities) are often associated with specific hormonal profiles (e.g. gonadal and/or adrenal function). Given the stability of certain behavioral/endocrine traits and the known costs and benefits of certain hormonal profiles (McEwen and Seeman, 1999), we hypothesized that a behavioral trait that develops in infancy and is relatively stable into adulthood and associated with hormonal production (i.e. willingness to move in a novel environment; Gentsch et al., 1982; Meaney et al., 1991; Piazza et al., 1991; Dellu et al., 1996; Cavigelli and McClintock, 2003) will lead to differential disease progression and mortality rates.

Given the well-established bidirectional influence of ovarian steroids, prolactin and glucocorticoids on behavior and tumor cells, we focused on individual differences in the development of spontaneous tumors in a rodent model. Hormones have a wide array of influences on tumor development, with different effects depending on the kind of tumor, the stage of tumor development and the timing and duration of hormonal exposure. Tumor initiation may be regulated by both ovarian steroids and glucocorticoids. Dexamethasone decreases apoptosis in mammary cancer cells in vitro, increasing risk of spontaneous mutations and, if operable in vivo, accelerating spontaneous tumor

* Corresponding author. Fax: +1 814 863 7525.
E-mail address: s-cavigelli@psu.edu (S.A. Cavigelli).

0018-506X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2006.06.001

initiation (Moran et al., 2000; McClintock et al., 2005). A lengthened luteal phase is another risk factor for mammary tumorigenesis because of increased mammary cell proliferation and decreased apoptosis during this phase (Bernstein and Ross, 1993; Schedin et al., 2000). Unopposed estrogen, seen during the follicular phase, affects mammary tissue differentiation during puberty and alters the risk factor for mammary tumorigenesis (Schedin et al., 2000). Once formed, tumors can regress during stress and glucocorticoid exposure (Justice, 1985; Sapolsky and Donnelly, 1985; Romero et al., 1992; Zhu et al., 2003), perhaps by decreasing production of one of the most potent angiogenic factors, vascular endothelial growth factor (VEGF; Lohrer et al., 2001). Estrogen promotes mammary tumor growth, but only in ER positive tumors, and typically only when prolactin is also present (Russo and Russo, 1998).

The Sprague–Dawley strain provides a powerful model to understand spontaneous mammary and pituitary tumor development. In these rats, spontaneous mammary tumors are non-virally derived, similar to human mammary tumors (Bischoff, 1969; Justice, 1985) and unlike common mouse models (Murphy, 1966). Benign tumors reflect failure of apoptosis over an extended period and set the stage for the spontaneous carcinogenesis requiring repeated mutations and tumor cell proliferation (Vogelstein and Kinzler, 1993). This lengthy process allows ample opportunities for individual differences in behavioral and endocrine function to affect health and disease trajectories (LeFevre and McClintock, 1988; Segerstrom, 2003; McClintock et al., 2005).

In a recent life span study of Sprague–Dawley rat brothers, we found that those reluctant to explore a new environment in infancy (i.e. neophobic) sustained that temperament into adulthood, had elevated corticosterone responses to novelty in adulthood and died earlier than their more exploratory brothers (Cavigelli and McClintock, 2003). In this small set of males, pathology at death included diverse tumors and some other processes, precluding identification of specific endocrine routes by which temperament could influence a specific disease process. To control for disease at the end of life, we studied female rats prone to two specific spontaneous neoplasias—mammary and pituitary tumors (Prejean et al., 1973; Löhrke et al., 1982; Schedin et al., 2000; Nakazawa et al., 2003). Understanding relationships among behavior, hormones and tumor development in females is essential given significant sex differences in all these variables. As compared to males, female rats are more exploratory, show fewer signs of harm avoidance (Fernandes et al., 1999; Ray and Hansen, 2004), have higher basal and reactivity corticosterone production (Tinnikov, 1999; cf. Rivier, 1999) and develop more pituitary and mammary tumors at the end of life (Prejean et al., 1973).

Much of the above work on mammary tumors has been done in vitro or with induced tumors or xenographs. Here, we tested the hypothesis that a neophobic temperament in infant females increases mortality rate as it did in males and extended the hypothesis to the onset and progression of spontaneous mammary tumor formation. We compared the following processes for females identified as neophobic or neophilic during infancy: frequency of spontaneous mammary and pituitary tumors, age when mammary tumors were first palpable, natural life span of females with tumors, ovarian function from late puberty to reproductive senescence and glucocorticoid responses during senescence. To minimize genetic variance among temperament types, we studied *within* family differences.

In summary, we address the following questions: (1) is exploration of a novel environment a stable trait over the female life span? (2) Do infant female behavioral responses predict mortality rates in rats afflicted with spontaneous mammary or pituitary neoplasias? (3) Are ovarian function and/or glucocorticoid dynamics associated with infant temperament, providing potential mechanisms for differential disease progression and/or mortality rates among temperament types? If Sprague–Dawley sisters are like their brothers, we expect those with a neophobic temperament to have elevated glucocorticoid responses to novelty (Cavigelli and McClintock, 2003). If the neophobic infant trait is associated with differential mortality rates in females, we expect to find differences in ovarian function reflecting differential exposure to ovarian steroids over the life span.

Methods

Overall protocol

Eighty-one female pups were selected from 14 independently bred Sprague–Dawley litters (parents from Charles River Laboratories, Wilmington, Massachusetts; selection criteria for exploratory temperament described below). Six sisters were selected for within-family comparisons because of known between-family differences in behavior, corticosterone responses and life span (Cavigelli and McClintock, unpublished data). Females lived throughout their lives in solid bottom plastic cages (43.5 × 23.5 × 20.5 cm) on a 14L:10D lighting schedule (lights on at 20:00 h) with food and water ad libitum. Cages were cleaned twice a week. Females were not allowed to breed. Until 22 days of age, females lived with their mother and littermates. Their mothers and brothers were then removed, and they continued living with their sisters until 27 days. As part of another study on peripubertal social experiences, housing conditions were manipulated during 28–46 days of age; 1/3 lived with two sisters, 1/3 with two unfamiliar females and 1/3 alone. This manipulation did not affect variables examined in this report and is therefore not discussed further. At 47 days of age, all females were housed in groups of 3 sisters per cage for the rest of their lives. Each trio included one low exploration, one intermediate exploration and one high exploration sister (based on testing at 20 days described below; trios were counterbalanced for peripubertal social experience).

Daily vaginal cytology samples collected from each female from late-puberty through reproductive senescence were used to analyze ovarian cycle phase durations (LeFevre and McClintock, 1988). Repeated blood samples collected at 15 months of age were used to assess corticosterone response dynamics. Health status, including mammary tumor development, was monitored biweekly beginning at 10 months of age, and necropsies conducted at the end of their natural lives. All methods detailed below were approved by the University of Chicago Institute for Animal Care and Use Committee and adhered to methods specified in the *Guide for the Care and Use of Laboratory Animals*.

Temperament: behavioral response to a complex novel environment

We measured rats' willingness to explore a complex novel environment at 20 days of age and again at 11 months of age. The first test was conducted just prior to typical weaning and moving above ground in the field (Calhoun, 1962); the second test was conducted in middle adulthood, prior to any external signs of illness. We designed the 'Exploration Arena' to mimic the novel room situation used to assess temperament in young children (details in Rheingold, 1969; Garcia-Coll et al., 1984; Cavigelli and McClintock, 2003) by modifying

previous arenas (e.g. Einon and Morgan, 1976). Unlike the classic 'Open Field' arenas to test 'emotionality' (Hall, 1934), we included rat-sized objects, opaque walls and a cover to minimize anxiety-provoking aspects (i.e. open spaces) for the thigmotactic rat. By minimizing aspects threatening to all individuals, we sought to maximize the full range of individual responses to benign novelty. Each rat was allowed to explore the arena for 5 min.

For behavioral coding, nine square areas (940 or 1655 cm^2 for infant or adult arena) were defined by a 3×3 grid. Exploration in the arena was quantified by the number of times a rat walked from one of the areas to an adjacent area (locomotion score). In a confirmatory factor analysis, this movement was associated with other exploratory behaviors in infancy, including sniffing and touching the objects, and in late adulthood with sniffing and climbing on them. It was not associated with escape-related behavior such as inspecting or rearing up on the walls of the enclosure. Thus, the locomotion score in this arena probably reflects exploration and not high levels of undirected motor activity.

Selection criteria

Locomotion scores (our index of exploration) varied within and among litters (within litter ranges for the extremes: 0–34 vs. 0–76 squares entered). Females were categorized and selected based on their performance relative to the mean performance of all females within their family. The two most active sisters were identified as 'neophilic', the two least active were identified as 'neophobic' and those with values closest to the family mean as 'intermediate'. These categories are slightly different from those used in the previous study of males, in which we excluded the least active (i.e. non-responding) males from each litter because they weighed less than their littermates. In this previous study, the male pups that showed intermediate levels of activity in the test arena were identified as 'neophobic' since the least exploratory were excluded.

Pathology and life span

Females were allowed to live their natural life span. Health of aging females was closely monitored by researchers and the institutional veterinarian. To preclude suffering, 47 of the 81 females were sacrificed when they displayed symptoms indicating that they were within 1 week of death (Cavigelli and McClintock, 2003). Decisions to sacrifice were made independently by a veterinarian making weekly health checks and an observer; neither knew the animals' temperament. Equal numbers of neophobic, intermediate and neophilic females were sacrificed (N=16, 16, 15).

Mammary tumors

To determine age of mammary tumor detection, we used a comprehensive technique for repeatedly palpating all mammary tissue; three checkers could reliably detect mammary neoplasia as small as 3 mm in diameter. Regular checks began just prior to 10 months of age and were repeated every 2–3 weeks until death. We defined onset of tumor detection as the first date at which a growth was reliably palpable. Checkers were not informed as to which females had been palpated with tumors in the previous weeks.

At necropsy, the ovoid mammary tumors were well encapsulated and easily excised. Mean (±SEM) mammary tumor size across all females was 78.4±15.6 g, approximately 20% of the mean aged female body weight (i.e. approximately 400 g). Random samples revealed that they were histologically complex, with multiple tumor subtypes within a single solid tumor. Tumor diagnoses included malignant cancer (e.g. in situ ductal carcinoma (comedo and cribiform), invasive ductal carcinoma and carcinosarcoma) as well as benign tumors (e.g. fibroadenoma, lactacting adenoma and papillary cystadenoma). Histological diagnosis was validated by concordance of two surgical pathologists specializing in breast cancer pathology in the Department of Pathology at the University of Chicago.

Pituitary tumors

Necropsies were performed to determine presence of a pituitary tumor and to excise mammary tumors. Pituitary tumors were defined by pituitary gland enlargement (0.263±0.017 g, as compared to normal range of pituitary gland: 0.009–0.074 g), extensive vasculature and confirmed histologically by concordance of the two surgical pathologists. Other gross morphological abnormalities were also noted indicating disease processes at the time of death (i.e. enlarged or abnormally small organs and other tumors).

Hormonal function

Ovarian cycles from puberty through reproductive senescence

Ovarian cycles were monitored daily from 55 to 450 days to measure cycle length and estimate the number of days each female spent in estrogenized or non-estrogenized "luteal" phases of each cycle (e.g. proestrus and estrus with cornified vaginal epithelium cells and no leukocytes followed by metestrus and diestrus with leukocytes and few cornified cells). To assess ovarian cycles, cells of the vaginal wall were collected by saline lavage during the middle of the dark (active) period. Samples were quantified according to the relative proportion of three cell types: cornified epithelial cells, nucleated epithelial cells and leukocytes. Rats were in the post-ovulatory ('luteal') phase when leukocytes represented more than 20% of the cells in a sample for two or more consecutive days (LeFevre and McClintock, 1988).

Given potential sensitive periods for sex steroid influences on mammary tumorigenesis and progression (Bernstein and Ross, 1993; Hilakivi-Clarke et al., 2002), we sampled ovarian function for 21 days during distinct phases of reproductive development: late puberty (55–75 days, when hormone receptors first develop in the mammary epithelium and females' alveolar buds were differentiating over sequential estrous cycles into an accumulation of lobules (Bernstein and Ross, 1993; Masso-Welch et al., 2000)), early adulthood (95–115 days), middle adulthood (185–205 days), the transition into reproductive senescence (275–295 days) and mid-reproductive senescence (430–450 days). Because glucocorticoid production is affected by ovarian cycles and estrogen (Viau and Meaney, 1991), the latest time point began 20 days prior to blood sampling for glucocorticoid assessment at which point many tumors were palpable.

At each age, we determined how many females were in one of four ovarian states: regular cycles, constant estrus, irregular cycles and persistent diestrus or anestrus (coding methods in LeFevre and McClintock, 1988). During senescence, with regular prolonged cycles and constant estrus, females spent proportionally more time in estrus and/or proestrus. Thus, these two states are associated with greater estrogen production than the later stages of reproductive senescence when the ovaries become atrophic (irregular cycles and persistent diestrus/anestrus).

Corticosterone response to restraint stressor at late middle age

To determine if late adult corticosterone reactivity, following tumorigenesis, differed between neophobic and neophilic females, we collected repeated blood samples following brief physical restraint at 15 months of age. Restraint consisted of 30 min in a Plexiglas tube, which is considered a psychological stressor for these animals. Tube diameters were adjusted for individual female body weights, such that none was constricted.

Blood sampling was performed at the end of the rats' active period (starting between 20:00 and 21:00 h). At this time, females were removed from their home cage in a pre-determined randomized order, carried to an adjacent room where blood sampling was conducted, placed into a restraint tube and bled within 4 min of home cage removal. After the first sample, the rats remained in the tube for 30 min, at which point a second sample was collected and the rats removed from the tube and placed in a clean solid-bottom plastic cage. Repeated samples were collected at 60, 90 and 150 min from the initial sample. The first sample was intended to capture unstimulated corticosterone concentrations. The 60-min sample, collected 30 min from the end of physical restraint, was timed to capture the near-maximal corticosterone response to this challenge. And the 150-min sample was collected to capture recovery levels—how quickly the corticosterone response is terminated and corticosterone removed from the system after a 30-min novelty. Females were processed on nine sequential evenings, with equal numbers of neophobic, intermediate and neophilic sisters bled each night and sampling time balanced across female temperaments.

Blood was collected from the tip of the tail. Approximately 3 mm of the tail tip was removed with a scalpel blade and bleeding induced by tail palpation. Blood was collected into EDTA tubes (Microtainers from Becton Dickinson and Company) and kept on ice until centrifuged to collect plasma. Plasma was diluted (1:200 with assay kit diluent) and frozen at −80°C until assayed. Corticosterone was measured using a commercial radioimmunoassay kit (Rat and Mice Corticosterone kit, MP Biomedicals). All diluted samples were run in duplicate across nine assays. Sisters were included on the same assay. Intra-assay and inter-assay variability for a low and high control were 9.8 and 8.7% and 13.3 and 12.7%.

Statistical analyses

Summary statistics are presented as mean ± SEM. Parametric ANOVAs were used in most comparisons among temperament. Non-parametric statistics were employed when data were not normally distributed (e.g. sign test, log survivor analysis). Life span was compared among neophobic, intermediate and neophilic females using the Mantel–Cox log-rank χ^2 test for log survivor analysis because this statistic assumes no difference in rates of death over time between comparison groups. Because we expected the largest differences between neophobic and neophilic females (temperament, life span, endocrine function), we graphed data for only these two types of females. For the most part, results for intermediate females were intermediate to their neophobic and neophilic sisters; we include results for intermediate females in the text.

Consulting veterinarians concurred that cause of death in aging rats likely involves a complex interaction of multiple pathologies, and thus determining specific cause of death for all animals was beyond the scope of this study. To simplify analysis of the ongoing disease processes at the end of life and control for the two major types of neoplastic processes at the time of death, life span analyses were conducted within the two groups of females that had either mammary (N=18) or pituitary tumors (N=18) at death. Some of their sisters had both a pituitary and mammary tumors (N=38), while others had rare types of pathology—e.g. invasive thoracic and/or abdominal cavity tumors (N=5), cerebral hemorrhage (N=1), no detectable pathology at the time of death (N=1).

To assess ovarian cycle patterns for each temperament, we compared relative representation in the four ovarian stages among neophobic, intermediate and neophilic females with χ^2 tests. To determine if baseline, reactivity or recovery levels of circulating corticosterone differed among the three temperament types, we used ANOVAs to compare corticosterone concentration at each sampling time, with post hoc paired t tests to compare neophobic and neophilic values. Because families had different overall corticosterone profiles, mean family corticosterone concentration at each time point was used as a covariate. Several females had died or were too ill for blood collection at 15 months; because temperament types were initially balanced within families, analyses of corticosterone data were limited to those sister trios in which all females were present for the blood collection (N=54). Because estrous cycle profoundly affects corticosterone levels during the acrophase of the daily rhythm (Atkinson and Waddell, 1997; Cavigelli et al., 2005), estrous cycle stage on the day of blood sampling was used as a covariate in the original analysis. Direction and significance of results did not change when estrous phase was included as a covariate or not, and so raw corticosterone values are presented.

Results

Temperament: behavioral response to complex novel environment

Neophobic female infants explored the new environment approximately 6 times less than did their neophilic sisters (moving into only 8±2.5 vs. 47±2.5 adjacent squares). This temperament difference was maintained into middle adulthood (11 months), although it became less pronounced (moving into 59±2.2 vs. 68±3.1 adjacent squares; sign test: $z(24)=2.25$, $P<.05$). As infants and adults, intermediate females moved midway between their sisters (into 30±3.3 and 65±2.6 adjacent squares in the arena).

As infants, females responded to the testing arena in a very similar manner as did their brothers (described in Cavigelli and McClintock, 2003). The most active females and males in a litter (i.e. neophilic) showed similar amounts of locomotion (female vs. male mean±SEM: 47±2.5 vs. 43±2.8), as did the least exploratory females and males within each litter (i.e. neophobic females and non-responding males: 8±2.5 vs. 8±2.9). Unlike the non-responding males from the previous study, the neophobic females were not significantly smaller (and thus not more developmentally delayed) than the two more exploratory types and thus could be included in these analyses.

As adults, however, females became significantly more exploratory than their brothers, both overall and within each infant temperament type (overall females vs. males: 65±1.5 vs. 49±1.7 moves to adjacent grid squares, $t(143)=6.99$, $P<.0001$; adult females vs. males that were most exploratory as infants: 68±2.9 vs. 54±2.4, $t(48)=3.73$, $P<.001$; adult females vs. males that were least exploratory as infants: 59±2.2 vs. 44±3.5, $t(41)=3.74$, $P<.001$).

Pathology and life span

Females with only a pituitary tumor (both neophobic and neophilic infant temperament) died earlier than those with only mammary tumors (pituitary vs. mammary median and maximum life span: 581 and 932 vs. 766 and 1128 days Mantel–Cox log-rank, $\chi^2=4.25$, $P<.05$). Nonetheless, within each type of disease, females with a neophobic temperament as infants died more quickly than their neophilic sisters.

Mammary tumors

Females that had been neophobic as infants developed palpable mammary tumors much earlier than did neophilic females (Mantel–Cox log-rank, $\chi^2=4.29$, $P<.05$, hazard ratio=3.45). All but one (i.e. 80%) of the neophobic females had a palpable tumor by 390 days, when only 38% of the neophilic females did. Age of first mammary tumor detection was a strong predictor of life span ($r=0.64$, $P<.01$). At any given age, neophobic females were more likely to die with a mammary tumor than their neophilic sisters (Fig. 1a; Mantel–Cox log-rank, $\chi^2=4.04$, $P<.05$, hazard ratio=3.99). Neophobic females had a median life span of 573 days vs. 850 days for neophilic females. The maximum life span of neophobic females was 781 days of age, almost 1 year less than neophilic females (1126 days). The intermediate females' life spans were similar to those of their neophobic sisters (median=561, maximum=785 days).

Pituitary tumors

Rats with only pituitary tumors manifest similar life span differences: females that had been neophobic as infants died more rapidly than neophilic females (Fig. 1b; Mantel–Cox log-rank, $\chi^2=5.29$, $P<.05$, hazard ratio=4.83). Neophobic females had a median life span of 493 days vs. 640 days for the neophilic females. The maximum life span of females neophobic as infants was 620 days, almost 1 year less than neophilic females (932 days). The intermediate females' life spans were more like their neophilic sisters (median=670, maximum=934 days).

Infant temperament did not have detectable effects on the progression or incidence of disease in this sample. Females with neophobic and neophilic temperaments as infants experienced the same latency between first mammary tumor palpation and death (Mantel–Cox log-rank, $\chi^2=1.50$, ns). Thus, although neophobic rats succumbed to mammary disease at an earlier age, once any mammary tumor was detected, the disease progressed at similar rates among all rats. Neophobic, intermediate and neophilic

females also developed similar numbers of mammary and pituitary tumors (28%, 28% and 44% with mammary tumors, and 28%, 28% and 44% with pituitary tumors, $\chi^2=9.08$, ns). Neophilic females were slightly more prone to have either a mammary or a pituitary growth in their life time than neophobic and intermediate females (i.e. 44% vs. 28% with tumors). This might be attributable to their longer life span and therefore having more time to develop such neoplasia.

Hormonal function

Ovarian cycle from puberty through reproductive senescence

During the 21 days at the end of the pubertal process (55–75 days of age when females are transitioning from irregular to regular cycles), females with a neophobic temperament were twice as likely as their neophilic sisters to remain in an irregular cycle state (52% vs. 22%, $\chi^2=5.08$, $P<.05$). Thirty percent had at least one prolonged luteal phase cycle lasting between 9 and 15 days during this age, whereas none of the neophilic sisters did (Fischer Exact Test, $P<.01$; maximum cycle length difference log survival analysis, Mantel–Cox log-rank, $\chi^2=6.92$, $P<.01$). Once females entered the phase of regular cycles during young adulthood (95–115 days), the ovarian axis stabilized, and the majority of neophobic and neophilic rats had regular four- and five-day cycles (70% of neophobic and 84% of neophilic females, $\chi^2=1.5$, ns). Moreover, females with both temperaments spent 33% of their days in an estrogenized state. At 6 months of age, however, temperament differences re-emerged; females identified as neophobic during infancy were beginning to progress into irregular cycles, while the majority of neophilic females sustained regular cycles (65 vs. 42% with irregular cycles, $\chi^2=2.8$, $P<.10$). Likewise, at 9 months of age, reproductive aging appeared accelerated in the neophobic females. Fifty percent had progressed to acyclic constant estrus and pseudopregnancy, whereas only 12% of the neophilic females had entered these acyclic stages. Late in the life span, accelerated reproductive senescence was evident in females that had been neophobic as infants. At the time of blood sampling for corticosterone analyses, neophobic females were showing more signs of ovarian aging and were in low-estrogen cycle phases as compared to their neophilic sisters. Twice as many neophobic females had progressed into the low-estrogen states of long irregular cycles or persistent diestrus/anestrus as compared to their neophilic sisters ($\chi^2=11.00$, $P<.01$; Fig. 2). Whereas the majority of neophilic females (58%) were still in the higher estrogenized states of long regular cycles or constant estrus, the majority of neophobic females (87%) were in the low-estrogen phases (irregular or persistent diestrus/anestrus).

Corticosterone response to restraint stressor at late middle age

At 15 months of age, after mammary tumors were first palpated, all females showed the expected aged corticosterone response profile, including a lengthened recovery period (Sapolsky et al., 1986). In addition, as expected, given opposite effects of testosterone and estrogen on adrenal function (Handa et al., 1994; McCormick et al., 2002), females had significantly higher levels of overall corticosterone production than did their brothers (repeated measure ANOVA: $F(1,142)=226$, $P<.0001$).

Females that had been neophobic or neophilic as infants did not differ in their baseline corticosterone levels at the circadian nadir (paired $t(17)=1.12$, ns). However, in response to the novel experience of physical restraint and blood sampling procedure, neophobic females secreted less corticosterone than did neophilic females—their peak levels (at 60 min) were approximately 15% lower (paired $t(17)=3.39$, $P<.01$; Fig. 3). Recovery values (at 150 min) were no different between neophobic and neophilic sisters (paired $t(17)=1.03$, ns). For the most part, intermediate females secreted intermediate levels of corticosterone as compared to neophobic and neophilic females.

Discussion

Willingness to explore a novel environment was a behavioral trait identifiable in female Sprague–Dawley rats as early as infancy. Female pups' levels of exploration were indistinguishable from those of their brothers reported previously (Cavigelli and McClintock, 2003). As with their brothers, this trait was still evident in adulthood, although the behavioral difference between early identified neophobic and neophilic females was reduced in adulthood. This trait appears to represent a variance along a continuum rather than a categorical difference as females with an intermediate temperament also had intermediate biological values. Most notably,

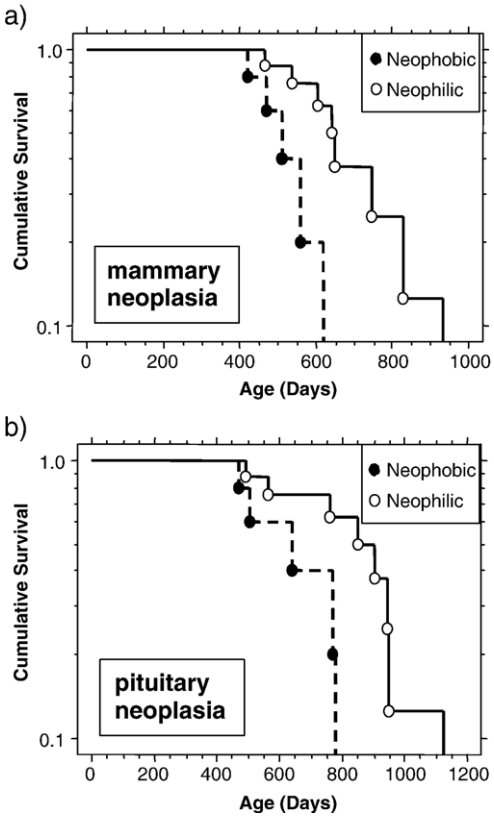

Fig. 1. Among females with spontaneous tumors, females identified as neophobic during infancy died at a faster rate than neophilic ones: (a) females with mammary neoplasia ($N=5$ neophobic, 8 neophilic) and (b) females with pituitary neoplasia ($N=5$ neophobic, 8 neophilic).

this temperament predicted the life span of female rats developing mammary or pituitary tumors. Females neophobic as infants developed palpable mammary tumors and died at a younger age than their sisters that were neophilic as infants. Tumors contained a variety of benign and malignant neoplasias, indicating that temperament may affect a number of different tumorigenic and carcinogenic (or "cancer promoting") mechanisms, including the suppression of apoptosis, increase in the rate of spontaneous mutation and/or loss of effective DNA repair mechanisms. Notably, the interval between tumor palpation and death was the same for neophobic and neophilic females, pinpointing tumor initiation as the stage affected by temperament rather than its rate of progression or of a female's ability to sustain a large tumor burden. Neophobic females dying with a pituitary tumor (as opposed to a mammary tumor) also had shorter life spans, suggesting possible common neuroendocrine mechanisms linking infant temperament with development of the two most common tumors in Sprague–Dawley female rats. It is probably specific endocrine profiles at different developmental periods that predict later tumorigenesis, as opposed to stable differences in either behavior or endocrine function throughout life.

We have presented the first evidence that neonatal temperament predicts the time at which spontaneous mammary tumors are first palpable and the age at death for females with mammary and pituitary tumors. Delayed menopause and high estradiol levels in middle age are risk factors for mammary cancer in humans (de Waard and Thijssen, 2005; Schairer et al., 2005) as are high prolactin levels (Harvey, 2005; Tworoger et al., 2004). In our rats that developed spontaneous mammary tumors during reproductive senescence, neophobic females had accelerated, not delayed, ovarian aging and had progressed into low-estrogen stages (i.e. prolonged irregular cycles and persistent diestrus/anestrus) earlier than neophilic females. Therefore, elevated unopposed estrogen during reproductive senescence probably does not account for the accelerated mortality rates in neophobic females that developed spontaneous mammary tumors. Neophobic sisters had more irregular and lengthened low-estrogen "luteal" phases during late puberty. Naturally occurring variance in progesterone production from the corpora luteum during puberty may affect the differentiation and proliferation of mammary tissue, potentially affecting the timing of adulthood susceptibility to tumorigenesis (Bernstein and Ross, 1993; Schedin et al., 2000). Because the life span of the corpora luteum is prolonged by prolactin secretion, these results suggest another possible hormonal mechanism linking temperament and survival with a tumor. Prolactin, which is released during psychosocial stress and which affects rate of mammary tumor development (Inano et al., 1999; Clevenger et al., 2003; Ueda et al., 2006), represents a highly plausible mechanism. Given that increased prolactin secretion can occur alongside decreased corticosterone secretion (Pohorecky et al., 2004), it is possible that neophobic females secrete greater amounts of prolactin and that these increased levels may account for earlier tumor onset.

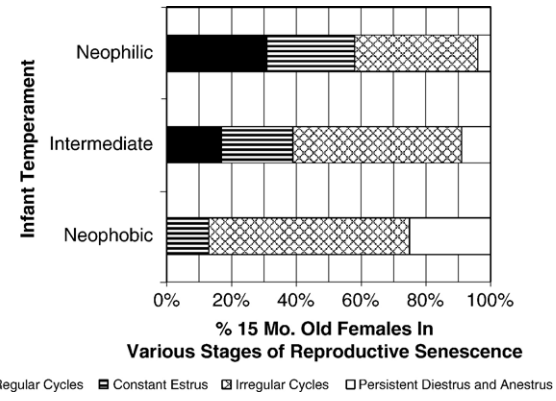

Fig. 2. Percent of 15-month-old females identified prior to weaning as neophilic, intermediate or neophobic in each of the four ovarian cycling phases: long regular cycles, constant estrus, irregular cycles and persistent diestrus or anestrus. Most neophilic females were experiencing the more estrogenized phases (long regular cycles and constant estrus), whereas most neophobic females are in the low-estrogen, more aged, phases of ovarian function (irregular cycles and persistent diestrus or anestrus) (N=24 neophobic, 23 intermediate, 26 neophilic).

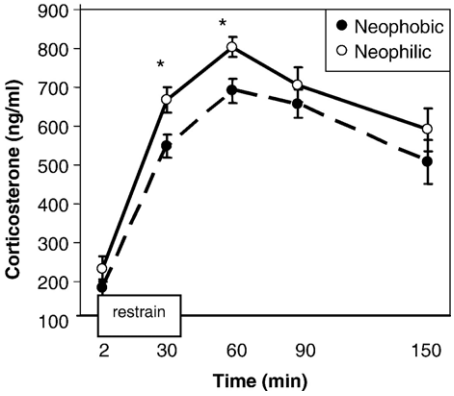

Fig. 3. As compared to females identified as neophobic during infancy, neophilic ones had a greater acute increase in circulating corticosterone levels following a novel restraint experience with similar baseline (0 min) and recovery (150 min) corticosterone levels (N=18 neophobic, 18 neophilic).

During reproductive senescence and following first mammary tumor palpation, females who minimally explored the novel environment as infants had lower, not higher, peak corticosterone levels in response to novel restraint stress compared to their more exploratory (neophilic) sisters. These results are the opposite of those found for both young and old male Sprague–Dawley rats. Lower corticosterone responses in senescent neophobic females could reflect lower levels of voluntary behavior in these females (Borer et al., 1992). However, given differences in ovarian function at this age, we propose a more parsimonious interpretation: a neophobic temperament during infancy is associated with accelerated aging of a variety of systems, including the gonadal axis. At 15 months, females that were neophobic as infants were in the final, low-estrogen stages of reproductive senescence. Because estrogen and corticosterone are positively correlated within individuals (Viau and Meaney, 1991; McCormick et al., 2002), low corticosterone in the neophobic females is consistent with their accelerated ovarian aging into low-estrogen states. At the time of blood sampling, their neophilic sisters were still having more estrogenized regular long cycles and constant estrus. Thus, lower glucocorticoid responses to a novel challenge in older neophobic vs. neophilic females most

likely reflects accelerated ovarian aging, as opposed to different psychological or physiological responses to the challenge. Under this hypothesis, neophobic males would also experience accelerated reproductive aging, lower testosterone and hence higher corticosterone production (Handa et al., 1994) in late middle age (e.g. Cavigelli and McClintock, 2003). These results call for further analysis of the interplay between gonadal and adrenal axes aging in males and females of different temperaments not only during reproductive senescence, but throughout adulthood corresponding to the different stages of disease.

There is ever-increasing evidence that stressors and glucocorticoids are associated with a variety of malignant disease, particularly tumor growth and metastasis as studied in vitro or with induced tumors (Justice, 1985; Sapolsky and Donnelly, 1985; Romero et al., 1992; Moran et al., 2000; Zhu et al., 2003; Parker et al., 2004; Antoni et al., 2006). The role of corticosterone in the multistep process of tumor development, however, is likely to be different for different types of neoplasias and malignant disease, as well as at different stages of the process. In this study, we did not find that female temperament, which was associated with glucocorticoid reactivity levels after tumorigenesis, had a significant impact on survival time following first tumor palpation. These results suggest that glucocorticoid production after spontaneous mammary tumorigenesis may not affect tumor growth or virulence as has been shown with in vitro or induced tumors (Justice, 1985; Sapolsky and Donnelly, 1985; Romero et al., 1992; Moran et al., 2000; Zhu et al., 2003). On the other hand, infant temperament predicted the age at which mammary tumors were first palpable, suggesting that early differences in temperament and possibly endocrine function may influence the rate of tumorigenesis. These results suggest that an important process that should be considered when looking at tumor development as it relates to endocrine profiles and/or temperament is the rate at which tumors first develop as opposed to tumor growth or organism survival following tumorigenesis.

Overall, these physiological results suggest that life-long interactions among ovarian steroid and glucocorticoid hormone production and receptor levels, as well as pubertal progesterone and prolactin levels may be important candidates for future cellular and genetic studies linking temperament and tumorigenesis. However, these hormones likely play different roles in the process of mammary tissue differentiation which sets the stage for hyperplasia and tumorigenesis. It is important to note that physiological processes associated with differential life span among siblings (i.e. *within* families) may be quite different from those associated with differential life span among unrelated individuals (i.e. *across* families). Future studies can clarify these processes by using both within and between family analyses of tumorigenesis, temperament and endocrine function. The most productive route for understanding mechanisms of individual differences in tumorigenesis will involve further attention to individual differences in interacting endocrine systems as well as the temporal dynamics of multiple neuroendocrine responses associated with stress (see Antoni et al., 2006 for review).

Interestingly, we found no evidence that social isolation or disruption during 2 weeks of puberty affected mortality rate with mammary or pituitary tumors. Peripubertal environmental and hormonal experiences represent a potent organizing influence on adult behavior and physiology (e.g. Sachser, 1993; Laviola and Terranova, 1998; Francis et al., 2002; Romeo, 2003). One reason we did not observe influences of peripubertal social manipulation may be that our outcome measures were collected during the end of the life span. The influence of a short pubertal social manipulation on adult function may have been minimized by other experiences accumulated over the life span or the influence of early social experiences may have been dampened by the distribution of these conditions across a variety of temperamental traits, thereby diluting effect size.

This study has identified a specific fundamental difference in temperament associated with tumorigenesis. This finding may shed light on currently conflicting results in human studies on personality and cancer which focus primarily on survival once a tumor has been identified (Jadoulle et al., 2004). Human studies may need to consider more basic behavioral traits than those already considered. More basic traits will more likely be associated with specific physiological processes, and a more basic trait may 'clean' up the currently conflicting picture. In particular, because the behavioral difference among females diminished over time, it may be that an early behavioral trait predicts either life-long differences in neuroendocrine function or different endocrine trajectories, which may be the more important factor predicting the onset of specific pathological processes and life span.

In summary, by categorizing and comparing rat sisters according to specific pathological processes at death, we have been able to implicate specific neuroendocrine mechanisms that may account for why siblings with different temperaments have different mammary and pituitary tumorigenesis trajectories. In particular, temperamental differences present among preweanling sisters, and sustained into adulthood, predicted survival rates for those that developed either a mammary or pituitary tumor. Endocrine mechanisms implicated were progesterone, prolactin and glucocorticoids operating in puberty and/or reproductive senescence. Most notably, we have shown that female temperament may have the greatest effect on when tumors first develop, as opposed to the rate at which tumors proceed following tumorigenesis. In addition, we found that infant temperament affected rates of reproductive senescence which may have caused significant differences in corticosterone reactivity in late life. Finally, the mammary tumors in this study were spontaneously developed during middle adulthood/reproductive senescence, as opposed to being triggered by carcinogens, genetic engineering or xenografts. Thus, this study may serve as a powerful model for identifying individual differences in rates of tumorigenesis that impact life span.

Acknowledgments

We thank T. Brawn, J. Clarke, K. Eisenman, J. Hoffman, A. Lindner, E. Shaw-Taylor, M. Tsakalis, T. Whitney, H. You and A. Wiley for their expert assistance. This research was supported by NICHD F32 HD08693 (to SAC), NIA PO1 AG018911 (to MKM) and NIMH R37 MH41788 (to MKM).

References

Antoni, M.H., Lutgendorf, S.K., Cole, S.W., Dhabhar, F.S., Sephton, S.E., McDonald, P.G., Stefanek, M., Sood, A.K., 2006. The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. Nat. Rev., Cancer 6, 240–248.

Atkinson, H.C., Waddell, B.J., 1997. Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. Endocrinology 138, 3842–3848.

Bernstein, L., Ross, R.K., 1993. Endogenous hormones and breast cancer. Epidemiol. Rev. 15, 48–65.

Bischoff, F., 1969. Carcinogenic effects of steroids. Adv. Lipid Res. 7, 165–244.

Borer, K.T., Bestervelt, L.L., Mannheim, M., Brosamer, M.B., Thompson, M., Swamy, U., Piper, W.N., 1992. Stimulation by voluntary exercise of adrenal glucocorticoid secretion in mature female hamsters. Physiol. Behav. 51, 713–718.

Calhoun, J.B, 1962. The ecology and sociology of the Norway rat. U.S. Public Health Serv. Pub. 1008.

Cavigelli, S.A., McClintock, M.K., 2003. Fear of novelty in infant rats predicts adult corticosterone dynamics and an early death. Proc. Natl. Acad. Sci. U. S. A. 100, 16131–16136.

Cavigelli, S.A., Monfort, S.L., Whitney, T.W., Mechref, Y.S., Novotny, M., McClintock, M.K., 2005. Frequent serial rat fecal corticoid measures reflect circadian and ovarian corticosterone rhythms. J. Endocrinol. 184, 153–163.

Clevenger, C.V., Furth, P.A., Hankinson, S.E., Schuler, L.A., 2003. The role of prolactin in mammary carcinoma. Endocr. Rev. 24, 1–27.

de Waard, F., Thijssen, J.H., 2005. Hormonal aspects in the causation of breast cancer: epidemiological hypotheses reviewed, with special reference to nutritional status and first pregnancy. J. Steroid Biochem. Mol. Biol. 97, 451–458.

Dellu, F., Mayo, W., Vallée, M., Maccari, S., Piazza, P.V., Le Moal, M., Simon, H., 1996. Behavioral reactivity to novelty during youth as a predictive factor of stress-induced corticosterone secretion in the elderly—A life-span study in rats. Psychoneuroendocrinology 21, 441–453.

Einon, D.F., Morgan, M., 1976. Habituation of object contact in socially-reared and isolated rats (*Rattus norvegicus*). Anim. Behav. 24, 415–420.

Fernandes, C., Gonzalez, M.I., Wilson, C.A., File, S.E., 1999. Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. Pharmacol. Biochem. Behav. 64, 731–738.

Francis, D., Dioro, J., Plotsky, P., Meaney, M., 2002. Environmental enrichment reverses the effects of maternal separation on stress reactivity. J. Neurosci. 22, 7840–7843.

Garcia-Coll, C., Kagan, J., Reznick, J.S., 1984. Behavioral inhibition in young children. Child Dev. 55, 1005–1019.

Gentsch, C., Lichtshteiner, M., Driscoll, P., Feer, H., 1982. Differential hormonal and physiological responses to stress in Roman high and low-avoidance rats. Physiol. Behav. 28, 259–263.

Hall, C.S., 1934. Emotional behavior in the rat: defecation and urination as measures of individual differences in emotionality. J. Comp. Psychol. 18, 385–403.

Handa, R.J., Nunley, K.M., Lorens, S.A., Louie, J.P., McGivern, R.F., Bollnow, M.R., 1994. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. Physiol. Behav. 55, 117–124.

Harvey, P.W., 2005. Human relevance of rodent prolactin-induced non-genotoxic mammary carcinogenesis: prolactin involvement in human breast cancer and significance for toxicology risk assessments. J. Appl. Toxicol. 25, 179–183.

Hilakivi-Clarke, L., Cabanes, A., Olivo, S., Kerr, L.R., Bouker, K.B., Clarke, R., 2002. Do estrogens always increase breast cancer risk? J. Steroid Biochem. Mol. Biol. 80, 163–174.

Inano, H., Suzuki, K., Onoda, M., Kobayashi, H., Wakabayashi, K., 1999. Radiation-induced tumorigenesis of mammary glands in pituitary transplanted rats ovariectomized before onset of estrous cycle. Cancer Lett. 138, 93–100.

Jadoulle, V., Ogez, D., Rokbani, L., 2004. Cancer, a defect of the psyche? Bull. Cancer 91, 249–256.

Justice, A., 1985. Review of the effects of stress on cancer in laboratory animals: importance of time of stress application and type of tumor. Psychol. Bull. 98, 108–138.

Laviola, G., Terranova, M.L., 1998. The developmental psychobiology of behavioural plasticity in mice: the role of social experiences in the family unit. Neurosci. Biobehav. Rev. 23, 197–213.

LeFevre, J., McClintock, M.K., 1988. Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. Biol. Reprod. 38, 780–789.

Lohrer, P., Gloddek, J., Hopfner, U., Losa, M., Uhl, E., Pagotto, U., Stalla, G.K., Renner, U., 2001. Vascular endothelial growth factor production and regulation in rodent and human pituitary tumor cells in vitro. Neuroendocrinology 74, 95–105.

Löhrke, H., Hesse, B., Goertller, K., 1982. Spontaneous tumours in male and female specific pathogen-free Sprague–Dawley rats (outbred stock Sut: SDT). Z. Versuchstierk. 24, 225–230.

Masso-Welch, P.A., Darcy, K.M., Stangle-Castor, N.C., Ip, M.M., 2000. A developmental atlas of rat mammary gland histology. J. Mamm. Gland Biol. Neoplasia 5, 165–185.

McClintock, M.K., Conzen, S.D., Gehlert, S., Masi, C., Olopade, F., 2005. Mammary cancer and social interactions: identifying multiple environments that regulate gene expression throughout the life span. J. Gerontol. B 60, 32–41.

McCormick, C.M., Linkroum, W., Sallinen, B.J., Miller, N.W., 2002. Peripheral and central sex steroids have differential effects on the HPA axis of male and female rats. Stress 5, 235–247.

McEwen, B.S., Seeman, T., 1999. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. Ann. N. Y. Acad. Sci. 896, 30–47.

Meaney, M.J., Viau, V., Bhatnagar, S., Betito, K., Iny, L.J., O'Donnell, D., Mitchell, J.B., 1991. Cellular mechanisms underlying the development and expression of individual differences in the hypothalamic–pituitary–adrenal stress response. J. Steroid Biochem. Mol. Biol. 39, 265–274.

Moran, T.J., Gray, S., Mikosz, C.A., Conzen, S.D., 2000. The glucocorticoid receptor mediates a survival signal in human mammary epithelial cells. Cancer Res. 60, 867–872.

Murphy, E.D., 1966. Characteristic tumors. In: Green, E.G. (Ed.), Biology of the Laboratory Mouse. McGraw-Hill, New York.

Nakazawa, M., Tawaratani, T., Uchimoto, H., Kawaminami, A., Ueda, M., Ueda, A., Shinoda, Y., Iwakura, K., Kura, K., Sumi, N., 2003. Spontaneous neoplastic lesions in aged Sprague–Dawley rats. Exp. Anim. 50, 99–103.

Parker, J., Klein, S.L., McClintock, M.K., Morison, W.L., Ye, X., Conti, C.J., Peterson, N., Nousari, C.H., Tausk, F.A., 2004. Chronic stress accelerates ultraviolet-induced cutaneous carcinogenesis. J. Am. Acad. Dermatol. 51, 919–922.

Piazza, P.V., Maccari, S., Derminière, J.-M., Le Moal, M., Mormède, P., Simon, H., 1991. Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proc. Natl. Acad. Sci. U. S. A. 88, 2088–2092.

Pohorecky, L.A., Baumann, M.H., Benjamin, D., 2004. Effects of chronic social stress on neuroendocrine responsiveness to challenge with ethanol, dexamethasone and corticotropin-releasing hormone. Neuroendocrinology 80, 332–342.

Prejean, J.D., Peckham, J.C., Casey, A.E., Griswold, D.P., Weisburger, E.K., Weisburger, J.H., 1973. Spontaneous tumors in Sprague–Dawley rats and Swiss mice. Cancer Res. 33, 2768–2773.

Ray, J., Hansen, S., 2004. Temperament in the rat: sex differences and hormonal influences on harm avoidance and novelty seeking. Behav. Neurosci. 118, 488–497.

Rheingold, H.L., 1969. The effect of a strange environment on the behavior of infants. In: Foss, B.M. (Ed.), Determinants Infant Behav., vol. IV. Methuen, London, pp. 137–166.

Rivier, C., 1999. Gender, sex steroids, corticotropin-releasing factor, nitric oxide, and the HPA response to stress. Pharmacol. Biochem. Behav. 64, 739–751.

Romeo, R.D., 2003. Puberty: a period of both organizational and activational effects of steroid hormones on neurobehavioural development. J. Neuroendocrinol. 15, 1185–1192.

Romero, L.M., Raley-Susman, K.M., Redish, D.M., Brooke, S.M., Horner, H.C., Sapolsky, R.M., 1992. Possible mechanism by which stress accelerates growth of virally-derived tumors. Proc. Natl. Acad. Sci. U.S.A. 89, 11084–11087.

Russo, I.H., Russo, J., 1998. Role of hormones in mammary cancer initiation and progression. J. Mammry Gland Biol. Neoplasia 3, 49–61.

Sachser, N., 1993. The ability to arrange with conspecifics depends on social experiences around puberty. Physiol. Behav. 53, 539–544.

Sapolsky, R.M., Donnelly, T.M., 1985. Vulnerability to stress-induced tumor growth increases with age in rats: role of glucocorticoids. Endocrinology 117, 662–666.

Sapolsky, R.M., Krey, L.C., McEwen, B.S., 1986. The adrenocortical axis in the aged rat: impaired sensitivity to both fast and delayed feedback inhibition. Neurobiol. Aging 7, 331–335.

Schairer, C., Hill, D., Sturgeon, S.R., Fears, T., Mies, C., Ziegler, R.G., Hoover, R.N., Sherman, M.E., 2005. Serum concentrations of estrogens, sex hormone binding globulin, and androgens and risk of breast hyperplasia in postmenopausal women. Cancer Epidemiol., Biomarkers Prev. 14, 1660–1665.

Schedin, P., Mitrenga, T., Kaeck, M., 2000. Estrous cycle regulation of mammary epithelial cell proliferation, differentiation, and death in the Sprague–Dawley rat: a model for investigating the role of estrous cycling in mammary carcinogenesis. J. Mammry Gland Biol. Neoplasia 5, 211–225.

Segerstrom, S.C., 2003. Individual differences, immunity, and cancer: lessons from personality psychology. Brain Behav. Immun. 17, S92–S97.

Tinnikov, A.A., 1999. Responses of serum corticosterone and corticosteroid-binding globulin to acute and prolonged stress in the rat. Endocrine 11, 145–150.

Tworoger, S.S., Eliassen, A.H., Rosner, B., Sluss, P., Hankinson, S.E., 2004. Plasma prolactin concentrations and risk of postmenopausal breast cancer. Cancer Res. 64, 6814–6819.

Ueda, E., Ozerdem, U., Chen, Y.H., Yao, M., Huang, K.T., Sun, H., Martins-Green, M., Bartolini, P., Walker, A.M., 2006. A molecular mimic demonstrates that phosphorylated human prolactin is a potent anti-angiogenic hormone. Endocr.-Relat. Cancer 13, 1–18.

Viau, V., Meaney, M.J., 1991. Variations in the hypothalamic–pituitary–adrenal response to stress during the estrous cycle in the rat. Endocrinology 129, 2503–2511.

Vogelstein, B., Kinzler, K.W., 1993. The multistep nature of cancer. Trends Genet. 9, 138–141.

Zhu, Z., Jiang, W., Thompson, H.J., 2003. Mechanisms by which energy restriction inhibits rat mammary carcinogenesis: in vivo effects of corticosterone on cell cycle machinery in mammary carcinomas. Carcinogenesis 24, 1225–1231.