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Heterogeneity of Neuroendocrine Stress Responses in Aging Rat Strains

Tracy M. Segar¹, John W. Kasckow^{2,3,*}, Jeffrey A. Welge¹, and James P. Herman¹

¹University of Cincinnati Department of Psychiatry, 2170 East Galbraith Road, Cincinnati, OH 45237

²VA Pittsburgh Health Care System MIRECC and Behavioral Health, Pittsburgh, PA 15206

³Western Psychiatric Institute and Clinics, University of Pittsburgh Department of Psychiatry, 3811 O'Hara St, Pittsburgh, PA 15213

Abstract

Hyperactivity of the hypothalamo-pituitary-adrenocortical (HPA) axis is linked with age-related decrements in cognition and neuronal survival. However, the nature and extent of age-related HPA axis deficits varies considerably across and indeed, within strains. The current study was designed to assess variance in HPA axis function using two rodent models commonly used in aging studies: Fischer 344 (F344) and F344/Brown-Norway F1 hybrid rats (F344/BN). We examined both basal and stress-induced ACTH and corticosterone (CORT) release in two stress contexts thought to differ in intensity: novel environment ('mild') and restraint ('intense'). Variability of the data was tested with a modification of the Brown-Forsythe test of homoscedasticity. The results indicated that F344 rats exhibit greater peak HPA responses. Furthermore, in most cases variability was increased in aged rats relative to young and middle-aged rats of the same strain, indicative of the emergence of individual differences in stress responsivity amongst older rats. The results suggest that these older rat strains may be useful models to further assess individual differences in neuroendocrine aging.

Keywords

aging; stress; HPA; ACTH; corticosterone; homoscedasticity

1. Introduction

The hypothalamo-pituitary-adrenocortical axis is responsible for secretion of glucocorticoids into the systemic circulation (e.g., [4]). Glucocorticoid secretion is a tightly regulated process, involving episodic drive by stress atop of a diurnal secretory rhythm [22, 23]. The catabolic nature of glucocorticoid signaling mandates that secretion be kept securely regulated. There is evidence that glucocorticoid control is impaired in the aging process,

*Corresponding author: John W. Kasckow, MD, PhD, VA Pittsburgh Health Care System, Behavioral Health (116A), 7180 Highland Drive, Pittsburgh, PA 15206, Phone: 412-365-5393, Fax: 412-365-5164, kasckowjw@upmc.edu.

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which may be associated with catabolic events occurring as a consequence of aging [31]. However, the aging literature is confounded by numerous contradictory reports regarding the status of the HPA axis; in some studies, profound deficits in feedback and basal hypersecretion are evident, whereas other studies show no such deficits or in some cases, opposite effects (e.g., [5, 7, 15, 19, 42]). In many cases, inter-study variability can be associated with the lack of standardized animal models or methods; however, there is even disagreement in HPA data from laboratories using the same animal strain and identical or near identical methods (e.g., [2] vs. [8]).

In rodents, aging is associated with elevated basal glucocorticoid secretion [16, 30] and prolonged stress-induced glucocorticoid release [32]. Impaired shut-off of the glucocorticoid stress response may be associated with loss of glucocorticoid feedback inhibition of the HPA axis [33]. However, the reproducibility and consistency of aging studies in rodents has been spotty at best. Several studies have been unable to visualize elevated glucocorticoids in aged rats of various strains, including the strain in which basal hypersecretion was first noted (Fischer 344) [1, 6, 13, 39, 40]. In addition, studies of pituitary and adrenal function suggest considerable within and between strain variation (e.g., [3, 8, 13, 36]). Thus, it is evident that the issue of an optimal rat model for HPA axis aging remains wanting.

While many rodent strains have been used for aging research [17], two of the most commonly used rodent strains are the Fischer 344 (F344) and the F344/Brown Norway (F344/BN) F1 hybrid rats. Examination of these strains has revealed the F344/BN strain to be a robust aging model; animals of this strain have a substantially elongated life-span relative to F344 and other aging models, largely due to a relative dearth of systematic pathologies [38]. However, studies of the HPA axis have revealed that the F344/BN animal displays remarkable stability in HPA axis function over time; notably, while age-related changes are observed at all levels of the axis, both basal secretion and stress responsivity remain largely intact and well-controlled [13]. Our past data have suggested that the F344/BN rat strain exhibits 'successful' HPA aging, and may not be optimal for understanding how deleterious changes in the HPA system may affect physiological and cognitive processes in aging [13]. Therefore, the current study evaluates age-related differences in HPA responsiveness in the F344 and F344/BN strains, focusing on both response magnitude and variability.

2. Methods

2.1. Subjects

Male Fischer 344 (F344) (4, 12 and 24 mo) and F344/Brown Norway (F344/BN) F1 hybrid rats (4, 12 and 30 mo) were acquired from the NIA colony maintained at Harlan Labs (Indianapolis, IN). The 'aged' groups were based on studies defining the 'aged' distinction upon the survival curves of the respective strains (time at which one-half of the original cohort remained alive) [38]. All animals were housed three per cage at the University of Cincinnati, with ad lib food and water in a constant temperature and humidity controlled environment on a 12:12 light/dark cycle with the lights on at 0600h. Animals were housed for 9 days between delivery and the start of the study. This was the same for all ages.

All procedures were approved by the University of Cincinnati IACUC. Rats were tested in open field novelty (OFN) and 60 min restraint stress. Subjects were allowed to recover at least seven days between each test. Testing was conducted between 0700h and 1200h. At the end of the experiment, rats were killed by rapid decapitation and adrenal and thymus gland harvested. In addition, brains and pituitaries were removed, and the thoracic and abdominal cavities examined for gross pathology. On the basis of this examination, two 30 month old F344/BN rats were removed from the study due to pituitary or adrenal tumors.

2.2. Open field (novel environment) Exposure

Methods are slightly modified from those previously described [10]. Each subject was placed individually into a novel Plexiglas enclosure (20cm h × 36cm w × 47cm l) for 5 min. Behavior was videotaped using a ceiling mounted camera. Cages were placed on a white background to increase the brightness/novelty of the test session. Subjects were transported to an adjacent room for blood sampling when the test session was completed. Cages were sanitized between sessions. For behavioral scoring, the image of each cage on the monitor was divided into 4 equal sized quadrants and the number of entries into a different quadrant was recorded by two scorers blind to age and strain. An entry was scored when the head and both shoulders entered the quadrant. Data from the test session were compared between the scorers and averaged if they were within 10%. Discrepancies greater than 10% were rescored. To determine 'peak' stress levels of glucocorticoids, subjects underwent the blood sampling 30 min after novelty exposure by "nicking" the tail under light restraint. Blood (200-250 ul) was collected into 1.5 ml Eppendorf tubes containing EDTA.

2.3 Restraint Stress

A full acute stress time-course was obtained using a restraint stress paradigm. Various diameter restraint cages were constructed from Plexiglas to accommodate differences in body size between the young, middle aged and old rats, ensuring a snug fit (such that animals could not move freely in the apparatus). A Plexiglas tab that could be moved to accommodate differences in body length was inserted at the base of the tail to further minimize movement within the restraint cage. Blood was sampled immediately after placement in restrainers, 30 min after the initiation of restraint and immediately before removal from the restrainer (60 min). Rats were then returned to their home cages for one hour, at which point they were briefly replaced in restrainers for the final sampling. Approximately 200-250 µl was collected each time blood was obtained, enough to allow dual- or triple-point determinations of ACTH and corticosterone.

2.4. Hormone assays

Plasma samples were collected as described above and stored at -20° C. Plasma CORT was assessed by radioimmunoassay using an MP Biomedicals, Inc. (Costa Mesa, CA) kit. Plasma ACTH concentrations were determined by radioimmunoassay using a specific antiserum generously donated by Dr. William Engeland (University of Minnesota) at a dilution of 1:210,000 and [¹²⁵I] ACTH (Amersham Biosciences, Piscataway, NJ) as labeled tracer [12]. Plasma samples were processed in two groups classified by the stressor: OFN or restraint.

2.6. Data analysis

Behavioral data, bodyweights, adrenal weights and thymus weights were analyzed with a 2 (strain: F344 vs. F344/BN) × 3 (age: young, middle, old) ANOVA. Hormone time-course data were analyzed with repeated-measures ANOVA. Significant main effects and interactions were followed by Newman Keuls post hoc tests when appropriate. Data were also analyzed for differences in homoscedasticity. For these analyses, raw data were converted to absolute difference scores from the median of each group and analyzed as above with the 2 × 3 ANOVA. This procedure is similar to the Brown-Forsythe test of equality of variance except that this method allows post hoc tests to be performed to determine specific group differences [14]. There were 6, 6 and 10-11 subjects per group for the young (4 mo), middle (12 mo) and aged (24 mo F344 and 30 mo F344/BN) respectively. Significance was set at $p \leq 0.05$ for all analyses.

3. Results

3.1. Neuroendocrine changes following novelty (open field) testing

Figure 1a depicts ACTH levels at 30 min following OFN. Statistical analyses did not reveal any significant differences. Figure 1b shows the CORT levels for all groups following novelty stress. ANOVA revealed a significant main effect of strain, $F(1,39) = 6.11$, $p < 0.05$. The main effect of strain was the result of an elevated corticosterone response to novelty in the F344 strain. However, there were no differences with regards to age or age X strain. Table 1 depicts line crosses during the open field testing. There was a significant strain effect (F344/BN greater than F344) and its interaction with age which are both explained by the 4 mo F344/BN group being significantly higher than all other groups.

3.2. Neuroendocrine changes following restraint stress

Figure 2a depicts the changes in ACTH, respectively, at various times following initiation of restraint. There was a main effect of time [$F(3,108) = 213.2$, $p < 0.05$], reflecting the expected influence of stress. Repeated measures ANOVA on ACTH levels revealed a main effect of strain [$F(1,36) = 11.0$, $p < 0.05$] reflecting elevated ACTH levels in the F344/BN strain. There was also an interaction between strain and age [$F(2,36) = 4.2$, $p < 0.05$] with a general trend for ACTH to decrease with age in the F344 group.

Figure 2b illustrates the effects of age and acute stress on corticosterone secretion. Repeated measures ANOVA revealed a significant main effect of time [$F(3,111) = 67.8$, $p < 0.05$] reflecting the expected impact of restraint stress. There was also a time by strain interaction, [$F(3,111) = 3.81$, $p < 0.05$], consistent with temporal differences in the overall stress response with F344 rats showing greater peak levels than F344/BN animals. However, there were no significant interaction effects between time, strain and age.

3.3. Homoscedasticity

ACTH and CORT levels were also tested for differences in variability between groups following novelty stress and restraint stress. Data were transformed into absolute difference scores from the group median and analyzed with the same ANOVA parameters as above [14]. This test is a modification of the Brown-Forsythe test, allowing testing of group differences.

There was no significant difference in ACTH values (data not shown) for novelty and restraint. Figure 3a illustrates the distribution of the corticosterone data for the novelty test. There was a significant effect of both strain ($F(1,39) = 6.31$) and age ($F(2,39) = 3.44$) (p 's < 0.05) on transformed corticosterone values in the novelty test. Post-hoc testing indicated that the aged groups exhibited significantly more variability than young or middle-aged animals. In addition, the aged F344 cohort was significantly more variable than the F344/BN group.

Figure 3b depicts the spread of the corticosterone data for the full restraint stress profile. The ANOVA on the transformed corticosterone values following restraint stress revealed a main effect of age, $F(2,37) = 4.1$, $p < 0.05$, an age x strain interaction, $F(2,37) = 4.7$, $p < 0.05$, and a main effect of time, $F(3,111) = 13.0$, $p < 0.05$. Newman Keuls post hoc tests on the main effect of age revealed that the older rats (24 and 30 mo) had higher variability than the 4 mo old rats across strains. More importantly, however, post hoc testing on the age x strain interaction revealed that the 24 mo F344 rats had higher variance than the 4 mo and 12 mo old F344 rats, again indicative of enhanced variability with age in this strain. In contrast, there were no significant age differences in the F344/BN strain.

3.4. Adrenal, thymus and bodyweights

ANOVAs on the final bodyweights (Table 2) taken before the final restraint stress revealed main effects of strain $F(1,37) = 16.6$, age, $F(2,37) = 26.0$, and their interaction, $F(2,37) = 3.9$, (p 's all <0.05). As previously documented, the F344/BN strain weighed more than the F344 strain across all ages. In addition, body weight did not differ between middle aged and aged F344 rats, whereas 30 month F344/BN rats weighed significantly more than 12 month-old rats of the same strain.

Adrenal and thymus weights (Table 3) are presented unadjusted and adjusted for terminal bodyweights. There was a significant effect of age, $F(2,37) = 5.2$, $p < 0.05$, on adjusted adrenal weights. The ANOVA revealed that when combined, the aged groups had higher adrenal weights than the middle aged groups. The ANOVA on adjusted thymus weights also revealed a main effect of age, $F(2,36) = 181.1$, $p < 0.05$, consistent with the well-described, age-related thymic involution. There was also an age by strain interaction, $F(2,36) = 8.1$, $p < 0.05$, which was carried by greater thymus size in the young F344/BN group compared to the young F344 group.

4. Discussion

Our findings suggest that aging appears to be associated with increased variability in the HPA axis stress response in rats. Thus, these rat strains could serve as models for further investigations aimed toward understanding what mechanisms account for these apparent changes with aging. The increased variance in HPA function, however, was not correlated with enhanced behavioral changes in these animals.

Unlike our previous report, aged F344/BN animals did not show significant decrements in ACTH release following restraint. However, in this cohort, corticosterone secretion was increased in the aged (as well as middle aged groups). These data indicate that the adrenal of the aged F344/BN rat is hypersensitive to ACTH, which was a fundamental finding of the prior study [13]. In combination, these studies indicate that aging engenders enhanced adrenal corticosteroid production in this strain.

The relatively large corticosterone responses observed in F344 rats are likely to be characteristic of this strain. Previous work indicates that F344 rats are hyperresponsive to both acute and chronic stress relative to several other rat strains, including Sprague-Dawley and Brown Norway strains. In addition, behavioral studies suggest that the F344 strain exhibits increased grooming and decreased locomotion in an open field relative to these strains, consistent with the notion that F344 rats have higher trait anxiety than other strains [24, 34, 35].

Stress testing further supports enhanced stress sensitivity in the F344 model, as both novelty- and restraint-induced corticosterone secretion was higher in this strain. Basal ACTH and corticosterone levels were comparable in the two strains, indicating that the two groups are not starting from different baselines. Notably, the greatest strain differences are evident at the peak of the corticosterone response; indeed, the F344/BN group appeared to recover more slowly from stress exposure, at least in the middle-aged and aged cohorts.

Prior results from our group suggest that the F344/BN F1 hybrid strain exhibits considerable HPA axis adaptation. This takes the form of increased PVN CRH peptide levels, which is apparently compensated by decreased pituitary proopiomelanocortin and CRH-1 receptor expression [9]; and decreased pituitary ACTH release, which is compensated by enhanced adrenal sensitivity to ACTH [13]. The net result is an axis that is fundamentally changed by the aging process, yet maintains its capacity to secrete and control circulating

glucocorticoids, the end-product of the neuroendocrine cascade. Thus, this strain represents an example of ‘successful’ HPA axis function, at least in terms of regulating glucocorticoid environment. Given the deleterious effects of glucocorticoids on numerous organ systems, ranging from bone to brain, it is possible that control of glucocorticoids may contribute to health and longevity in this strain. It is also possible that HPA stability in this strain may be related to general retardation of the aging process itself; the robustness of the F344/BN strain may work against uncovering age-related physiological or behavioral differences.

Our plasma ACTH levels were measured at one fixed time for the restraint, and at four fixed times for the restraint. Furthermore, age or strain or individual differences resulting from these measures could be due either to altered activation of the HPA system and/or alterations in metabolism or disposition of the hormones. We acknowledge that without an even finer-grained temporal analysis, the present study cannot distinguish these two possibilities. One other limitation of the current study was that repeated sampling was employed in the hormonal measurements following restraint which could have confounded the levels obtained later in the samples.

In addition, we stated that animals were housed for 9 days between delivery and the start of the study and that this was the same for all ages. Thus, we could not control for comparable life experiences such as group housing, presence or absence of littermates or different caretakers. Obviously, if the prior environment was more variable for 1 strain and/or a particular age relative to the others, this could have also been a factor which could have confounded the results.

The concept of heterogeneity or ‘individual differences’ is well known in many areas of the aged human population including cognitive functioning (e.g., [18, 43]), learning and memory [11, 21, 26, 41, 44] and variables thought to underlie the higher brain functions such as brain morphology [25, 37]. Directly related to the current study are human studies that have measured individual differences in stress responsivity [20, 37]. These studies support the concept of ‘individual differences’ in aging research and suggest that to truly understand the role of glucocorticoids in various aging processes, it is important to incorporate individual differences in response patterns as a variable. Indeed, this concept is even more salient when current estimates indicate a wide variation in the aging process as only 20% of elderly Americans can be classified as ‘aging successfully’ (using the criteria of Rowe and Kahn[28, 29]). Thus, within-strain comparison of aged glucocorticoid impaired and aged non-impaired subjects is a logical next approach to understanding the nature of glucocorticoid action in brain and body aging.

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Figure 1.

The top of figure 1 depicts ACTH blood levels 30 min following initiation of novelty stress in the two strains of rats. The ANOVA did not reveal any significant effects of strain, age or strain x age for ACTH secretion. The bottom of Figure 1 reveals CORT levels observed following novelty stress examining the effects of age and strain. The ANOVA revealed that the F344 strain had higher overall CORT values than the F344/BN strain.

Figure 2.

The top of Figure 2 reveals ACTH levels resulting from the final restraint. Analysis of the data revealed that all time points were different from each other and also that the F344/BN strain had higher overall ACTH levels than the F344 strain. In the bottom of Figure 2, CORT levels following the final restraint are shown. Analysis of the data revealed that the F344 strain had higher CORT levels than the F344/BN strain at the 60 min time point but that the F344/BN rats had higher levels at the 120 min time point.

Figure 3.

Figure 3a is a scatterplot of CORT values after the novelty experiment. Analysis of the transformed data revealed that the F344 strain was more variable than the F344/BN strain (*) and that the aged rats were more variable than the young rats (**). CORT values following the restraint stress are shown in Figure 3b. Data analysis of the transformed values for the restraint stress profile revealed that aged animals of both strains were more variable than the 4 mo old animals of both strains (*). In addition, the F344 aged animals were more variable than the 4 and 12 mo old animals of the same strain (**). (†) All time points were different from each other except the 60 and 120 min time points.

Table 1

Line Crosses During Open Field Testing

	Line Crosses	
	F344 ($\pm$ SEM)	*F344/BN ($\pm$ SEM)
4 mo	6.58 ($\pm$ 2.44)	26.50 ($\pm$ 1.45)
12mo	12.58 ($\pm$ 3.09)	15.25 ($\pm$ 5.01)
24/30 mo	11.86 ($\pm$ 0.86)	13.9 ($\pm$ 2.16)

There was a significant strain effect (F344/BN greater than F344) and its interaction with age which are both explained by the 4 mo F344/BN group being significantly (*) higher than all other groups.

Table 2

Bodyweights

	Final Bodyweights (g)	
	F344 ($\pm$ SEM)	F344/BN* ($\pm$ SEM)
4 mo	323.7 ($\pm$ 13.4)	342.7 ($\pm$ 10.7)
12mo	421.3 ($\pm$ 12.8)	505.3 ($\pm$ 12.0)
24/30 mo	434.5 ($\pm$ 32.2)	588.3** ($\pm$ 22.2)

* F344/BN weighed more than the F344 strain overall

** aged F344/BN weighed more than 12 mo of same strain

Table 3

Adjusted and unadjusted total adrenal and thymus weights

	adjusted total adrenal (mg × 10 ³ /bw)		adjusted thymus (mg × 10 ³ /bw)	
	F344 (± SEM)	F344/BN (± SEM)	F344 (± SEM)	F344/BN (± SEM)
4 mo	0.10 (± 0.00)	0.11 (± 0.01)	0.50** (± 0.05)	0.62** † (± 0.03)
12 mo	0.10 (± 0.01)	0.09 (± 0.00)	0.18 (± 0.01)	0.11 (± 0.02)
24/30 mo	0.14* (± 0.01)	0.11* (± 0.01)	0.18 (± 0.01)	0.15 (± 0.02)

	unadjusted total adrenal (mg × 10 ³)		unadjusted thymus (mg × 10 ³)	
	F344 (± SEM)	F344/BN (± SEM)	F344 (± SEM)	F344/BN (± SEM)
4 mo	32.1 (± 2.0)	38.7 (± 1.1)	161.3 (± 18.0)	211.9 (± 14.5)
12 mo	40.6 (± 3.5)	45.0 (± 1.8)	75.2 (± 6.3)	57.0 (± 7.8)
24/30 mo	59.3 (± 5.0)	63.3 (± 4.2)	73.2 (± 6.5)	82.1 (± 7.9)

* aged animals (combined across strain) had higher adrenal weights than middle aged

** young animals (combined across strain) had higher thymus weights than both the middle aged and old groups

† young F344/BN thymus weights were higher than young F344