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Pharmacology, Biochemistry and Behavior 67 (2000) 809–819

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

Lewis/Fischer rat strain differences in endocrine and behavioural responses to environmental challenge

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Accepted 23 August 2000

Abstract

The Lewis (LEW) and Fischer (F344) rat strains provide a comparative model of hypothalamic–pituitary–adrenal (HPA) function in which LEW is relatively hypoactive at homeostasis and hyporeactive to environmental challenge. The present study describes a comparison of LEW and F344 rats, males and females, in terms of their corticosterone (CORT) or behavioural responses to a range of behavioural tasks, where each of the tasks used contains a stressor component and has been demonstrated to be sensitive to corticotropin releasing factor (CRF) and/or CORT manipulation: acoustic startle response (ASR), elevated plus maze, schedule-induced polydipsia, and fear-conditioned suppression of drinking. Our aim was to determine to what extent the LEW trait of HPA axis hyporesponsiveness is associated with strain differences in behavioural responsiveness to environmental challenge. As expected, young (2–3 months)–mature (5–10 months) LEW males and females exhibited a lesser CORT response to restraint and novel confinement than did F344 males and females, although in old adulthood (18 months) the CORT stress response was equable in LEW/F344 males and actually higher in LEW than in F344 females. In young–mature adults, the ASR was greater in LEW males than in the other groups; all groups spent a low proportion of time on the open arms of the elevated plus maze; polydipsia was greater in F344 females than in the other groups; and fear-conditioned suppression of drinking was greater in F344 males and females than in LEW males and females. Therefore, relative hyporeactivity of the HPA axis in LEW rats is clearly not associated with uniform behavioural hyporeactivity, including CRF-dependent behaviours. Rather, this study suggests further evidence that environmental reactivity reflects a number of distinct emotional states and underlying neural circuits.

Keywords: Lewis rat; Fischer rat; Stressor; HPA axis; Emotional behaviour; CRF

Inbred rat strains demonstrate different neuroanatomical, physiological, and behavioural phenotypes. By comparison of different combinations of these phenotypes between specific strains it is possible to gain important understanding of the neurobiological regulation of behavioural phenotype, both normal and dysfunctional. Lewis (LEW) rats and Fischer 344 (F344) rats are inbred and histocompatible strains that have been the subject of comparative investigation based on the LEW strain's high susceptibility to experimental immune/inflammatory disease. This is associated with hypoactivity in the regulatory feedback loop between the immune system and the hypothalamic–pituitary–adrenal (HPA) axis, via which corticosteroids normally limit the intensity of the immune/inflammatory response. LEW rats exhibit a defect in the hypothalamic response to immune/inflammatory challenge, specifically in the form of reduced synthesis and secretion of corticotropin releasing factor (CRF), leading to reduced plasma corticotropin (ACTH) release from the pituitary and corticosterone (CORT) from the adrenal cortex (males [15]; females [43–45]). This is the case in comparison with both outbred and inbred strains, including F344 among the latter; in fact the F344 HPA axis is hyperresponsive relative to at least some outbred strains [15]. As well as their attenuated HPA axis stress response, LEW rats exhibit an attenuated circa-

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0091-3057/00/$ – see front matter
PII: S0091-3057(00)00426-3

dian profile of the HPA axis relative to F344 and other strains, such that basal peak CORT levels are relatively low whilst basal nadir levels are approximately the same as those in other strains (males [15,30]; females [41]).

The LEW HPA axis is not only hyporesponsive to immune/inflammatory challenge but also to factors in the external environment that typically act as stressors in the rat. Open field exposure, forced swimming, physical restraint, and acoustic startle all induce an activation of the HPA axis in LEW rats that is attenuated relative to that of other strains, including F344 (males [15,36]; females [43]). Given the essential role of the limbic (L)–HPA system in the regulation of emotional and cognitive interaction with the environment (e.g. Refs. [13,22]), detailed comparative investigation of endocrine, behavioural, and neurobiological responses to environmental challenge in the LEW/F344 genetic model is warranted. It is widely recognised that dysfunction in the LHPA system is involved in the pathophysiology of a range of neuropsychiatric diseases, including affective disorders and drug abuse [7,12,14,31,33]. The small number of comparative studies of LEW/F344 behaviour that have been conducted recently describe a complex of strain-dependent similarities and differences. For example, it has been reported that LEW and F344 strains exhibit similarly high anxiety-like behaviour but that the LEW strain exhibits relative locomotor hyporeactivity in a test environment, either spontaneously [5] or following amphetamine [46]. As such, the LEW/F344 genetic model contributes to a growing body of evidence that emotional reactivity in the rat — behavioural, endocrine, and autonomic — is highly heritable, varies along a number of independent dimensions, and that this variability is due in large part to different measures reflecting different emotional states and their respective neural circuits [2,35].

The aim of the present study was to compare the endocrine or behavioural responses of LEW and F344 rats, males and females, in a range of standard tasks with an inherent stressor component. The behavioural tasks were selected on the basis of previous demonstrations of their sensitivity to CRF and/or CORT, and because together they allowed for the challenge of different emotional systems, including anxiety (elevated plus maze), frustration (schedule-induced polydipsia), and fear (conditioned emotional response, CER), and of different types of behavioural response, including reflex (acoustic startle response, ASR), spontaneous appetitive (elevated plus maze) and conditioned consummatory (CER). We compared adults of the two strains using both males and females, allowing not only for LEW/F344 comparison but also for direct identification of strain–sex interactions.

Basal CORT levels were determined, as was CORT response to restraint, confinement, or confinement combined with acoustic startle pulses. This was carried out with young, mature, and old adults, with the aim of demonstrating in our own laboratory the marked LEW/F344 differences reported by others. In terms of behaviour we compared young–mature adult LEW and F344 rats in the following paradigms: (1) The ASR, a reflex elicited by acute auditory stimulation and demonstrated to be potentiated by CRF and CORT administration [1,19,23,48]; here our main aim was to determine whether the relatively high ASR reported for LEW males [20] also applies to females. (2) The elevated plus-maze anxiety test, in which a high proportion of time spent on the closed arms is interpreted as evidence of high anxiety [11,32], and which is increased by CRF and decreased by CRF-blockade [16,40]; here our aim was to extend the analysis of Chaouloff et al. [5] to females and to include risk assessment behaviours. (3) Schedule-induced polydipsia, a behavioural trait displayed by hungry rats during scheduled food delivery and interpreted as adjunctive or displacement behaviour; removal of endogenous CORT by adrenalectomy blocks the development of polydipsia and CORT substitution reinstates it [8,24,27]; this paradigm had not previously been studied using a comparative-strain approach, as was the case for: (4) The CER, suppression of drinking behaviour in the presence of a stimulus previously paired with a mild electric shock, and demonstrated to be enhanced by CRF [9]. Of course, any consistent interstrain differences across behavioural tasks could be idiosyncratic to LEW/F344 and would need to be tested against additional strains.

Therefore, this study presents a comparison of LEW and F344 rats, males and females, in terms of their CORT or behavioural responses to a range of environmental challenges, where each of the varied behavioural tasks studied has been demonstrated to be sensitive to CORT and/or CRF manipulation. Our aim was to determine to what extent, if any, the relative hyporesponsiveness of the LEW LHPA system to environmental challenge was associated with LEW/F344 strain differences in behavioural responsiveness to environmental challenge.

1. Method

1.1. Animals

The experimental subjects were aged 2–3 months (young adult), 5–10 months (mature adult), or 18 months (old adult) at testing. They were first or second generation offspring, born in-house, of male and female F344 rats (F344/NHsd; mean weight: 500 g/260 g) or LEW rats (LEW/SsNHsd; mean weight: 530 g/310 g) (Harlan, Horst, The Netherlands). Subjects were socially housed in standard polycarbonate cages under a reversed light–dark cycle (lights on: 1900–0700 h). The animal room temperature was maintained at $23 \pm 1^{\circ}C$ and humidity at $50 \pm 5\%$. Rats received ad libitum food (NAFAG Food Pellets No. 890; NAFAG ECOSAN, Gossau, Switzerland) and water in their home cage, except during food deprivation for the schedule-induced polydipsia paradigm and water deprivation for the CER paradigm. Subjects were handled prior to testing, and each subject

was tested in one paradigm only unless stated otherwise. All experiments were carried out in accordance with the Swiss federal legislation for animal experimentation.

1.2. Endocrine and behavioural tests

1.2.1. CORT levels under basal and stress conditions

In young adults, plasma CORT levels were measured at basal level and after restraint. Six subjects per strain × sex and naive of experimental procedures were blood sampled in squads of four subjects, and per cage a maximum of two subjects were studied, with both included in the same squad. The procedure began at 1300–1600 h when subjects were expected to be at or approaching the basal nadir of their CORT circadian profile: this was not established in-house but was extrapolated from the published reports of basal nadir CORT values in LEW and F344 occurring in the last 3–4 h of the dark phase and first 3–4 h of the light phase [41]. Restraint tubes were constructed from transparent plastic with a 5-cm diameter; they were equipped with air holes, closed at one end by a wall of the same material, and adjustable at the open end with an O-shaped fixture that allowed the length of the tube to be set to the exact length of the animal and provided access to the tail for blood sampling. Each subject was removed from the home cage and within 60 s had been placed in the restraint tube and a first blood sample obtained via a small incision at the tip of the tail. Blood samples (50–100 μl) were collected into heparinized tubes (Microvette; Sarstedt, Sevelen, CH) and placed on ice between collection and centrifugation. This first sample was designated as time −30 min and provided the estimate for basal CORT. Restraint was conducted for 30 min after which a second blood sample, time 0 min, was collected and the subject removed from the restraint tube and returned to the home cage. Postrestraint blood samples were then collected at time 30, 60, and 120 min followed on each occasion by immediate return to the home cage.

In the case of mature and old adults, basal CORT was determined in tail-vein blood samples several days in advance of stressor exposure and at two time periods of the diurnal cycle: shortly before onset of the light phase (1830 h: anticipated basal nadir (e.g. Ref. [41])) and shortly after the onset of the dark phase (0730 h: basal peak). For analysis of stress responses, mature adult subjects ($N=10$ per strain × sex) were placed in the ASR chamber for 30 min, one occasion with and on a separate occasion without exposure to the startle-eliciting auditory pulses (see below for description of the apparatus). Tail-vein blood samples were collected at 0 and 30 min following removal from the chamber. This procedure provided evidence on CORT responses of subjects to the ASR chamber per se and to the chamber plus auditory pulses, this latter procedure providing CORT titres to complement the ASR behavioural data obtained with a separate group of subjects (see below). In the old adult subjects ($N=12$ per strain × sex), the restraint paradigm (tube ∅ = 6 cm) was used as described for young adults with the exception of basal CORT blood sampling. Each of these subjects had been tested previously in one of the behavioural paradigms as a mature adult.

All blood samples were centrifuged at 2500 rpm for 15 min at 4°C and the plasma removed and stored at −25°C. Sample and quality control preparation involved 1:400 dilution followed by binding-protein heat denaturation in a 90°C water bath for 10 min. Total CORT was determined using a specific in-house radioimmunoassay, comprising CORT antiserum (07-120016; ICN Biomedicals, Costa Mesa, CA), CORT standard over the range 12.5–500 pg/tube (Sigma C2505; Fluka Chemie, Buchs, CH), and [1,2,6,7-^{3}H] CORT as tracer (TRK 406; Amersham Pharmacia Biotech, UK). Separation of antibody bound and unbound CORT was performed after a 16–20-h incubation at 4°C using dextran-coated charcoal. The sensitivity of the RIA was 4 pg/tube, and the interassay precision was 10% at low CORT and 6% at high CORT concentrations, respectively ($N=4$).

Turning to behavioural tasks, young and mature adults were not anticipated to differ from each other in terms of behavioural performance, and the age of rats at testing on each behavioural task was determined by logistical factors rather than experimental design.

1.2.2. Acoustic startle response

Naive subjects ($N=8$ per strain × sex) were tested in the ASR paradigm as young adults. The apparatus consisted of four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) each containing a transparent Plexiglas tube (∅ = 8.2 cm, length = 20 cm) mounted on a Plexiglas frame within a ventilated enclosure. Acoustic pulses were presented via a speaker mounted 24 cm above the tube. A piezoelectric accelerometer mounted below the frame detected and transduced motion within the tube. Startle amplitudes were defined as the average of 100 × 1-ms stabilimeter readings collected from stimulus onset. Rats were run in squads of four. Each rat was put into the chamber for a 5-min acclimatisation period with a 68-dB[A] background noise level that continued throughout the session. After acclimatisation, four startle pulses of 120 dB[A] broadband burst for 30 ms were presented with an average intertrial interval of 15 s (range 10–20 s). The same apparatus was used as a stressor to monitor CORT response in mature adults (see above).

1.2.3. Elevated plus maze

Naive subjects ($N=6$ per strain × sex) were tested in the elevated plus maze as mature adults. The elevated plus-maze consisted of two open (i.e. uncovered) arms (each 45 × 10 × 0.5 cm) and two closed arms (each 45 × 10 × 40 cm) extending from a common central platform (10 × 10 cm). The entire apparatus was elevated to a height of 62 cm above floor level. The floor of the maze was constructed of white painted wood while the side and end walls of the

enclosed arms were made of black painted wood. In order to increase the likelihood of any subject entering into an open arm, testing was conducted under dim light of ca. 12 lx as measured on an open arm. Subjects were brought into the test room in their home cages and allowed 30 min to acclimatise to the testing room prior to placement in the elevated plus maze. Testing commenced by placement of a subject onto the central platform facing an open arm and continued for 5 min. Behavioural measures included the percent time spent on the open arms as well as measures of risk assessment behaviour [37]: Stretched attend posture: the rat stretches forward and retracts to its original position without forward locomotion; closed arm returns: rat exits a closed arm with the forepaws only and then returns into the same arm; head dipping: rat scans over the sides of the maze towards the floor, either exposed (on open arm) or protected (on central platform or closed arm). Behaviour was recorded by a video camera mounted on the ceiling and analysed by a computerised behaviour analysis system (Ethovision, Noldus Information Technology, Netherlands) or an observer blind to the identity of the animals.

1.2.4. Schedule-induced polydipsia

Naive subjects ($N = 10$ per strain $\times$ sex) were tested on schedule-induced polydipsia as mature adults. They were food deprived (1 h access to food in the home cage per day) for 7 days prior to the start of and during the experiment, with body weight maintained at 85–90% of normal. Schedule-induced polydipsia was assessed in the same chambers as those used in the CER procedure (see below), in which a food dispenser was mounted on the left side of the water bottle. Rats were given 14 daily 30-min sessions during which one 45 mg food pellet was delivered automatically every 60 s. Water consumption was measured by weighing the water bottle before and after each session and calculated as millilitre water per kilogram body weight.

1.2.5. Conditioned emotional response

Naive subjects ($N = 8$ per strain $\times$ sex) were tested on CER as young adults. The apparatus consisted of four test cages (Coulbourn Instruments, Allentown PA, model E10-10), each set in a ventilated sound-attenuating isolation cubicle (Coulbourn Instruments, model E10-20). A drinking bottle with a tube opening of 3 mm diameter could be inserted into the chamber through a 3×4 cm hole located in the centre of the right wall of the chamber, 1.5 cm above the grid floor. When the bottle was not present, the hole was covered by a metal lid. Licks at the drinking bottle were detected by an infrared optical lickometer (Coulbourn Instruments, model E24-01). The unconditioned stimulus (US) was a scrambled shock, set at 0.75 mA and 1 s duration, delivered through the cage floor from an electric shocker and scrambler (Coulbourn Instruments, models E13-14, E13-13). The conditioned stimulus (CS) was a 10-s light flashing at a rate of 2 Hz, generated from a 28-V, 40-mA house light located on the right wall of the chamber 26 cm above the grid floor, which also provided background chamber illumination. The four test cages were connected to an IBM-compatible PC for experimental control and data recording. Five days prior to the beginning of the CER experiment, subjects were put on a 23-h water deprivation schedule, which continued throughout the experiment. Water in the test apparatus was given in addition to the daily 1-h ration. The CER procedure consisted of the following stages, with conditioning, re-baseline, and test sessions conducted 24 h apart: Baseline (days 1–5): on each of 5 days, rats were placed individually into the experimental chambers and allowed to drink for 20 min. The latency to the first lick during each baseline session was recorded. Conditioning (day 6): with the bottle removed, each rat received two light-shock pairings 5 and 10 min after the start of the session, with the 1-s shock immediately following light termination. After the second pairing, rats were left in the experimental chamber for an additional 5 min. Re-baseline (day 7): each rat was given a drinking session as in baseline training, and the latency to first lick was recorded. Test (day 8): each rat was placed in the chamber and allowed to drink from the bottle. When the rat completed 175 licks, the light was presented and continued for 15 min. For each subject the times to complete 25 licks before (licks 151–175) and after stimulus onset (lick 176–200) were recorded (time A and B, respectively). The suppression ratio [Time A/(Time A + Time B)] served as the dependent measure, with a ratio of 0.5 indicating no suppression and a ratio approaching 0 indicating high suppression.

1.3. Statistical analysis

Data were analysed using analysis of variance (ANOVA), calculated with the SuperANOVA and StatView software system (Abacus Concept, Berkeley, CA). The level of significance was set at $P < .05$. Post hoc tests were conducted using the Duncan multiple range test.

2. Results

2.1. CORT levels under basal and stress conditions

In young adult subjects, plasma CORT values were analysed by 3-way ANOVA with strain and sex as between-subject factors and time as within-subject factor (five levels, baseline (-30 min) and 0, 30, 60, and 120 min following restraint). There were significant main effects of strain [$F(1,20) = 8.9$, $P < .008$], sex [$F(1,20) = 14.5$, $P < .002$], and time [$F(4,80) = 79.3$, $P < .001$], as well as a strain $\times$ time interaction [$F(4,80) = 12.8$, $P < .001$]. As given in Fig. 1, plasma CORT was higher in females than males overall, F344 males had higher plasma CORT levels than LEW males overall, and F344 females had higher plasma CORT levels than LEW females overall. Two-way ANOVAs

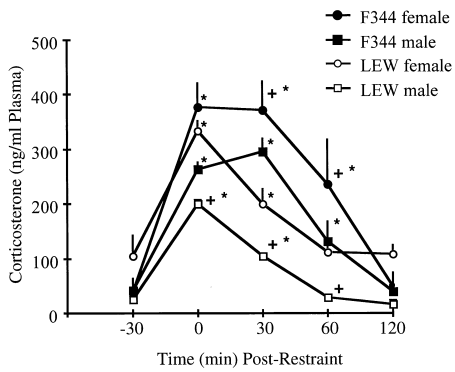

Fig. 1. Concentration profiles (ng/ml) of plasma CORT in young adult male and female LEW and F344 rats relative to restraint stress between time −30 min (basal nadir) and 0 min. Values represent the mean ± S.E.M. of six rats per group. + Denotes a significant sex-specific strain effect; * denotes a significant elevation of CORT relative to basal nadir levels.

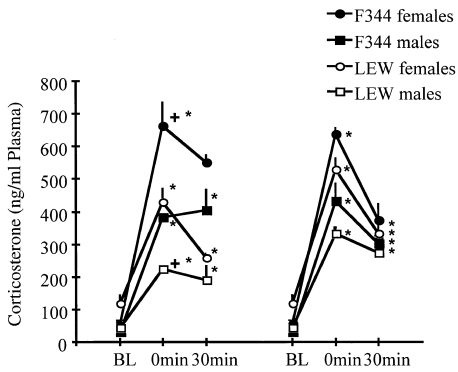

Fig. 2. Concentration profiles (ng/ml) of plasma CORT in mature adult male and female LEW and F344 rats during challenge. Left-hand-panel: confinement in the ASR apparatus; right-hand-panel: confinement in the ASR apparatus and exposure to four 120 dB[A] startle pulses. Basal nadir (BL) samples were obtained several days prior to the ASR challenge. Values represent the mean ± S.E.M. of 10 rats/group. + Denotes a significant sex-specific strain effect; * denotes a significant elevation of CORT relative to basal nadir levels.

at individual time points indicated that there was a non-significant tendency to an interaction between strain and sex at time −30 ($P<.10$), and also a significant strain × sex interaction at time 120 min [$F(1,20)=5.5$, $P<.03$], primarily attributable to the high CORT levels in LEW females following poststress recovery. There was a significant effect of strain at times 0 min [$F(1,20)=4.7$, $P<.05$], 30 min [$F(1,20)=34.1$, $P<.001$], and 60 min [$F(1,20)=6.2$, $P<.03$], in each case attributable to the relatively low stress response in LEW subjects (Fig. 1). LEW subjects also had a blunted stress response relative to F344 subjects in terms of time taken to return to baseline, with time 60 min levels not differing significantly from basal values in LEW rats whereas in F344 this was not the case until 120 min.

In mature adults, a four-way ANOVA with strain and sex as between-subject factors and stressor (startle box with or without startle pulses) and time (baseline, 0 min and 30 min following stress) as within-subject factors revealed significant main effects of strain [$F(1,38)=34.7$, $P<.001$], sex [$F(1,38)=38.0$, $P<.001$], stressor [$F(1,38)=31.5$, $P<.001$], and time [$F(2,184)=4.4$, $P<.001$], as well as a significant interaction of strain × stressor [$F(1,38)=9.6$, $P<.01$]. As given in Fig. 2, both stressors significantly increased plasma CORT concentrations. The peak CORT response was higher in F344 than in LEW rats in the case of confinement. F344 rats responded equally to both stressors, whereas LEW subjects demonstrated an increased peak CORT response to the combined stressor. A two-way ANOVA of basal CORT values indicated a significant main effect of strain [$F(1,36)=5.1$, $P<.01$], and a nonsignificant tendency to an interaction between strain and sex ($P<.10$), reflecting relatively high basal CORT in mature LEW females, as was also the case in young adult LEW females (see above). During confinement in the absence of startle pulses, there was a significant effect of strain at times 0 min [$F(1,36)=3.5$, $P<.04$] and 30 min [$F(1,36)=33.8$, $P<.001$], attributable to the relatively low stress response in LEW subjects (Fig. 2).

In old adults, basal CORT titres during the diurnal cycle were analysed by a three-way ANOVA with strain and sex as between-subject factors and the within-subject factor of diurnal cycle (basal peak and basal nadir). There were significant main effects of strain [$F(1,43)=4.2$, $P<.05$], sex [$F(1,43)=49.5$, $P<.001$], and diurnal cycle [$F(1,43)=22.5$, $P<.001$], as well as a diurnal cycle × strain interaction [$F(1,43)=4.3$, $P<.05$]. As can be seen in Fig. 3 (left panel), LEW females had higher basal nadir CORT levels than F344 females, due to the absence of a reduction from the basal CORT titres pertaining at the onset of the dark phase. Plasma CORT titres following restraint in these 18-month-old subjects were analysed by three-way ANOVA, which revealed significant interaction of strain and time [$F(2,88)=5.6$, $P<.001$] and sex and time [$F(2,88)=4.9$, $P<.01$] (Fig. 3, right panel). A two-way ANOVA indicated

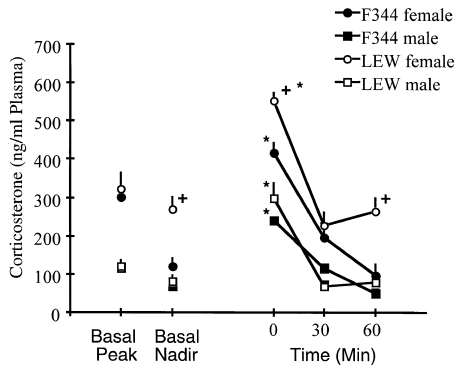

Fig. 3. Concentration profiles (ng/ml) of plasma CORT in old adult male and female LEW and F344 rats. Left-hand-panel: diurnal basal peak and basal nadir concentrations of plasma CORT (peak basal values of LEW and F344 males are superimposed). Right panel: plasma CORT in the same subjects relative to 30-min restraint ending at time 0 min. Values represent the mean ± S.E.M. of 12 rats/group. + Denotes a significant sex-specific strain effect; * denotes a significant elevation of CORT relative to basal nadir levels.

that the peak stress CORT response (time 0) was higher in LEW females than in F344 females. In LEW and F344 females and LEW males, CORT titres returned to basal values within 30 min postrestraint, and in F344 males within 60 min.

2.2. Acoustic startle response

Fig. 4 gives the startle amplitude of male and female LEW and F344 rats in response to the four startle pulses. A three-way ANOVA with between-subject factors of strain and sex and within-subject factor of startle pulse [1–4] revealed significant main effects of strain [$F(1,29)=17.6$, $P<.001$] and sex [$F(1,29)=9.1$, $P<.01$], as well as a significant strain × sex interaction [$F(1,29)=5.2$, $P<.05$]. As can be seen, male LEW rats displayed a stronger startle reflex than F344 males and F344 and LEW females, with the latter three groups demonstrating similar ASRs. In addition, there was a significant effect of startle pulse [$F(3,87)=5.5$, $P<.01$], but no interaction with any of the other factors ($P>.1$), reflecting similar habituation of the startle reaction with repeated pulse presentation in all groups.

2.3. Elevated plus maze

All dependent variables were analysed by two-way ANOVAs with strain and sex as between-subject factors. There were no differences between the groups in the percentage of time spent on the open arms (all $P>.2$; Fig. 5). However, in three of the four measures of risk assessment behaviours, ANOVA revealed a significant effect of strain: protected head dips [$F(1,20)=14.5$, $P<.01$], stretch-attend postures [$F(1,20)=11.6$, $P<.01$], and closed arm returns [$F(1,20)=14.2$, $P<.01$]. As can be seen in Fig. 5, LEW rats demonstrated more of these behaviours than did F344 rats.

2.4. Schedule-induced polydipsia

Fig. 6 presents, in 2-day blocks, the water consumption induced by scheduled food delivery in subjects under food

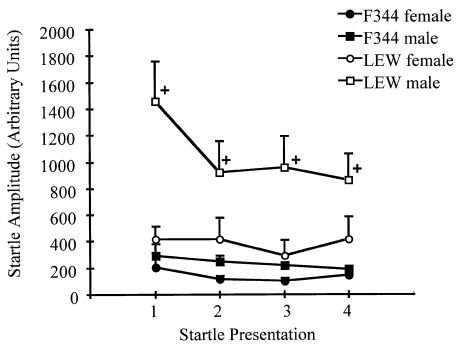

Fig. 4. Amplitude of ASR, in arbitrary units, to four 30 ms acoustic startle pulses of 120 dB[A], in young adult male and female LEW and F344 rats. Values are means ± S.E.M. of eight rats/group. + Denotes a significant difference between LEW males and each of the other strain × sex groups.

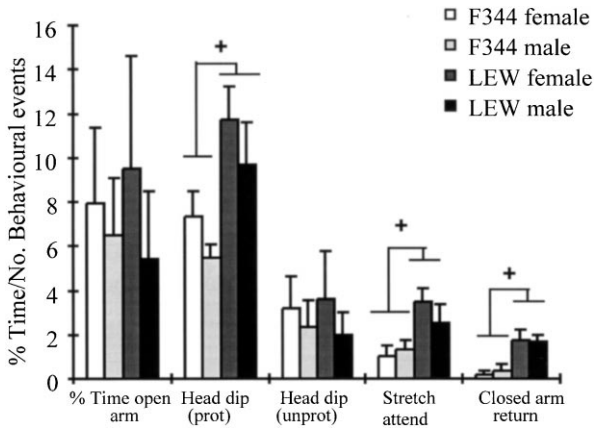

Fig. 5. Summary of behaviour of mature adult male and female LEW and F344 rats on the elevated plus maze. Values are means ± S.E.M. for six subjects/group, for percent of time spent on the open arms and number of occurrences of risk assessment behaviours. + Denotes a significant difference between LEW and F344 strains.

deprivation. An ANOVA with between-subject factors of strain and sex and a within-subject factor of time (seven 2-day blocks) revealed a significant main effect of time [$F(6,168)=42.2$, $P<.001$] and significant interactions of sex and time [$F(6,168)=5.0$, $P<.001$] and strain, sex, and time [$F(6,168)=2.7$, $P<.05$]. All treatment groups developed schedule-induced polydipsia, and, as demonstrated by Fisher's PLSD test, F344 females developed higher levels of fluid intake than LEW females and F344 and LEW males, with the latter three groups demonstrating similar levels of fluid intake.

2.5. Conditioned emotional response

The latency to the first lick during the baseline sessions was analysed by a three-way ANOVA with between-subject factors of strain and sex and within-subject factor of base-

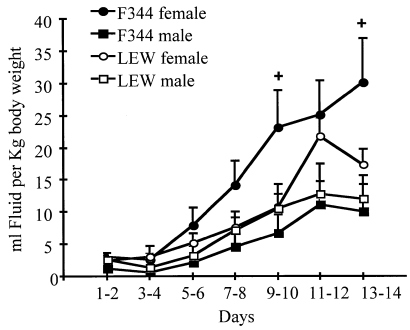

Fig. 6. Development of polydipsia as induced by scheduled food delivery in food-deprived mature adult male and female LEW and F344 rats. Values are mean ± S.E.M. water consumption in millilitre per kilogram body weight during the 30-min daily test for 10 subjects/group and per 2-day block. + Denotes a significant difference between F344 females and each of the other strain × sex groups.

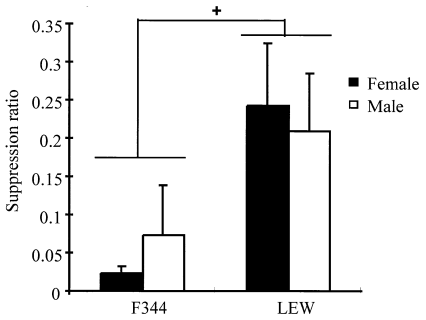

Fig. 7. Conditioned emotional suppression of drinking by a flashing-light CS in young adult male and female LEW and F344 rats. The suppression ratio represents the additional amount of time required to complete 25 licks in the presence of the CS and is calculated such that in the absence of suppression the ratio has a value of 0.5, and the greater the conditioned suppression the lower the value of the suppression ratio. Values are mean ± S.E.M. suppression ratios for eight subjects/group. $^{+}$Denotes a significant difference between LEW and F344 strains.

line session [1–5]. This analysis revealed a significant main effect of strain [$F(1,29) = 6.3$, $P < .05$], reflecting longer latencies in F344 rats (339 ± 40 s, mean ± S.E.M.) as compared with LEW rats (177 ± 27 s), in both males and females. Furthermore, a significant reduction in overall latencies (in seconds) to the first lick across baseline sessions was obtained (Baseline 1: 484 ± 63; Baseline 2: 325 ± 60; Baseline 3: 213 ± 50; Baseline 4: 175 ± 48; Baseline 5: 82 ± 19) [$F(4,116) = 13.9$, $P < .001$]. The latency to the first lick during the re-baseline session was analysed by two-way ANOVA with between-subject factors of strain and sex. There was a nonsignificant trend for the latency to the first lick to be greater in LEW rats than in F344 rats [$F(1,29) = 3.4$, $P < .08$]; compared with baseline session 5, the latency increased from 125 ± 33 to 701 ± 77 s in F344 subjects, and from 42 ± 14 to 935 ± 97 s in LEW subjects. Fig. 7 presents the suppression ratio during the test session for male and female LEW and F344 rats, where a lower value depicts higher suppression of drinking, i.e. a higher CER. A two-way ANOVA with between-subject factors of strain and sex revealed a significantly increased CER, i.e. suppression of drinking, in F344 rats compared with LEW rats [$F(1,29) = 7.2$, $P < .01$].

3. Discussion

The present study compares the responsiveness of LEW and F344 rats, male and female, to a range of environmental challenges, at both the endocrine and behavioural levels. With regards to CORT responses to restraint, confinement, or confinement and startle-eliciting auditory pulses, this study confirms that in young and mature adults, LEW male and female rats exhibit an attenuated peak-stress CORT response and a truncated period of poststress CORT elevation, compared with F344 male and female rats. In old adults, however, we have demonstrated that ageing leads to a relative increase in the CORT stress response, and in LEW females to the extent that their peak-stress CORT response to restraint exceeds that of old F344 females. This ageing-related increase in LEW female CORT response was associated with higher basal nadir CORT, and this latter characteristic was also present in young and mature adult LEW females. In terms of behavioural responses, as measured in young–mature adults, strain difference was ubiquitous across all tasks although on some tasks the strain effect was restricted to one sex. LEW males demonstrated a greater ASR relative to LEW females and F344 males and females. On the elevated plus maze, both LEW and F344 males and females spent high amounts of time on the closed arms, and LEW rats demonstrated higher levels of risk-assessment behaviours. Development of schedule-induced polydipsia was greater in F344 females relative to LEW females and males and F344 males. F344 males and females demonstrated a greater CER than did LEW rats, as measured using suppression of drinking behaviour by a discrete auditory stimulus paired with foot shock 48 h previously.

Our finding that basal nadir CORT levels were elevated in adult LEW females compared with F344 females is in line with previous reports [6,41]. Whilst it is possible that this difference is due to female strain differences in levels of corticosteroid-binding proteins, it has been reported that LEW males actually exhibit lower levels of such proteins than do F344 males [15] and also Wistar males [29]; LEW and F344 females remain to be compared in terms of corticosteroid-binding proteins. That young and mature adult male and female LEW rats demonstrated attenuated CORT responses in various stress paradigms is in broad agreement with a number of previous studies (males [15,20,36]; females [43]). Therefore, LEW rats, male and female, respond to restraint and confinement with an increase in plasma CORT, but the peak CORT stress response to each type of stressor is lower and of shorter duration than is the case in F344 rats of the same sex; as a direct consequence of this poststress return to basal levels occurs sooner in LEW rats. Furthermore, activation of a maximal CORT stress response appears to require greater challenge load in LEW than in F344 rats: mature adults of the latter strain demonstrated no further increase in CORT peak response when confinement was accompanied by acoustic startle pulses whereas LEW rats did respond incrementally to these two stressor types.

In old adulthood, in addition to the elevated basal nadir CORT levels in LEW females relative to F344 females, there was no strain difference in basal peak CORT levels, which contrasts with the consistent reports of elevated basal peak CORT in F344 compared to LEW in young adulthood [15,30,41]. The CORT response of old LEW male and female rats to restraint increased markedly relative to that measured in young adults, whereas that of F344 rats was stable between young and old adulthood. The peak CORT response to restraint was actually greater in LEW females

than in F344 females in old adulthood. There is evidence derived from several rat strains that ageing is characterised by a reduced ability to maintain homeostasis including enhanced and prolonged HPA stress responses. Basal CORT levels begin to increase from months 12–15 onwards and increased peak-stress response and prolonged poststress recovery from months 15–18 onwards [25,26,38]. These ageing-related increases in the HPA axis re/activity are associated with reduced densities of mineralocorticoid receptor (MR) and glucocorticoid receptors (GR) in the hippocampus and hypothalamus, and have been attributed to attenuated proactive and reactive negative feedback in the LHPA axis [14]. The well-described LHPA hyporesponsiveness in the LEW strain and our current findings of exaggerated age-related hypercorticoidism in the LEW strain might both be associated with strain differences in MR and/or GR activity in those structures involved in the regulation of CRF synthesis and secretion. It has been reported for young adult LEW males that, at least relative to Wistar rats, they display increased MR binding capacity in the hippocampus and hypothalamus and that this mediates increased proactive LHPA negative feedback [29]. An enhanced ageing-related reduction in MR function in these brain regions in LEW females relative to F344 females could account for our CORT findings. However, in young adult LEW vs. F344 females, hippocampal MR binding capacity was actually found to be lower in LEW than F344 with no significant difference in MR mRNA expression, whilst hippocampal GR capacity was equable in LEW and F344 females but GR mRNA expression was higher in the LEW female hippocampus [42]. Clearly, further investigation of the LEW/F344 LHPA model is required to elucidate the contribution of MR and GR function to the aetiology of the well-established reduced CRF synthesis and HPA axis hyporesponsiveness per se, and to the ageing-related attenuation of this phenotype in the LEW strain as reported here.

Turning to our behavioural findings, LEW male rats exhibited a markedly enhanced ASR relative to LEW females and F344 males and females, which both confirms and expands upon a study describing the higher acoustic and tactile startle responses of LEW males relative to F344 and Sprague–Dawley males [20]. The neural circuitry mediating the ASR includes the caudal pontine nucleus (PnC) of the reticular formation in the brain stem [21]. Activity in the PnC is modulated by input from the central nucleus of the amygdala, and this provides the major neural circuit for potentiation of the ASR by fear or anxiety [18]. This pathway uses CRF as a neurotransmitter and injections of CRF into the PnC increase the ASR [1], while microinjections of CRF receptor antagonists into the PnC block expression of fear-potentiated startle [19]. Whilst Glowa et al. [20] conclude it to be paradoxical that LEW males should demonstrate high ASR when ASR is increased by CRF, this is only the case if one assumes that the CRF hypore/activity present in the paraventricular nucleus of the hypothalamus is characteristic of all CRF systems in the LEW brain. It is possible that the LEW/F344 difference in CRF activity is restricted to the LHPA axis and, furthermore, that this well-described difference is associated with LEW CRF hyperactivity in other brain regions, such as those mediating behavioural expression of fear. Such a dual role for CRF could contribute to the similar CORT responses and very dissimilar behavioural responses observed in LEW and F344 males in the ASR paradigm. Of course it also needs to be recognised that CRF is not the only neurotransmitter that might account for the high ASR in LEW males [21], and that LEW/F344 differences have been described for several neurotransmitter systems, including a lower basal release of dopamine, serotonin, and glutamate in the LEW strain, in other contexts [4,28,39,47]. That the LEW hyper-ASR was restricted to males of the strain is also an important factor of relevance to elucidating underlying neural circuitry.

In the elevated plus maze, both strains spent a small proportion of the 5-min test time on the open arms compared to that generally reported for outbred strains (e.g. Ref. [11]). This would suggest that both LEW and F344 subjects were highly fearful of one or more aspects of the novel, illuminated, narrow, and elevated open arms, a finding very similar to the study of Chaouloff et al. [5], in which neither LEW nor F344 rats entered the open arms of the elevated plus maze or the white compartment in the black/white box test of anxiety. The increased levels of risk assessment behaviour demonstrated by LEW subjects in the present study are difficult to interpret against this background of low and equable time spent on the open arms. Anxiolytic compounds tend to decrease risk assessment behaviours and increase time spent on the open arms [11], so that one possible interpretation of our data would be that LEW subjects were more anxious. On the other hand, it is possible that the increased levels of LEW risk assessment reflect a transition stage from initial high anxiety to reduced anxiety and the onset of risk assessment, whereas F344 were more anxious and demonstrated such a transition to a lesser extent [37]. The evidence from manipulative studies is that fear relative to exploration is increased by CRF and decreased by CRF-blockade [16]. Further comparison of LEW, F344, and outbred strains is required at the behavioural and neuroendocrine levels if the comparative approach is to be used to contribute evidence to the role of CRF in behaviour on the elevated plus maze and anxiety.

Polydipsia is interpreted as an adjunctive behaviour induced by scheduled food delivery. That such behaviour is regulated by CORT is evidenced by adrenalectomy blocking polydipsia development and subsequent CORT substitution reinstating it [8,24,27]. Our demonstration that F344 females developed higher levels of polydipsia than F344 males and LEW females and males provides additional comparative evidence that CORT is involved in the mechanism underlying development of polydipsia, but would also suggest that the mechanism is more sensitive in females.

In the CER paradigm, CER in the form of drinking suppression was greater in F344 rats compared with LEW rats. The most direct interpretation of this strain difference is that F344 rats are more fearful of the foot shock US than are LEW rats and therefore acquire and express higher levels of tone-foot shock conditioning, measured as suppression of drinking. In addition to its CRF projections to the PnC and modulation of ASR, the central nucleus of the amygdala also sends CRF projections to brainstem regions containing noradrenergic cell bodies, primarily the locus coeruleus and nucleus tractus solitarius, and these projections contain CRF receptors. This circuitry is associated intimately with the expression of fear and anxiety: CRF administration increases CER [9] and injection of CRF receptor antagonist into the locus coeruleus attenuates novelty-induced suppression of exploratory behaviour and fear-induced behavioural freezing [3,49]. Therefore, whilst we might conclude that, in addition to reduced hypothalamic CRF activity, LEW rats exhibit reduced amygdaloid CRF activity, we proposed exactly the opposite as a putative explanation for elevated ASR in male LEW subjects (see above). In fact, there is at least one other interpretation of the reduced CER in LEW subjects and this is that their reduced CER expression is due to impaired consolidation of information acquired in a stressful situation due to attenuated CORT-receptor binding in the poststress period. There is growing evidence for an important role for poststressor combined MR + GR occupancy in the mediation of information consolidation following environmental challenge (e.g. Refs. [10,13]). In a recent LEW/F344 study we described how LEW rats demonstrated a higher CER in the form of freezing than did F344 rats during acquisition of tone-foot shock conditioning, but that expression of freezing CER to context and to CS at 24 and 48 h postconditioning, respectively, was lower in LEW than in F344 rats [34]. Whereas F344 rats expressed levels of CER at test that were very similar to those attained during conditioning, LEW rats expressed a markedly reduced freezing CER to both context and discrete CS. What we are hypothesising here, therefore, is that high levels of fear-motivated behaviour during conditioning might be mediated by high activity in one CRF system (i.e. amygdala–locus coeruleus) and that low levels of expression of conditioning might be mediated by low activity in a second CRF system (i.e. LHPA axis).

The present study provides evidence on the relative endocrine and behavioural responsiveness of LEW and F344 males and females to environmental challenge. In terms of CORT, our findings substantiate the existing data on LEW LHPA hyporesponsiveness to environmental stressors and provide a robust demonstration that this is restricted to young–mature adulthood, with old LEW females actually being CORT hyperresponsive relative to F344 females. Behaviourally, our present evidence and that from other recent studies, indicates that the LEW rat is not consistently hyporesponsive to environmental challenge of behaviour. That is, the endocrine phenotypes in the LEW/F344 model do not predict the behavioural phenotypes in a simple manner. Our findings are consistent with the hypothesis that LEW rats are not deficient in CRF excitatory neurotransmitter activity in neural circuits mediating certain behavioural responses to environmental challenge. However, they are also consistent with the hypothesis that when an organism is deficient in CRF then another factor(s) assumes the neurobehavioural function normally ascribed to CRF. This latter hypothesis was proposed recently in a study of CRFko mice lacking the CRF gene [17]. Relative to the wild-type, CRFko mice demonstrated a deficient stress-related activation of the HPA axis, whereas behavioural responses to stress-related tasks were unaffected. The LEW/F344 rat model parallels the CRFko/wild-type mouse model in terms of both endocrine and behavioural parameters. As such, this study provides further evidence that environmental reactivity in the rat reflects a number of distinct emotional states regulated by distinct neural circuits. Recognition of this situation, with multivariate analysis of traits at the neuroanatomical, neurochemical, physiological, and behavioural levels, is required for a full utilisation of the comparative approach to the study of environmental reactivity, both normal and dysfunctional.

Acknowledgments

This research was supported by a grant from the Swiss Federal Institute of Technology Zürich. The authors thank Doris Schulte Wermeling and Pascal Zenklusen for technical assistance, Animal Services for husbandry, and Bonnie Strehler for assistance with manuscript preparation.

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