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Stress hormones and emotion-regulation in two genetic animal models of depression

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Summary

Children of depressed parents often exhibit emotion-regulation deficits, characterized by either excessive withdrawal or approach strategies toward the mother. The current study examined behavioral and physiological emotion-regulation in preweanling pups (postnatal day 17–19) belonging to two different genetic animal models of depression, Wistar-Kyoto (WKY) and Flinders Sensitive-Line (FSL) rats. The study also examined the effects of stress on the two animal models, hypothesizing an interactive effect of hereditary vulnerability and exposure to stress. Chronic-stress was simulated by providing limited bedding to the dam and litter for a week, in the early postnatal period. Acute-stress was generated by exposure to an adult male rat, an ethologically valid stressor. Emotion-regulation of the pups was examined using a Y-maze preference test and radioimmunoassay of Hypothalamic-Pituitary-Adrenal (HPA) axis hormones (corticosterone & adrenocorticotropic/ACTH). WKY and FSL pups exhibited reduced approach-behavior toward the dam, an emotion-regulation profile reminiscent of avoidant attachment evident in many children of depressed parents. In contrast, the two animal models did not show similar HPA axis activity. FSL pups exhibited markedly lower ACTH levels compared to controls, while WKY pups did not differ from controls. With regard to the stress manipulations, the limited-bedding condition had no effect, while the acute-stressor induced overall effects on all groups, with more pronounced
1. Introduction

Parental depression has been shown to adversely affect the development of the offspring’s emotion-regulation, along with effects on their social and cognitive development (Downey and Coyne, 1990; Gelfand and Teti, 1990; Field, 1992; Goodman and Gotlib, 1999). Infants of depressed mothers, typically described as withdrawn, and exhibiting “depressed-like” behavior, are at increased risk for future psychopathology (Field, 1992). The current study attempts for the first time to investigate emotion-regulation in preweanling pups belonging to two genetic animal models of depression, Flinder’s Sensitive Line (FSL) and Wistar-Kyoto (WKY) rats (Paré, 1989; Overstreet, 1993; Paré and Redei, 1993; Paré, 2000; Overstreet et al., 2005). Genetic animal models are a relatively new type of model, exhibiting hereditary vulnerability similar to that found in depressed humans (Nestler et al., 2002). FSL rats were found to exhibit abnormalities in central neurochemical systems connected to depression (e.g., dopaminergic, seratoninergic & HPA axis) and behavioral deficits resembling those of depressed humans (Yadid et al., 2000; Overstreet et al., 2005). Similarly, the WKY strain exhibits characteristic depressive-like behavior (Paré, 1989; Lahmame et al., 1997; Lopez-Rubalcava and Lucki, 2000), while showing neurochemical and hormonal abnormalities (e.g., dopaminergic, noradrenergic and HPA axis) (Solberg et al., 2001; Jiao et al., 2003; Malkesman et al., 2006). Overall, the two animal models were found to meet all three types of validity criteria for animal models of psychiatric disorders: face, construct and predictive validity (Willner, 1986).

The first phase of the current study aimed to investigate the similarities between emotion-regulation of pups from the WKY and FSL strains and that of children of depressed parents. Children of depressed parents often exhibit emotion-regulation disturbances, manifested by either excessive withdrawal or approach strategies towards the mother (Gianino and Tronick, 1988; Field, 1994, 1995). In line with these findings, we hypothesized that WKY and FSL pups will show abnormal emotion-regulation patterns, evident in either an increased or decreased tendency to approach the dam. The pups’ behavioral emotion-regulation was explored using a Y-maze preference test, creating a mildly stressing situation and investigating the pups’ preference for the anesthetized dam versus a nutritive stimulus (Kavushansky and Leshem, 2004). In the second phase of the study, we focused on the HPA axis as a physiological correlate of behavioral emotion-regulation. The HPA axis is a prominent mechanism by which the brain reacts to acute and chronic-stress and was implicated in mood-disorders (Owens and Nemeroff, 1991; Brown et al., 1999; McEwen, 2000; Muller et al., 2004). Furthermore, factors associated with parental depression, such as dysfunctional maternal care and exposure to stress, were shown to affect this axis (Meane et al., 1989; Francis and Meaney, 1999). Previous studies in our laboratory showed that on postnatal day (PND) 35 (a pre-pubertal age) FSL rats demonstrated significantly lower levels of CORT and ACTH (compared to controls), while WKY rats demonstrated an opposite profile (Malkesman et al., 2006). The current study extends these initial findings, focusing on a younger pre-weaning age (PND 16–18), while investigating links between prominent behavioral and physiological emotion-regulation components. We hypothesized that pups from the “depressive-like” strains will exhibit disturbances in HPA hormones similar to findings in depressed human children (Nestler et al., 2002; Nemeroff and Vale, 2005).

An additional aim of the study was to explore the interactive effects of stress and hereditary vulnerability on the pups’ emotion-regulation. Diathesis-stress theories postulate that stress interacts with hereditary vulnerability in the establishment of mental disorders, a postulation also supported by animal research (Monroe and Simons, 1991; Nestler et al., 2002; Newport et al., 2002). In accordance, early-life stress constitutes a major risk-factor for the development and persistence of affective disorders and establishment of insecure attachment (Goldberg, 2000; Heim and Nemeroff, 2001; Nemeroff and Vale, 2005). In the current study, the effect of chronic-stress on emotion-regulation was investigated by testing the subjects either in standard rearing conditions or after exposure to a “mild chronic-stress” (Gilles et al., 1996) paradigm: limiting the amount of available bedding...
material in the cage, a persistent stressor for the dam and the pups (Avishai-Eliner et al., 2001). This paradigm (limited bedding) is unique in that it constitutes a handling manipulation with long-term effects that does not involve separation of dam and offspring. In order to collect comprehensive data on the pups’ emotion-regulation under varying stress conditions, we also included an acute-stress condition, exposure to an adult male rat. Adult male rats may harm unrelated pups and rat pups respond to adult male cues with physiological and behavioral indicators of stress (e.g., CORT elevations, c-fos expression in relevant brain areas, immobility and reduced vocalization) (Mennella and Moltz, 1988; Takahashi, 1994; Tanapat et al., 1998; Wiedenmayer and Barr, 2001). We hypothesized that the behavior and HPA activity of the 'depressed-like' strains will show an interaction between hereditary and rearing conditions, with the 'depressed' strains exhibiting more significant deficits in the chronic-stress condition (compared to the control growing condition). In contrast, the control strains were hypothesized to show only a limited difference between the two rearing conditions. Finally, the 'depressed-like' strains were expected to exhibit disturbances in reactivity to the acute stressor, compared to controls.

2. Materials and methods

2.1. Subjects

WKY, Wistar, FSL and SD rat pups (200 litters; \( N = 13–14/7–12 \) per strain and growing-condition in the Y-maze test and HPA analyses, respectively) were reared in the colony of the Developmental Psychobiological Laboratory in Bar-Ilan University. Cages of pregnant dams were checked daily before noon and parturition day was noted as PND 0. Dam and pups were maintained in individual polycarbonate cages (26 \( \times \) 42 \( \times \) 18 cm; width \( \times \) length \( \times \) height), with ambient temperature of 22 \( \pm \) 2 \( ^{\circ} \)C and a 14:10 light–dark cycle (lights on between 0500 h and 1900 h). Food and water were available ad lib. On PND 2/3 the litter size was culled to 6, with equal sex distribution when possible, since litter size and the sex of the pups can alter the pattern of maternal behavior (Kumaresan et al., 1967; Grota and Ader, 1969; Moore, 1985, 1992). Additionally, the litters were divided randomly to two groups (see the procedure section). Two pups from each litter participated in the experiments in order to avoid the interfering effect of inter-litter variability (Abbey and Howard, 1973; Zorrilla, 1997). The study was approved by the Institutional Animal Care and Use Committee and adhered to the guidelines of the National Institute of Health (NIH) for the Care and Use of Laboratory Animals.

2.2. Materials

Drugs: Anesthetic solution was prepared at the day of the experiment with a 2.5/1 Ketamine to Xylazine ratio (Sigma-Aldrich; St. Louis, MO), for administration at a dose of 30 mg/kg body weight.

**Y-maze preference test:** The maze was constructed from interconnected sub-units: start box (11 \( \times \) 8 \( \times \) 4 cm), maze arm (4.5 \( \times \) 24 \( \times \) 4 cm) and two goal boxes (each; 10 \( \times \) 23 \( \times \) 4 cm). The walls and floor of the maze were built from white Plexiglas and the ceiling from transparent Plexiglas (Kavushansky and Leshem, 2004). The apparatus allows free exploration of all maze compartments (start box, arms and goal boxes).

**Data analysis system:** Videotape recordings of the Y-maze preference test were analyzed using the **Observer 5.0** data analysis system (Noldus Information Technology b.v., Wageningen, The Netherlands).

2.3. Procedure

On PND 2/3 the litters from each strain were divided to two groups (Gilles et al., 1996): (1) **Limited-bedding** group (simulating mild chronic-stress): litters and dams were transferred to standard cages equipped with a steel grid (2.5 cm above the cages floor); the grid served as the cage floor while allowing the passage of excrements. The only bedding material available were paper towels (total: 112.5 \( \times \) 22.5 cm) that the dam shreds and uses to make a nest (Gilles et al., 1996). The paper towels were replaced at PND 4/5. (2) **Control group:** litters and dams were transferred to standard growing cages (bedding from wood shavings). Bedding was replaced on PND 4/5. On PND 8/9 all litters and dams (limited-bedding and control) were returned to standard growing conditions.

2.3.1. Acute stressor and radioimmunoassay (RIA) for HPA axis hormones

On PND 16–18 (at 9:00–11:00 am) four pups were removed from the home cage and divided into experimental groups in accordance to Wiedenmayer and Barr (2001): (1) **Exposure to a stressor, adult male rat:** two pups were transferred to a new cage (26 \( \times \) 42 \( \times \) 18 cm; width \( \times \) length \( \times \) height) divided by a metal grid to two equal sections. The compartment in which the pups were put had bedding from the home cage (to simulate the nest
area). The pups were given 15 min to adjust and afterwards an unfamiliar adult male rat was put into the empty compartment for five minutes. (2) Control condition, without exposure to a stressor: two pups underwent an identical procedure, without exposure to an adult male.

Five minutes after removal of the adult male rat the two sibling pups were removed and trunk blood was collected by decapitation. Special care was taken in order to avoid pre-decapitation stress—while decapitation took place the other animal was left outside the room and after decapitation gloves were changed and all equipment cleaned. Trunk blood was collected into chilled tubes containing EDTA solution and the tubes were gently shaken. Samples were centrifuged for 10 min at 4 °C at 2000 g rpm and plasma was stored at −80 °C until determination of CORT & ACTH basal levels. On the day of assay, frozen plasma samples were thawed and plasma CORT & ACTH levels were measured using commercial RIA kits (rat CORT RIA kit; Diagnostic Plasma CORT & ACTH levels were measured using assay, frozen plasma samples were thawed and nation of CORT & ACTH basal levels. On the day of assay, frozen plasma samples were thawed and plasma CORT & ACTH levels were measured using commercial RIA kits (rat CORT RIA kit; Diagnostic Products Corporation, USA—sensitivity of 5.7 ng/ml; ACTH RIA kit with sensitivity of 5.7 pg/ml—Immuno Biological Laboratories, Hamburg, Germany). These kits are valid for measuring these hormones in rats, according to the manufacturer’s information and previous studies from our laboratories.

2.3.2. Y-maze preference test
Preference tests took place between 10:00 and 12:30 (based on, Stollof and Supinski, 1985; Rakover-Atar, 2000). The apparatus was cleaned using a 70% ethanol solution. Next, the dam was anesthetized using a 1 ml/kg body weight intraperitoneal (i.p.) injection of the anesthetic solution (see materials section) and placed in a goal box, lying on her side. A plate (5 cm diameter) containing milk (10% fat content warmed to 33 °C) was placed in the other goal box (position of dam/milk in the goal boxes was randomized). Next, the pups were taken out of the home cage, weighed, and divided into two groups (acute-stress/control condition; identical procedure to that described earlier). Based on Kavushansky and Leshem (2004) each pup underwent a Y-maze preference test: the pup was placed in the start box and allowed to explore the maze freely for four minutes. Next, the pup was transferred to a cage for 1 min and the maze was cleaned using a dry paper-towel (spreading scents evenly in the maze). During the 1 min interval the location of the dam and the milk-plate in the maze were switched between the goal boxes and the pup underwent a second preference test. At the end, the maze was cleaned using a 70% ethanol solution and the second pup went through a similar procedure.

Video-tapes of the Y-maze test were later used to analyze the pups’ behavior using the Observer 5.0 data analysis system. Two clusters of behaviors were analyzed: (1) position of the pup in the maze (location): during the time period that the pup freely explored the maze, its position/location was determined (whether in the start box, maze’s arms or one of the two goal boxes). (2) behaviors of the pup with the dam: The behaviors of the pups were divided to three behavioral clusters (see also; Kavushansky and Leshem, 2004); (a) Passive behavior—contact with the dam, while lying or dozing. Passive behavior was scored only if the contact was with the pup’s trunk and not just a single organ, such as the snout, tail, etc. (b) Active behavior—active touching/pushing of the dam with the pup’s snout or forelimbs, sniffing or nibbling the dam, climbing on her body, etc. (c) Nursing—nipple attachment lasting more than 3 s.

2.4. Data analysis
ACTH and CORT RIA: ACTH and CORT results were analyzed by two-way multivariate analysis of variance (MANOVA), with strain (WKY compared to Wistar/FSL compared SD) and growing-condition (limited-bedding compared to standard growing conditions) as between-subjects measures. Since variability between siblings is much lower than variability between non-related pups, acute-stress (acutely-stressed pup compared to the control sibling pup) was used as a within-subjects measure (i.e., within-litter measure; Abbey and Howard, 1973; Zorrilla, 1997). Significant interactions with the acute-stress measure were followed-up by paired-samples t-tests.

Y-maze preference test: Position of the pups was analyzed using MANOVA, with strain and growing-condition as between-subject measures, and with acute-stress and location as within-subject (within-litter—for the acute stress variable) measures. Behaviors of the pups with the dam were analyzed using a similar MANOVA. Significant interactions with the acute-stress measure were followed up by post-hoc paired-samples t-tests.

3. Results
3.1. WKY compared to wistar control pups
3.1.1. HPA axis hormones
The MANOVA for ACTH (see Fig. 1) revealed no strain, growing-condition or strain × growing-condition
effects \[F(1, 27) = 2.653, \text{n.s.}; F(1, 27) = 0.193, \text{n.s.}; F(1, 27) = 0.001, \text{n.s.};\] respectively. In contrast, the acutely-stressed pups had higher ACTH levels than the control sibling pups \[F(1, 27) = 22.763, p < 0.001\]; in the within-litter comparison. As shown in Fig. 1, there was also a significant acute-stress × strain interaction \[F(1, 27) = 4.593, p < 0.05\]; only Wistar control pups exhibited significantly higher ACTH levels in the acutely-stressed pup compared to its not-stressed sibling \[t(17) = 5.681, p < 0.001\], while WKY pups showed no significant ACTH response \[t(12) = 1.720, \text{n.s.}\]. There were no additional significant acute-stress interactions (not presented).

The MANOVA for CORT (see Fig. 2) revealed no strain, growing-condition or strain × growing-condition effects \[F(1, 30) = 0.989, \text{n.s.}; F(1, 30) = 2.436, \text{n.s.}; F(1, 30) = 1.728, \text{n.s.};\] respectively. There was a non-significant trend for an acute-stress effect, with acutely-stressed pups tending to have higher CORT than controls \(p < 0.067\). However, there was no differential acute-stress effect for the ‘depressed’ and control strains (no significant acute-stress interactions; see Fig. 2).

### 3.1.2. Y-maze preference test

**Pups’ preference for dam vs. nutritive-stimulus:** The MANOVA revealed a significant strain main-effect \[F(2, 37) = 6.182, p < 0.01\] (see Fig. 3); post-hoc one-way ANOVA’s indicated that WKY pups spent significantly less time than controls in the dam goal box \[F(1, 38) = 8.509, p < 0.01\], while showing comparable preference to the milk goal box (not presented). There were no significant growing-condition or strain × growing-condition effects \[F(2, 37) = 0.447, \text{n.s.}; F(2, 37) = 0.168, \text{n.s.};\] respectively. In addition, a significant acute-stress effect was found \[F(2, 37) = 4.783, p < 0.05\]; The acutely stressed pup spent significantly less time in the dam goal box, compared to the control pup \[F(1, 38) = 7.451, p < 0.01\], while no significant difference was found in time spent in the milk goal box (see Table 1). There were
no significant acute-stress interactions (not presented).

Pups’ behavior with the dam: The MANOVA revealed a significant strain main-effect \( [F(3, 36) = 5.202, p < 0.01] \); WKY pups spent more time with the dam in passive behaviors, less time nursing \( [F(1, 38) = 8.087, p < 0.01; F(1, 38) = 7.721, p < 0.01] \) and tended to spend less time in active behaviors (non-significant trend, \( p < 0.056 \)) compared to control pups (see Fig. 4). There were no significant growing-condition or strain\( \times \)growing-condition effects \( [F(3, 36) = 0.453, n.s.; F(3, 36) = 0.001; \) respectively]. The MANOVA also showed an acute-stress effect \( [F(3, 36) = 37.177, p < 0.001] \); The acutely stressed pups exhibited less passive behaviors, more active behaviors and less nursing with the dam, compared to the control pups \( [F(1, 38) = 31.949, p < 0.001; F(1, 38) = 91.465, p < 0.001; F(1, 38) = 29.507, p < 0.001;\) respectively] (see Table 1). There were no significant acute-stress interactions (not presented).

In summary, WKY pups’ behavior in the Y-maze preference test was significantly different than that of the control strain. WKY pups spent less time in the dam’s goal box, while spending similar time in the milk goal box (compared to the control pups). When interacting with the dam, WKY pups spent more time in passive behaviors, and less time nursing. HPA axis measurements revealed no significant differences in WKY pups’ ACTH and CORT levels, while suggesting a more moderate ACTH response to the acute-stress in the WKY strain (compared to controls).

While the limited-bedding paradigm (simulating early-life chronic-stress) showed no effects on the pups, the acute-stressor had several clear effects: (1) Behavioral measures: The acutely stressed pups spent less time in the dam goal box and while interacting with the dam exhibited more active behaviors, less passive behaviors and less nursing, compared to control pups. (2) Physiological measures: the acutely stressed pups had higher ACTH levels and tended to have higher CORT (non-significant trend), compared to their non-stressed siblings. WKY pups exhibited a more moderate ACTH response to the acute-stress than the control Wistar pups.

### 3.2. FSL compared to SD control pups

#### 3.2.1. HPA axis hormones

The MANOVA for ACTH (see Fig. 5) revealed a significant strain main-effect \( [F(1, 29) = 22.612, p < 0.001] \); FSL pups had lower levels of ACTH compared to SD control pups. There were no

### Table 1 WKY and FSL preweanling pups’ behaviors with the dam in the Y-maze preference test (compared to Wistar and SD control strains, respectively): Mean±SEM of acutely-stressed pup and control pup

<table>
<thead>
<tr>
<th>Measure/strain</th>
<th>% time in passive behaviors with dam (mean±SEM)</th>
<th>% time in active behaviors with dam (mean±SEM)</th>
<th>% time nursing (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control pup</td>
<td>Acute-stress pup</td>
<td>Control pup</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control pup</td>
<td>69.99 (±4.25)</td>
<td>29.13 (±3.73)</td>
<td>0.86 (±2.74)</td>
</tr>
<tr>
<td>N = 22</td>
<td>N = 20</td>
<td>N = 20</td>
<td>N = 22</td>
</tr>
<tr>
<td>Wistar</td>
<td>53.11 (±4.05)</td>
<td>35.22 (±3.55)</td>
<td>11.66 (±2.61)</td>
</tr>
<tr>
<td>N = 22</td>
<td>N = 20</td>
<td>N = 20</td>
<td>N = 22</td>
</tr>
<tr>
<td>Total</td>
<td>61.55 (±4.15)**</td>
<td>32.18 (±3.65)**</td>
<td>6.26 (±2.68)</td>
</tr>
<tr>
<td>FSL</td>
<td>52.05 (±4.33)</td>
<td>40.32 (±3.84)</td>
<td>7.621 (±2.29)</td>
</tr>
<tr>
<td>N = 23</td>
<td>N = 23</td>
<td>N = 23</td>
<td>N = 23</td>
</tr>
<tr>
<td>SD</td>
<td>43.85 (±4.78)</td>
<td>35.13 (±3.99)</td>
<td>21.01 (±3.00)</td>
</tr>
<tr>
<td>N = 23</td>
<td>N = 23</td>
<td>N = 23</td>
<td>N = 23</td>
</tr>
<tr>
<td>Total</td>
<td>47.95 (±4.56)</td>
<td>37.72 (±3.92)*</td>
<td>14.31 (±2.65)</td>
</tr>
</tbody>
</table>

Notes: *\( p < 0.05 \); **\( p < 0.001 \).

Strain comparisons (WKY/Wistar & FSL/SD) for pup behaviors with the dam, averaging over stress conditions, are presented in Figs. 4 and 8, respectively.
significant growing-condition or strain × growing-condition effects \[F(1, 29) = 0.806, \text{n.s.}; \]
\[F(1, 29) = 1.344, \text{n.s.} \] respectively. There was, however, a significant acute-stress effect, with pups exposed to acute stress showing higher ACTH levels than control pups \[F(1, 29) = 66.717, p < 0.001\]. In addition, we found a significant acute-stress × strain interaction \[F(1, 29) = 15.508, p < 0.001\]; FSL pups exhibited a more moderate response to the acute-stress \[t(15) = 2.982, p < 0.01\] (comparing acutely-stressed pup to the control pup), compared to SD control pups \[t(16) = 9.041, p < 0.001\] (see Fig. 5). There were no additional significant acute-stress interactions (not presented).

The MANOVA for CORT (see Fig. 6) revealed no significant strain, growing-condition or strain × growing-condition effects \[F(1, 27) = 1.109, \text{n.s.}; \]
\[F(1, 27) = 0.540, \text{n.s.}; F(1, 27) = 0.698, \text{n.s.} \] respectively) (see Fig. 6). There were no significant acute-stress main effect or interactions (not presented).

3.2.2. Y-maze preference test
Pups’ preference for the dam vs. nutritive-stimulus: The MANOVA revealed a strain main-effect \[F(2, 40) = 3.653, p < 0.05\]. As shown in Fig. 7, FSL pups spent less time in the dam goal box \[F(1, 41) = 7.412, p < 0.01\], while no significant differences were found in time spent in the milk goal box, compared to SD control pups. There were no significant growing-condition or strain × growing-condition effects \[F(2, 40) = 0.031, \text{n.s.}; \]
\[F(2, 40) = 1.079, \text{n.s.} \] respectively. There were no significant acute-stress main effect or interactions (see Table 1).

Pups’ behavior with dam: The MANOVA revealed a significant strain effect \[F(3, 40) = 6.576, p < 0.01\]. As shown in Fig. 8, FSL pups spent more time with the dam in passive behaviors and less time nursing than SD controls \[F(1, 42) = 5.880, p < 0.05; F(1, 42) = 13.146, p < 0.001\]. There were no significant growing-condition or strain × growing-condition effects \[F(3, 40) = 0.229, \text{n.s.}; \]
There was a significant acute-stress effect \( F(3; 40) = 26.699, p < 0.001 \); The acutely stressed pups exhibited less passive behaviors, more active behaviors and less nursing with the dam, compared to control pups \( F(1; 42) = 5.486, p < 0.05; \quad F(1; 42) = 64.554, p < 0.001; \quad F(1; 42) = 21.920, p < 0.001 \); respectively. The MANOVA also showed a significant acute-stress x strain interaction \( F(3; 40) = 7.943, p < 0.001 \), related to the active behavior component of the behavioral analysis \( F(1; 42) = 19.340, p < 0.001 \). However, follow-up paired t-tests revealed no significant differences for the FSL and SD control strains (for each strain comparing acutely-stressed pup to control pup \( t(22) = 1.304, n.s.; \quad t(22) = 1.061, n.s. \); respectively, see Table 1).

In summary, FSL pups exhibited a similar behavioral pattern to that of WKY pups in the Y-maze preference test; FSL pups spent less time in the dam’s goal box (with no significant differences in time spent in milk goal box), while spending more time in passive behaviors and less time nursing, compared to controls. In addition, FSL pups exhibited lower ACTH levels than SD controls.

The limited-bedding paradigm had had no significant effects on the pups, similar to findings in the WKY/Wistar strains. In contrast, the acute-stress affected both behavioral and physiological measures: (1) Behavioral measures: The acutely stressed pups exhibited less passive behaviors, more active behaviors and less nursing with the dam, compared to control pups. (2) Physiological measures: The acutely stressed pups exhibited higher ACTH levels than the control pups. In addition, FSL pups exhibited a more moderate ACTH response to the acute-stress than pups from the control strain (a similar pattern to that seen in the WKY strain).

4. Discussion

The current study investigated for the first time the emotion-regulation capacities of pups belonging to the WKY and FSL genetic animal models of depression. The findings indicate that preweanling pups from these strains exhibit a reduced tendency to approach the dam; WKY and FSL pups spent significantly less time with the anesthetized dam compared to controls, while spending a similar amount of time in the milk goal box. Furthermore, pups from both strains differed from controls in their behavioral profile, while interacting with the dam; WKY and FSL pups spent more time in passive behaviors and less time nursing compared to control pups. These behavioral profiles of emotion-regulation can be interpreted from the perspective of attachment theory. According to attachment theory, threatening conditions evoke infant behaviors aimed at maintaining and enhancing proximity to caregivers and eliciting their care.
(Bowlby, 1969, 1973, 1980; Cassidy and Shaver, 1999). From this viewpoint, WKY and FSL pups show behavioral patterns similar to avoidant attachment, characterized by deactivating/restricting the expression of attachment behavior. These findings fit those reported in children of depressed parents (Gelfand and Teti, 1990; Radka-Yarrow, 1991; Murray, 1992; Cicchetti et al., 1998), and a meta-analysis pointing toward an increased risk of avoidant and disorganized attachment in children to depressed parents (Martins and Gaffan, 2000). The findings are also in agreement with other results from our lab indicating reduced approach-behavior toward the dam of WKY and FSL pups as early as PND 10–11 (Braw et al., unpublished data).

The current study additionally investigated for the first time elements of the HPA axis in WKY and FSL preweanling pups (PND 16–18). The results indicate that weanling WKY pups do not differ in ACTH or CORT secretion from their control strains. The lack of differences at the preweanling age (PND 17) can be contrasted with findings at older ages; pre-pubertal (PND 35) and adult WKY rats show higher plasma levels of CORT and ACTH compared to controls (Solberg et al., 2001; Malkesman et al., 2006). Adult WKY rats also show increased plasma ACTH levels after both chronic and acute stress relative to several other rat strains (Paré and Redei, 1993; Redei et al., 1994). The lack of differences between WKY pups and controls at the preweanling period, along-side clear differences at older ages, suggests the possibility of a prolonging of the stress hypo-responsive period (SHRP). During PND 1–14 the HPA axis is characterized by a 'silent period' in which the animal is hypo-responsive to stress (Vazquez, 1998). The putative prolonging of the SHRP in WKY rats may point toward a general deficit in HPA axis function, leading at older ages to an impaired efficacy of the glucocorticoid negative feedback and heightened levels of HPA activity (Redei et al., 1994).

FSL pups, in contrast, exhibited hypo-secretion of ACTH accompanied by normal levels of CORT secretion, compared to controls. Such an HPA pattern fits, in general, the findings in older FSL rats; pre-pubertal FSL pups (PND 35) hypo-secreted both ACTH and CORT (Malkesman et al., 2006) and adult FSL rats hypo-secreted ACTH (but not CORT) (Owens et al., 1991). We have recently suggested that this low HPA activity may be associated with the phenomenon of hypocortisolism in human children (Malkesman et al., 2006). Hypocortisolism is a novel and paradoxical phenomenon that has emerged in recent years from neurobiological studies on the effects of stress (Heim et al., 2000). There are increasing indications for a relatively low cortisol secretion in individuals who were exposed to severe stress or are suffering from stress-related disorders. Hypocortisolism can be found among children growing up under less than optimal conditions of care and among maltreated, depressed children (Kaufman, 1991; Hard et al., 1996; Gunnar and Vazquez, 2001). Low cortisol and increased reactivity of the pituitary were additionally found in individuals with Post Traumatic Stress Disorder (PTSD) (Yehuda, 2005). The current study indicates that as early as PND 17, the FSL strain shows evidence of hyposecretion of HPA hormones, as previously reported in older FSL rats. Such findings putatively suggest alterations in the HPA axis at an early age, which may enhance these pups’ susceptibility to the effects of stress. These results also suggest the use of the FSL strain to model disorders accompanied by hypocortisolism.
In general, it appears that the values of corticosterone found in this study are relatively high, while ACTH values are relatively low. If the rats were in stress we would expect high levels of both CORT and ACTH. High CORT followed by low ACTH may sometimes be due to hypercorticosteronemia (hyperactivity of the adrenal) followed by negative feedback on the hypophisis. Various factors could account for this, e.g., strain, condition, etc.

An advantage of animal research lays in the possibility of manipulating factors associated with the establishment of emotion-regulation patterns (Willner, 1984, 1986, 1991). The current study exemplifies such possibilities by investigating the effects of chronic-stress on the pups’ emotion-regulation. However, our results do not show significant effects of the limited-bedding manipulation on the behavior of the WKY and FSL pups. This lack of effect, along with the fact that under standard laboratory rearing conditions the pups’ emotion-regulation was already altered at an early age, suggest a strong hereditary influence on the strains’ behavioral phenotype. Note, however, that the chronic-stress paradigm used (limited bedding) has not previously been shown to have behavioral, but only physiological effects (Gilles et al., 1996; Avishai-Eliner et al., 2001). Thus, it is possible that this particular manipulation is too mild to have a clear behavioral impact. The current findings, therefore, can not provide strong evidence for hereditary transmission pathways of attachment disturbances in the two animal models. Overall, it seems that the chronic-stress used in this study requires more research in order to assess, and increase its effectiveness. In contrast, the acute stressor (adult male) had clear behavioral and physiological effects. While time in the goal boxes was not affected by the acute-stressor, the acutely stressed pup exhibited more active behaviors and less passive and nursing behaviors with the dam, compared to its unstressed sibling. These changes can be seen as reflecting greater agitation by the stressed pups, showing difficulties in calming (i.e., in regulating emotion). Moreover, the acute-stress had strain-specific effects on the ACTH levels of the pups; pups from the depressed strains showed a more moderate ACTH response than control strains, indicating both differential behavioral and physiological stress reactivity.

Before closing, a theoretical issue in interpreting our data should be addressed. We discussed the behavior of the pups in terms of emotion-regulation. However, the increased time in passive behaviors with the dam and reduced time in the dam goal box could alternatively be viewed as increased fear. Emotion-regulation is closely related to the concept of stress, since emotion-regulation is exactly these “extrinsic and intrinsic processes involved in monitoring, facilitating and inhibiting heightened levels of positive and negative affect” (Thompson, 1994). Nevertheless, our findings suggest that pup behavior may be more related to emotion-regulation (a specific effect) than general negative affect (e.g., fear and anxiety). Briefly, generally accepted measures of anxiety, such as freezing/immobility (“time to leave start box”), defecation, and general activity level (“no. of line crossings”) did not differ between strains in the current study. While not eliminating the possibility that our findings are related to anxiety/fear, this behavioral profile suggests that behaviors are more related to the construct of emotion-regulation. The differential effects of acute stress (leading, for example, to changes in time spent with the dam, but not affecting time in milk goal box) further support this conclusion.

In summary, the two animal models show similar patterns of behavioral emotion-regulation, characterized by a reduced tendency to approach the dam and altered dam-pup interactions. The similarities to avoidant attachment suggest the use of these strains to study factors affecting the establishment of this disturbed attachment pattern in children of depressed parents. The current study additionally indicates that at PND 17 FSL pups hypo-secret ACTH, while WKY pups show normal activity of the HPA axis. Such findings add to previous studies conducted on older WKY and FSL rats, helping to clarify the development of the HPA axis in the two animal models (e.g., Owens et al., 1991; Solberg et al., 2001; Malkesman et al., 2006). The fact that in the preweaning period both animal models show normal secretion of CORT, the end-point of the HPA axis, may be connected to the similar behavioral phenomenology in the two models. The changes in the HPA activity of the two animal models may be related to the divergence in the behavioral profiles of the two strains at older ages (Malkesman et al., 2005; Braw et al., 2006; Malkesman et al., 2006). Overall, the current study indicates that the strains may be used to model certain pediatric conditions—the two strains may be used to model avoidant attachment and the FSL strain may be especially suitable to model pediatric depression accompanied with hypocorticolism.

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