Anxiety-like behaviors in pre-pubertal rats of the Flinders Sensitive Line (FSL) and Wistar-Kyoto (WKY) animal models of depression

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Abstract

Animal models have been used in understanding the neuro-biological basis of depression and predicting successful treatment strategies. The current study focused on two genetic models of depression, the Flinder’s Sensitive Line (FSL) and Wister-Kyoto (WKY). Our laboratory showed depressive symptomatology in pre-pubertal WKY and FSL rats, and the current study focused on the strains’ anxiety-like traits. Since human depression–anxiety comorbidity is very common at young ages, it is essential to establish whether FSL and WKY pre-pubertal rats also exhibit such comorbidity. In addition, the effect of different rearing environments was studied using a mild chronic-stress condition (limiting available bedding between post-natal days 2–9). Two well-validated tests of anxiety, the open-field and elevated plus-maze, were used on 40-day-old pups. FSL pups exhibited lower anxiety-like behavior when compared to controls, in traditional open-field and plus-maze measures. A different pattern was observed in the WKY strain, which exhibited heightened anxiety-like behaviors in the FSL strain and affecting WKY’s body-weight. Overall, the findings indicate differential expression of anxiety in pre-pubertal rats belonging to the ‘depressed’ strains, suggesting that these strains may be suitable for modelling different sub-groups of depression at young ages.

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Consistent associations have been found between parental depression and adverse child outcomes, spanning a wide age range [16,18]. Children of depressed parents exhibit difficulties in regulating emotional expression, controlling aggressive impulses and cooperating with others [13,16,57]. Physiological regulation is disturbed, as expressed in measurements of different neuro-biological systems; HPA axis activity, vagal tone measurements of the parasympathetic system and functioning of the frontal lobe [14,15,19]. In addition, these children commonly suffer from psychiatric disorders, such as anxiety and depression [see reviews, 11,16,18]. Various models have been proposed to account for the transmission of disturbances between generations, with time moving from primarily genetically based models to ‘multifactorial’ approaches [11,18]. These newer approaches imply an interaction of heredity and environment. However, despite this shift in focus we do not have an adequate understanding of the mechanisms that underlie the risk for these adverse outcomes [18].

Animal models, used in past decades in understanding the neuro-biological basis of depression, offer a way to increase knowledge on the mechanisms of inter-generational transmission of deficits [26,58]. In the current study, we investigated two genetic animal models of depression, Flinder’s Sensitive Line (FSL) and Wistar-Kyoto (WKY) rats. The FSL model of depression was created by selectively breeding Sprague–Dawley (SD) rats for hyper-sensitivity to cholinergic agonists, sensitivity found in depressed humans [10,24,36]. The second model, the WKY, was inbred from the Wistar strain [42]. Recently, the WKY strain has been proposed to be a genetic animal model of depression, exhibiting sensitivity to stress alongside characteristic depressive-like behaviors which do not originate in stress [28,30,42]. WKY
Previous research in our laboratory showed that pre-pubertal WKY and FSL rats exhibited heightened depressive-like behaviors, such as greater immobility in the swim-test andanhedonia in a saccharin preference test (in WKY rats) compared to controls [32,33]. At the same time, these models showed distinct behavioral and physiological patterns, leading to a suggestion to view the two strains as modeling different sub-groups of childhood depression [32]. In the current study, we aimed to elaborate upon these previous findings, focusing on the anxiety component in these two animal models. The monitoring of anxiety is important, in light of the high rates of depression-anxiety comorbidity in human populations; In clinical samples, the rate of comorbid anxiety disorders in depressed youth can run as high as 70%, and in community samples the average is 25–50% [4,7,21]. Comorbidity is also associated with increased severity of depression [5,31,52], and may even exert a potentiating effect [4,6]. To conclude, the first aim the study was the investigation of an anxiety component in the WKY and FSL strains at pre-pubertal age. Such investigation can shed light into the possibility of using the strains to model sub-groups of pediatric depression (with differential patterns of depression-anxiety comorbidity) and increase our knowledge on the two animal models.

The second aim of the study was the investigation of the effect of stress on the establishment of anxiety-like behaviors in the two animal models. In humans, early-life stress constitutes a major risk-factor for the development and persistence of mental disorders [22]. According to diathesis-stress theories, the stress interacts with hereditary vulnerability in the establishment of these mental disorders [34]. In the current study we investigated such possible interaction on the development of anxiety-like behaviors of WKY and FSL pre-pubertal pups. In order to achieve this aim the subjects were tested either in a standard rearing condition or after exposure to mild chronic-stress [17]. The use of two different rearing conditions has the added value of better simulating lives of children to depressed parents, since their lives are associated with increased exposure to stress [18]. The chronic-stress paradigm chosen is based on the finding that limiting the amount of available bedding material in the cage constitutes a persistent stressor for dam and pups [3]. This paradigm is unique in that it constitutes a handling manipulation with long-term effects, while avoiding the use of stress-arousing manipulations alien to the world of the rat. As far as we know, the current study is the first investigation of behavioral effects of this stress paradigm.

Two well-established tests of anxiety were used in the study, analyzing ethologically valid measures of anxiety. The two anxiety tests, the open-field and the elevated plus-maze, involve a forced confrontation of the animal with a novel environment creating an approach-avoidance conflict [20,23,50]. The tests study the animal’s unconditioned or spontaneous behavior, providing high degree of ecological validity and were extensively validated [29,46,49]. In the current study the tests were used for two aims: (1) exploring for the first time WKY and FSL pre-pubertal rats’ anxiety-like behavior. Our hypothesis was that pre-pubertal rats from the two animal models will show differential, perhaps opposing, anxiety patterns when compared to their control strains (fitting earlier findings of differential behavioral and physiological profiles of the models at young age, 32). In order to achieve this aim, the ‘depressed’ strains were compared to their control strains from which they were originally bred, Wistar and SD. (2) Investigation of the effect of stress on the establishment of anxiety-like behaviors in the two animal models. For this aim, pups were raised in one of two rearing conditions (chronic-stress and control). We hypothesized that, in accordance to diathesis-stress theories, the ‘depressed’ strains’ behavior will show an interaction between hereditary and rearing condition, exhibiting heightened anxiety in the chronic-stress condition. The control strains were hypothesized to show limited difference only between the two rearing conditions.

1. Materials and methods

1.1. Animals

Wistar, WKY, SD and FSL rats (N=200) that reached the age of 40 days participated in the study. Two siblings per litter were tested in order to achieve litter representation while controlling for litter effects [1]. Littermate results were averaged and entered as single datum to the analyses (N=12–13 per strain and growing-condition). The animals were reared in the “Developmental Psychological Laboratory” in Bar-Ilan University. Cages of pregnant dams were checked daily before noon and parturation day was noted as post-natal day 0 (PND 0). Dam and pups were maintained in individual polycarbonate cages (26 cm × 42 cm × 30 cm), in our colony with temperatures of 22±2 °C and a 14 h light:10 h dark cycle (lights on between 5:00 and 19:00). The animals had food and water ad lib. On PND 2/3 litter size was culled to 6 (with equal sex distribution when possible) and the litters were divided randomly to two groups, as described in the Section 1.1 [17]. The study protocol was approved by the Institutional Animal Care and Use Committee in Bar-Ilan University and adhered to the guidelines of the Society for Neuroscience and the American Psychological Association.

1.2. Materials

The open-field consisted of an arena (62 cm × 62 cm) enclosed by walls (30 cm high). The floor was built from blue polygal (opaque polycarbonate) and the walls from green polygal (modified version of that used in 25). The plus-maze consisted of two open arms, 50 cm × 10 cm, and two enclosed arms, 50 cm × 10 cm × 40 cm with an open roof, arranged such that the two arms of each type were opposite each other [based on 47]. The maze was elevated to a height of 70 cm. The walls of the enclosed arms were made from black Plexiglas, while the floors from white Plexiglas. Videotape recordings were analyzed using the “Observer” system (Noldus Information Technology b.v., Wageningen, The Netherlands).

1.3. Procedure

Two groups (per strain) were created on PND 2/3: 1. Mild chronic-stress group. 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 cm × 22.5 cm), towels that the dam needed and not to make a nest [17]. 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 at PND 4/5.
At PND 8/9 all litters were returned to standard growing-conditions until weaning (PND 21), when two pups per litter (siblings) were transferred to a separate cage. On the day of experiment (PND 40/41) at 14:00 h, the two sibling rats were transferred to the testing room and placed separately in two cages (identical to the home cage) with a thin layer of bedding. The first subject was removed from the cage (sex randomized), weighed and placed gently in the center of the open-field area. The rat was allowed to explore the open-field freely and its behavior was videotaped for 5 min. Afterwards it was transferred to the plus-maze and placed at the center, facing a closed arm. The rat’s behavior was videotaped for 5 min, afterwards it was returned to the home cage. The apparatus was cleaned using a 70% ethanol solution and the second rat underwent an identical procedure.

1.4. Data analysis

1.4.1. Preliminary procedures

Results from the two pup siblings were averaged and entered into analysis as single data. Problems were encountered with one recording and the results from the second pup were used as representative of the two pups. Recordings were later analyzed by two observers with inter-rater reliabilities on scoring open-field behaviors ranging from $r = 0.954$ to $r = 0.993$ ($p < 0.001$), while inter-rater reliabilities on scoring plus-maze behaviors ranged from $r = 0.926$ to $r = 1$ ($p < 0.001$). Open-field and plus-maze measures and were grouped into two behavioral classes: ‘anxiety-like behaviors’ and ‘activity’ (see also Section 3 which addresses methodological issues concerning the division) and included additional latency measures that our coding system provided (Noldus, ‘Observer’).

Open-field behavioral measures included: (1) Anxiety measures: % time in center square, % number of entries to center square (from total squares entered), % time freezing, latency to first freezing (freezing was scored when the subject was in an immobile state for at least 3 s [55]) (2) Activity measures: Total squares entered, number of rears (standing on two hind legs), latency for first rearing. Plus-maze measures included traditional parameters [based on 47], as well as two ‘risk-assessment’ behaviors, protected and unprotected head-dips. These behaviors are part of an ethological behavioral repertoire aimed at assessing danger by the animal and research has indicated that the addition of these indices increases the sensitivity of the test [9,51]. Plus-maze measures included: (1) Anxiety: % time in open arms, % number of entries to open arms (from total entries), number of unprotected head dips, latency to enter open arm. (2) Activity: Total entries to all arms.

In the next step, preliminary Pearson correlations were performed, in order to avoid the inclusion of measures with a large overlap in the multivariate analysis of variance (MANCOVA); measures with $r > 0.80$ correlation were reduced to a single measure. Two measures (percent time freezing and number of freezes; $r = 0.86$) showed such overlap in the open-field. In the plus-maze the following measures showed an overlap: percent time as closed arms (overlapping with percent time in open arms, $r = -0.93$), percent number of entries to closed arms from total entries (overlapping with percent number of entries to closed arms from total entries, $r = -0.99$), latency to make unprotected head dip (overlapping with latency to enter open arm, $r = 0.83$) and percent number of entries to center from total entries (overlapping with percent time in center, $r = 0.84$). See Table 1 for full list of measures used (after discarding overlapping measures) in the open-field and plus-maze.

1.4.2. General analyses

For all analyses, strains were separated into two groups, with each ‘depressed’ strain compared to its control strain (FSL compared to SD, WKY compared to Wistar). Body-weight was analyzed using a 2 × 2 ANOVA with strain and growing-condition (normal growing and chronic-stress/limited-bedding) as between-subject variables. Since the strains differed in body-weight, all further analyses were conducted with body-weight as covariate (MANCOVA).

1.4.3. Open-field and plus-maze analyses

The behavioral measures in the open-field were divided into two behavioral classes (anxiety-like behaviors and activity); each behavioral class was analyzed in a 2 × 2 MANCOVA with strain and growing-condition as between-subject variables. The MANCOVA was followed up by one-way ANOVAs on each of the measures, for each the between-subject comparisons. Plus-maze analyses were conducted in a similar fashion. In addition, two measures of latency were analyzed (latency to leave center section in the open-field and latency to leave center in the plus-maze) as indexes of immobility/stress-induced behavioral inhibition. The measures were analyzed in a 2 × 2 MANCOVA in a similar fashion to the anxiety-like behaviors MANCOVA.

2. Results

2.1. The effect of strain and growing-condition on WKY and Wistar (controls) pre-pubertal rats

2.1.1. General results

The 2 × 2 ANOVA for body weight revealed a significant main-effect of strain with WKY pups weighing less than Wistar control pups [$F(1,45) = 143.663$, $p < 0.001$]. The strain × growing-condition interaction was also significant [$F(1,45) = 5.995$, $p < 0.05$]; While Wistar pups weighed more after exposure to chronic-stress compared to normal growing-conditions, WKY pups weighed less after exposure to chronic-stress compared to normal growing-conditions. There was no significant main effect for growing-conditions [$F(1,45) = 0.636$, n.s.] (see Table 2).

2.1.2. Activity

The 2 × 2 MANCOVA for activity cluster in the open-field revealed a significant main effect of strain [$F(1,42) = 14.055$, $p < 0.001$]; post-hoc one-way ANOVA’s revealed that WKY pups performed fewer rearings (number) and their latency for Table 1

<table>
<thead>
<tr>
<th>Measures chosen for the open-field and plus-maze analyses</th>
<th>Open-field</th>
<th>Plus-maze</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral clusters</td>
<td>Anxiety-like behaviors</td>
<td>(1) % time in center square</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) % number of entries to center square (from total squares entered)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) % time freezing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4) Latency to first freezing</td>
</tr>
<tr>
<td></td>
<td>Activity</td>
<td>(5) Latency to leave center square</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(subject placed in the square at beginning of experiment)</td>
</tr>
<tr>
<td></td>
<td>(1) Total squares entered</td>
<td>(1) Total entries to maze sections</td>
</tr>
</tbody>
</table>
Table 2
Mean (±S.E.M.) behaviors of pre-pubertal WKY and Wistar pups, raised in early chronic-stress or normal growing-conditions

<table>
<thead>
<tr>
<th>Factor</th>
<th>Measure</th>
<th>Wistar Normal growing-condition, N = 12</th>
<th>Wistar Chronic-stress condition, N = 12</th>
<th>Total Wistar strain, N = 24</th>
<th>WKY Normal growing-condition, N = 13</th>
<th>WKY Chronic-stress condition, N = 13</th>
<th>Total WKY strain, N = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>Body-weight</td>
<td>153.98 (±4.47)</td>
<td>167.78 (±3.93)</td>
<td>160.88 (±3.25)</td>
<td>113.29 (±1.12)</td>
<td>106.29 (±5.09)</td>
<td>109.65 (±3.06)</td>
</tr>
<tr>
<td>Activity</td>
<td>No. of total line crossings (OF)</td>
<td>75.25 (±7.18)</td>
<td>84.33 (±11.25)</td>
<td>79.79 (±6.60)</td>
<td>58.66 (±1.95)</td>
<td>65.92 (±10.19)</td>
<td>62.44 (±7.68)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>No. of freezes (OF)</td>
<td>2.91 (±1.16)</td>
<td>5.84 (±1.41)</td>
<td>4.44 (±0.95)</td>
<td>3.92 (±1.42)</td>
<td>0.91 (±0.54)</td>
<td>2.53 (±0.85)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>% time freezing (OF)</td>
<td>3.53 (±1.46)</td>
<td>5.10 (±2.09)</td>
<td>4.31 (±1.26)</td>
<td>18.79 (±5.01)</td>
<td>11.22 (±3.68)</td>
<td>14.86 (±3.10)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>% no. of entries to center (OF)</td>
<td>7.61 (±1.94)</td>
<td>8.92 (±0.90)</td>
<td>8.27 (±1.06)</td>
<td>13.57 (±2.50)</td>
<td>12.70 (±2.41)</td>
<td>13.08 (±1.71)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>% time in center (OF)</td>
<td>0.81 (±0.25)</td>
<td>1.19 (±0.32)</td>
<td>1.00 (±0.20)</td>
<td>14.62 (±5.12)</td>
<td>6.09 (±2.23)</td>
<td>10.19 (±2.80)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>% no. of entries to open arms (PM)</td>
<td>8.44 (±1.70)</td>
<td>13.55 (±2.27)</td>
<td>11.00 (±1.49)</td>
<td>12.45 (±2.54)</td>
<td>10.26 (±2.32)</td>
<td>11.31 (±1.69)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>Latency to enter open arms (PM)</td>
<td>189.24 (±26.03)</td>
<td>145.33 (±31.10)</td>
<td>167.28 (±20.35)</td>
<td>143.92 (±32.83)</td>
<td>144.81 (±34.16)</td>
<td>144.38 (±23.25)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>% time in open arms (PM)</td>
<td>0.70 (±0.43)</td>
<td>1.88 (±1.58)</td>
<td>1.29 (±0.81)</td>
<td>13.30 (±4.63)</td>
<td>9.34 (±2.87)</td>
<td>11.24 (±2.65)</td>
</tr>
</tbody>
</table>

Weight is given in grams, latency in seconds; all other measures are pure number or percent (noted in table). Abbreviations: OF, open-field; PM, plus-maze.

* p < 0.1
* p < 0.05
*** p < 0.001
2.1.3. Anxiety-like behaviors

Figs. 1 and 2) revealed a significant main effect of strain to leave center square (in open-field and plus-maze, see Table 2). Wistar strains exhibited more percent time freezing than controls (non-significant trend; \( F(1,44) < 0.01 \); WKY pups exhibited more percent time freezing than controls \( F(1,42) = 12.864, p < 0.001 \), respectively). The strain × growing-condition interaction showed a non-significant trend \( F(2,41) = 3.075, p = 0.057 \). WKY pups tended to have longer latencies to leave center square (in the plus-maze) when raised in chronic-stress conditions, while Wistar pups exhibited an opposite pattern \( F(1,42) = 6.290, p < 0.05 \). There was no significant main effect for growing-condition \( F(2,41) = 1.069, p = 0.34 \) (see Fig. 2).

In summary, WKY pups exhibited lowered body-weight and activity levels compared to controls (activity differences were found in the open-field, but not in the plus-maze). Overall differences in anxiety-like behaviors were found in the open-field with WKY pups exhibiting a tendency toward immobility (measured in latencies to leave center square) and freezing. A similar trend for immobility was found in the plus-maze, although overall differences were not significant.

2.2. The effect of strain and growing-condition on FSL and SD (controls) pre-pubertal rats

2.2.1. General results

The 2 × 2 ANOVA for body-weight revealed a significant main effect of strain with FSL pups weighing less than controls \( F(1,47) = 25.071, p < 0.001 \). No significant difference main effect for growing-conditions or strain × growing-conditions interaction were found in the ANOVA \( F(1,47) = 0.003, n.s. \) (see Table 3).

2.2.2. Activity

The 2 × 2 MANCOVA for activity cluster in the open-field revealed a significant main effect of strain \( F(3,44) = 5.462, p < 0.01 \). FSL pups exhibited more rearing per cent in the plus-maze and their latency for first rearing was longer compared to controls \( F(1,46) = 5.146, p < 0.05 \). Total squares entered was not significantly different between the strains \( F(3,44) = 0.490, n.s. \). In the 2 × 2 ANOVA for activity cluster in the plus-maze there was a significant main effect for strain; FSL pups had more total entries to maze sections than controls \( F(1,46) = 10.300, p < 0.01 \). There were no significant main effect for growing-condition or strain × growing-condition interaction \( F(1,46) = 0.013, n.s. \) (see Table 3).

2.2.3. Anxiety-like behaviors

The 2 × 2 MANCOVA for anxiety-like behavioral cluster in the open-field (Fig. 3) showed a significant main effect of strain \( F(4,43) = 4.348, p < 0.001 \). FSL pup’s exhibited more percent number of entries to center square (from total squares entered) and spent more percent time in center square compared to controls \( F(1,46) = 10.206, p < 0.01 \). There were no significant differences in percent time freezing and latency for first freezing. A strain × growing-condition interaction effect was also significant \( F(4,43) = 4.470, p < 0.01 \). FSL pups’ latency for first
Table 3

<table>
<thead>
<tr>
<th>Measure</th>
<th>SD (N=12)</th>
<th>FSL (N=12)</th>
<th>SD (N=12)</th>
<th>FSL (N=12)</th>
<th>SD (N=12)</th>
<th>FSL (N=12)</th>
<th>SD (N=12)</th>
<th>FSL (N=12)</th>
<th>SD (N=12)</th>
<th>FSL (N=12)</th>
<th>SD (N=12)</th>
<th>FSL (N=12)</th>
</tr>
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<tbody>
<tr>
<td>General Body-weight</td>
<td></td>
<td></td>
<td>201.66 (±6.48)</td>
<td>194.84 (±6.04)</td>
<td>198.11 (±4.38)</td>
<td>165.87 (±5.45)</td>
<td>172.03 (±5.37)</td>
<td>168.71 (±3.81)**</td>
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<tr>
<td>Activity No. of total line crossings (OF)</td>
<td>105.91 (±11.52)</td>
<td>90.61 (±9.10)</td>
<td>97.96 (±7.29)</td>
<td>108.35 (±10.13)</td>
<td>122.33 (±4.88)</td>
<td>114.80 (±5.96)</td>
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<tr>
<td>Activity No. of rearings (OF)</td>
<td>27.50 (±3.90)</td>
<td>24.61 (±3.68)</td>
<td>26.00 (±2.64)</td>
<td>35.78 (±5.28)</td>
<td>40.25 (±5.39)</td>
<td>37.84 (±3.73)</td>
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<tr>
<td>Activity Latency for rearing (OF)</td>
<td>18.14 (±2.44)</td>
<td>20.91 (±3.97)</td>
<td>19.58 (±2.34)</td>
<td>50.94 (±12.37)</td>
<td>30.19 (±5.10)</td>
<td>41.36 (±7.24) *</td>
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<tr>
<td>Activity Total no. of entries (PM)</td>
<td>19.00 (±3.25)</td>
<td>16.61 (±2.05)</td>
<td>17.76 (±1.87)</td>
<td>25.53 (±2.06)</td>
<td>28.50 (±3.33)</td>
<td>26.90 (±1.88) **</td>
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</tr>
<tr>
<td>Anxiety No. of freezes (OF)</td>
<td>2.66 (±0.79)</td>
<td>4.66 (±1.65)</td>
<td>3.66 (±0.92)</td>
<td>11.33 (±2.26)</td>
<td>8.15 (±1.66)</td>
<td>9.68 (±1.39)</td>
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</tbody>
</table>

Body-weight is given in grams, latency in seconds; all other measures are number or percent (noted in table). Abbreviations: OF: open-field; PM: plus-maze.

* *p < 0.05.
** *p < 0.01.

freezing was longer in the chronic-stress condition than in normal growing-condition, while SD (controls) pups’ latency was shorter in the chronic-stress condition than in normal growing-condition [F(1,46) = 12.620, p < 0.001]. There were no significant differences in percent number of entries to center square, percent time in center square and percent time freezing. There was no significant main effect for growing-condition [F(1,43) = 0.944, n.s.] (see Fig. 3).

The 2 × 2 MANCOVA for the anxiety-like behaviors in the plus-maze (Fig. 3) showed a significant main effect of strain [F(4,42) = 2.810, p < 0.05]; FSL pups spent more percent time in open arms than controls, showed more percent number of entries to open arms (from total entries) and entered the open arm (latency) more quickly than controls [F(1,45) = 5.896, p < 0.05; F(1,45) = 11.357, p < 0.01; F(1,45) = 6.181, p < 0.05, respectively]. There was no significant difference in number of unprotected head dips. There was no significant main effect for growing-condition and strain × growing-condition interaction [F(4,42) = 1.323, n.s.; F(4,42) = 1.443, n.s., respectively]. The MANCOVA for latencies to leave center square (in open-field and plus-maze, see Fig. 4) revealed a tendency toward significant main effect of strain [F(2,46) = 2.888, p = 0.066]; FSL pups showed a tendency toward longer latencies to leave center (in the open-field) [F(1,47) = 4.074, p < 0.05]. There were no significant main effect for growing-condition or strain × growing-condition interaction [F(2,46) = 0.483, as.; F(2,46) = 0.650, as., respectively].

Fig. 3. Anxiety-like behaviors in the open-field and plus-maze: mean ± S.E.M. of 40–41-day-old rats of the FSL and SD strains (note that all measures are in percent, except number of protected head-dips which is a number). * *p < 0.05.

Fig. 4. Anxiety-like behaviors (latency measures) in the open-field and plus-maze: mean ± S.E.M. of 40–41-day-old rats of the FSL and SD strains (note that these measures are in seconds). * p < 0.05.
of anxiety and activity in tests of anxiety has been extensively discussed. The confounding vulnerability and exposure to early-life stress is a major concern an underlying construct. Second, we attempted to dissociate the possibility of even more drastic change in the anxiety profile of FSL rats, as they mature.

The current study found indications of heightened anxiety-like behaviors in pre-pubertal WKY rats, as indexed by a tendency toward freezing and immobility in the open-field. The plus-maze revealed a similar behavioral pattern, indicating that WKY pups show increased immobility (measured as latency to leave center) compared to controls. Together, the tests suggest that a behavioral tendency for freezing and immobility is the major characteristic of the strain, exhibited as early as PND 40. This behavioral pattern also fits findings in adult WKY rats [43,45]. Earlier studies interpreted the freezing and immobility as depressive-like behaviors, strengthening the WKY strain’s vulnerability as a model of depression [43–45]. Interpretation of freezing as a depressive-like behavior, accompanied by limited differences in other measures of anxiety, has led to the conclusion that “anxiety is not the stress response that best characterizes WKY behavior” (taken from [45]). We suggest that such a conclusion is unwarranted and confounds between indexes of anxiety and depression. Freezing in response to aversive stimuli is a well characterized and ethologically validated measure of anxiety in rodents [12,35,48]. The current study suggests that freezing and immobility may in fact represent the strain’s susceptibility to anxiety, a possibility not explored by previous studies (e.g., [43,45]).

The current study investigated anxiety-like behaviors of WKY and FSL pre-pubertal rats, employing established tests of anxiety. The study explored the possibility of using the WKY and FSL strains as animal models of pediatric depression, with differential patterns of anxiety comorbidity. An additional aim was the investigation of mechanisms effecting the establishment of anxiety-like behaviors in the two animal models of depression. To accomplish this goal, a chronic-stress condition was employed, exploring the interactive effects of hereditary vulnerability and exposure to early-life stress [17]. The results indicate that pre-pubertal rats of the two animal models differ in their behavioral patterns, showing almost opposite levels of anxiety-like behaviors. However, before detailed description of the results and their significance a few methodological issues should be briefly mentioned.

The investigation of anxiety, and especially in animal models of depression, raised the need to address two important issues. First, the multiple behavioral measures had to be grouped into theoretically logical and methodologically sound factors. In the current study, a two-factor solution was used in grouping the measures (‘anxiety-like behaviors’ and ‘activity’). The factors chosen have a strong support in the literature, while avoiding the complexity associated with a higher number of extracted components [50,56]. The use of an ‘approach-avoidance conflict’ factor was avoided, in light of Messier and Wall’s [56] review that calls into question the ability of these behaviors to measure an underlying construct. Second, we attempted to dissociate between anxiety-like behaviors and activity. The confounding of anxiety and activity in tests of anxiety has been extensively criticized [37,49] and is especially important since there are indications of activity differences between the strains [8,50, see also results]. These activity differences are not surprising in light of the fact that lowered activity is a characteristic of depression [2], but highlight the importance of monitoring possible activity differences in anxiety tests.

In summary, FSL pups exhibited lowered body-weight and heightened activity levels compared to controls. As for anxiety-like behaviors, the open-field and plus-maze revealed a consistent pattern of lowered anxiety-like behaviors of FSL pups, compared to controls.

2.3. General summary of results

WKY and FSL pre-pubertal rats had lower body-weights compared to controls, with almost opposing behavioral profiles. WKY pups showed evidence of lowered activity levels compared to controls, as opposed to the heightened activity of FSL pups. WKY pups also exhibited markedly freezing and immobility (anxiety-like behaviors) compared to controls, while FSL pups showed a pattern of lowered anxiety-like behaviors. Lastly, the chronic-stress growing-condition (limited-bedding) exhibited limited effect on all strains, showed no main-effect and interacted with WKY pups’ body-weight and FSL pups’ latency for freezing.

3. Discussion

The current study investigated anxiety-like behaviors of WKY and FSL pre-pubertal rats, employing established tests of anxiety. The study explored the possibility of using the WKY and FSL strains as animal models of pediatric depression, with differential patterns of anxiety comorbidity. An additional aim was the investigation of mechanisms effecting the establishment of anxiety-like behaviors in the two animal models of depression. To accomplish this goal, a chronic-stress condition was employed, exploring the interactive effects of hereditary vulnerability and exposure to early-life stress [17]. The results indicate that pre-pubertal rats of the two animal models differ in their behavioral patterns, showing almost opposite levels of anxiety-like behaviors. However, before detailed description of the results and their significance a few methodological issues should be briefly mentioned.

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FSL pre-pubertal rats exhibited an opposite profile of lowered anxiety-like behaviors compared to controls. In the open-field, FSL pups spent more time in the center square and entered the center square more often. A similar pattern is seen in the plus-maze, where FSL pre-pubertal rats spent more time in the open arms and entered the open arms more often (even entering the open arms more quickly) compared to controls. These behaviors are indicative of lowered anxiety, since rodents tend to avoid open spaces, environments associated with increased risk [50]. In addition, an interaction was revealed between strain and growing-condition on latency to for first freezing (in the open-field). This latency measure is seldom used in the literature, and therefore, additional research is needed to investigate its ability to act as a marker of environmental stress. One last point to be considered concerns the FSL strain’s anxiety-like behavior’s developmental trend. While WKY pre-pubertal rats exhibit a similar behavioral pattern as adults, studies suggest that FSL adults exhibit different anxiety-like behavioral profile than pre-pubertal FSL rats (investigated in the current study). FSL adults rats spent as much time in the open arms of the elevated plus-maze as did controls [40] and the increase in time spent in the open arms induced by diazepam was similar in FSL and controls [53]. These studies on adult FSL rats suggest a developmental trend with FSL rats maturing from low anxiety levels at pre-pubertal age to normal anxiety levels of anxiety as adults [37]. However, recent studies suggest that FSL rats exhibit anxiogenic behavior in other tasks, such as the social interaction task and the active avoidance task [39,41]. Such studies raise the possibility of even more drastic change in the anxiety profile of FSL rats, as they mature.

The chronic-stress paradigm used in our study (limited-bedding) was investigated for the first time for its effect on behavior rather than physiology [3,17]. This mild, ecologically valid [17], manipulation did not show a main effect, but...
interacted to some degree with the strain of the pups. FSL pups exhibited longer latency for first freezing in the chronic-stress condition while SD pups showed an opposite pattern (in the open-field). While this latency measure may be proven significant in the future, the fact that only a single behavioral measure was affected casts doubts on the paradigm’s effectiveness. This conclusion is strengthened when analyzing WKY/Wistar results, where the chronic-stress was able to affect only bodyweight and not behavioral measures. The lack of differences between growing-conditions (and stress component) may be seen as evidence for the resiliency of the strains to environmental challenge, and indicate a more hereditary influence on their anxiety-like behaviors. However, the limited effect of the chronic-stress paradigm casts doubt on such possibility and indicates a need for a stronger stress paradigm. Research is needed to establish whether changes should be made in order to increase the paradigm’s effectiveness, perhaps by prolonging the manipulation.

The current study suggests that the WKY and FSL genetic animals models of depression [36,42] exhibit differential patterns of anxiety-like behaviors at a pre-pubertal age. The results indicate heightened anxiety in WKY pre-pubertal pups compared to controls, while the FSL strain seems less anxious than its control strain. The abnormal levels of anxiety at a pre-pubertal age, accompanied by a depressive behavioral profile, indicate that pre-pubertal pups of these strains may be suitable to model different subgroups of pediatric depression. In fact, this possibility fits earlier findings in our laboratory, suggesting that the strains differ from one another in behavioral as well as physiological measures [8,32]. FSL pre-pubertal pups may be used to model depression with low anxiety levels, while pre-pubertal WKY pups may model depression with a marked anxiety component. The modeling of these sub-groups fits the increasing attention given to depression–anxiety comorbidity in pediatric populations and may help advance research in the field of child and adolescent psychiatry.

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References


Punt L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463:3-33.


Solberg LC, Olson SL, Turek FW, Redi E. Altered hormone levels and circadian rhythm of activity in the WKY rat, a putative animal model of depression. Am J Physiol Regul Integr Comp Physiol 2001;281:R786-94.


