Reward and anxiety in genetic animal models of childhood depression

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Abstract

One of the most important criteria for major depressive disorder in adults and in children and adolescents as well, is the loss of interest in or pleasure from typically enjoyable experiences or activities: anhedonia. Anxiety is frequently co-morbid with depression. We examined reward and anxiety in genetic animal models of childhood depression. Two different “depressed” lines were studied: the Flinders Sensitive Line (FSL) and their controls, Sprague–Dawley (SD) rats and the Wistar Kyoto (WKY) line and their controls, Wistar rats. Recently, we found that prepubertal rats (about 35 days old) from these lines exhibited increased immobility in the swim test, and abnormal social play observed after 24-h isolation. We hypothesized that FSL and WKY prepubertal rats will further show anhedonia in two different behavioral assays: the conditioned place preference test (CPP), examining the rewarding aspect of social interaction and the saccharin preference test. Behavior in the open field paradigm and freezing behavior in the CPP paradigm were also used as measures of anxiety. WKY, but not FSL, prepubertal rats, consumed less of the saccharin solution compared to their control line. FSL and WKY prepubertal rats found social interaction to be rewarding to a similar extent as their control lines, in the CPP test. Only the WKY rats showed anxiety in behavior in the open field and freezing behavior in the CPP paradigm. The results suggest that WKY prepubertal rats are anxious and sensitive to stress-induced anhedonia, while FSL prepubertal rats exhibit none of these symptoms.

Keywords: Anxiety; Reward; Childhood depression

1. Reward and childhood depression

One of the most important criteria for major depressive disorder, in adults, and in children and adolescent as well, is the loss of interest in or pleasure from typically enjoyable experiences or activities (DSM-IV, American Psychiatric Association). This phenomenon is known as anhedonia. In contrast to a second cardinal symptom of major depression, dysphoric mood, anhedonia can be assessed in animal experiments. Hedonic effects in laboratory animals can be studied by the brain stimulation reward technique as well as by measuring the animals’ natural preference for sweetened fluids such as sucrose or saccharin solutions [1,2]. One of the often-employed methods to assess reward is the conditioned place preference test (CPP).

2. Anxiety and depression

Co-occurrence of psychiatric disorders (co-morbidity) is common [36]. In persons with depression or an anxiety disorder, co-morbidity with the other disorder occurs in one quarter to one-half of individuals [37,38,39] and is associated with increased severity [40,41,42]. As in adults, there is signifi-
cant overlap in criteria between generalized anxiety disorder (GAD) and major depressive disorder (MDD) in children and adolescents as well: both include fatigue, difficulty concentrating, and sleep disturbance [43]. Irritability is an additional overlapping symptom unique to pediatric populations and is commonly present in other child psychiatric illnesses such as disruptive disorders and bipolar disorders as well [43]. Anxiety and depressive symptoms may exert a potentiating effect in co-morbid children and adolescents, depressive symptoms tend to be more severe in depressed youth with co-morbid anxiety compared to non-anxious depressed youth, as was the case in adults [43,44]. In clinical samples, the rate of comorbid anxiety disorders in depressed youth can be as high as 70% [45], and on average about 25–50% of subjects with pediatric depression have a co-morbid anxiety disorder [43].

In the current study we attempt, for the first time, to find out whether prepubertal rats of two different “genetic animal models” for depression demonstrate anhedonia and/or anxiety. The two lines are the Flünder Sensitive Line (FSL), which was selectively bred from the Sprague–Dawley (SD) line and the Wistar Kyoto (WKY) rat, derived from the Wistar line. Adults from both lines (FSL and WKY) have been shown to present depression-like symptoms such as reduced body weight and disturbed REM sleep. They also displayed depression-like symptoms on several behavioral tests, e.g., increased immobility in the swim test and greater degree of “anhedonia” in response to chronic mild stress and acute stress. The increased levels of swim-test immobility can be prevented by chronic (but not acute) antidepressant treatment [4,5]. Both lines have been shown to exhibit abnormalities in some central neurochemical systems (dopaminergic, both lines; noradrenergic, WKY; cholinergic, FSL; serotoninergic, FSL), compared to controls [6,5,7], in the few studies to date. Recently, we have found in our lab that prepubertal rats (about 35 days old) from the FSL and WKY lines exhibit increased immobility in the swim test, abnormal social play as observed after 24-h of isolation and different abnormalities of the hypothalamic–pituitary–adrenal (HPA) axis (Malkesman et al., submitted). Thus, although several behavioral similarities exist between the FSL and the WKY models, they are fundamentally different, as adults as well as juveniles. They are derived from different lines by different breeding approaches, and their patterns of neurochemical abnormalities are separate. Furthermore, as prepubertals, their abnormal patterns of social play and HPA hormone levels are also different, while FSL rats showed lower levels of HPA hormones and higher levels of social play compared to their controls—SD, the WKY prepubertal rats showed higher levels of HPA hormones and lower levels of social play compared to their controls—Wistar [Malkesman et al., submitted].

In order to examine anhedonia we used two behavioral tests: a modified version of the conditioned place preference test (CPP) for revealing the rewarding aspect of social inter-

action in those lines and their controls [8] and the saccharin preference test [9,1]. In order to examine anxiety, we used two behavioral assays: the open field test [34,35] and measuring freezing behavior in the CPP apparatus [10].

2.1. CPP

In this paradigm, the potentially rewarding stimulus is repeatedly paired with a set of distinct environmental cues, while a neutral control treatment is repeatedly paired with a different set of distinct environmental cues. If the treatment in question is rewarding for the animal, it will, during this repeated pairing, associate these rewarding effects with the distinct environmental cues paired with the treatment. Subsequently, it will then show a preference for these cues over the neutral cues when given a free choice between them [11,23]. Hence, when the rat increases the time it spends in an initially less-preferred environment following reward-place pairings, it is inferred that the contextual cues in that environment act as reward stimuli in themselves, i.e., they elicit approach, and possibly, the neurochemical equivalent of positive affect [8].

In the current study, the rewarding stimulus was the opportunity for social interaction with a peer after social isolation, in which episodes of social play or some characteristics behaviors of social play might occur. Social play, one of the earliest forms of non-mother-directed social behavior observed in mammals, has been observed to contain behavioral patterns related to social, sexual and aggressive behavior, displayed in an exaggerated and/or out-of-context fashion [12]. One of the characteristics of social play behavior is its reward value. There is some evidence that social interaction in rats is rewarding: Juvenile rats will learn to traverse a T-maze when access to a playmate serves as the reward at the end of the maze [13,14,15]; departure from the play goal-box is rarely observed even when an avenue of escape from a playmate is available (such as a wire screen on one wall) [15]. Dominant prepubertal rats do not hesitate to pass through a one-way door to gain access to a playmate [15].

Social play in most species exhibits a characteristic developmental time course, with the amount of play increasing during the early juvenile period (the peak in rats is about 35 days of age), remaining stable through youth, and diminishing as animals approach puberty [16]. Social environment (housing conditions) of the subject prior to the behavioral test is an important factor that determines social behavior. Rearing in a poor social environment early in ontogeny (e.g., social isolation) dramatically changes social behavior [17]. Rats are very sensitive to the effects of social isolation during the period between weaning and sexual maturation, when social play is most abundant. Early social isolation markedly increases behavioral manifestations of play [16,24]. Both short-term (not more that 24-h) social deprivation and long-term (several days) individual housing have been used to increase the incidence of play behavior during testing [18,16].
2.2. Saccharin preference

As mentioned above, hedonic effects in laboratory animals can be studied by the brain stimulation reward technique as well as by measuring the animals’ natural preference for sweetened fluids such as sucrose or saccharin solutions [1]. Rats normally exhibit very high preferences for saccharin solution when it is paired with water, but exhibit decreases when exposed to chronic mild stress. The preference for saccharin over water is used as a measure for the rat’s sensitivity to reward [20]. Williner et al. [49] proposed that reduced consumption of sweet solutions (sucrose, saccharin) by chronic mildly stressed (CMS) rats is a measure of anhedonia. Studies have shown that there are differences between stressors in affecting the preference of saccharin in rats. Rats showed opposite reactions after physical (foot shocks) and emotional (presence in the adjacent compartment during foot shock treatment of their cage mates) stress. Physically stressed animals displayed less preference for a sweet solution, while emotionally stressed animals displayed a slight increase in saccharin preference [9].

Rats also react differently to different concentrations of a saccharin solution. Pijlman et al. [9] reported that the strongest effects of stress on saccharin consumption and preference were observed with the concentration of 0.004%, in adult male Wistar rats. Pucilowski et al. [1] found that adult males from both Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) showed saccharin preference in a concentration of 0.02%.

2.3. Open field

The open field, which was originally described as a test of emotionality in rats, is now one of the most popular procedures in animal psychology [34,35]. In the open field procedure, the animal is placed in the center or close to the walls of the apparatus and the following behavioral items are recorded for a period ranging from 2 to 20 min (usually 5 min): horizontal locomotion, frequency of rearing or leaning and grooming. In such a situation, rodents spontaneously prefer the periphery of the apparatus to activity in the central parts of the open field, and indeed, mice and rats walk close to the walls, a behavior called thigmotaxis [39]. Anxiety behavior in the open field is triggered by two factors: individual testing (the animal is separated from its social group) and agoraphobia (as the arena is very large relative to the animal’s breeding or natural environment). These two factors may trigger anxiety behavior only in gregarious species and/or in species that show fear of open spaces into which they are forced. This is precisely the case with rodents that live in social groups and in small tunnels [39].

2.4. Freezing behavior

Freezing in response to aversive stimuli is a well-characterized and ethologically validated measure of fear in rodents [10]. Freezing is defined as the total time the animal was in an immobile state, often in a crouching position with wide open eyes and irregular respiration, after it had remained motionless for at least 1 s [19].

The CPP paradigm has characteristics of a stress test (e.g., open field test), since the animal in this paradigm is being habituated to the apparatus and given free access of movement throughout this novel environment—the apparatus. This kind of habituation and free movement may be stressful for part of the animals, especially if they are genetic animal models for depression. Therefore, we decided to assess a cardinal measure of anxiety in the CPP apparatus—freezing behavior.

3. Methods

3.1. Animals

Nulliparous SD, FSL, Wistar and WKY female rats were mated with males from the same line in their breeding colonies, in the Developmental Psychobiology laboratory at Bar-Ilan University, Ramat-Gan, Israel. The lines were likely to be inbred because of the relatively small number of original parents. After weaning on postnatal day 21, male pups (not participating in the CPP test) were housed in polypropylene cages (38 cm × 21 cm × 18 cm), three per cage, in a temperature-controlled vivarium (20–24 °C), under a 14-h light:10-h dark cycle (lights on at 05:00). Food and water were available ad libitum. The animals that participated in the CPP procedure were similarly weaned on day 21 and housed in the same vivarium, with ad lib food and water. These pups, however, were housed individually in polypropylene cages (29 cm × 12 cm × 11 cm). Each animal in this study participated in only one test.

The study protocol was approved by the Institutional Animal Care and Use Committee and adhered to the guidelines of the Society for Neuroscience and the American Psychological Association.

3.2. CPP

Place conditioning was conducted in a three-chambered stainless steel apparatus, consisting of a neutral center section (20.5 cm × 30.5 cm × 20.0 cm; width × length × height) and two cue-distinct end chambers. The end chambers (20.5 cm × 30.5 cm × 20.0 cm; width × length × height), originally identical, were altered to provide the following discriminable cues: the left (dark) side of the chamber was illuminated with a 6 W, 30 V red light bulb and had a smooth black plastic floor. The right (light) side was illuminated with a 6 W, 30 V white light bulb and had a steel bar grid floor with pine shavings in the drop pan. Thus, there were multisensory (i.e., light, tactile, odor) distinctions between the two end chambers (as in [38]). During conditioning, a removable wall served to restrict a subject’s egress from the chamber in which it was placed. All chambers were washed with water and ethanol (95%) between each subject’s sessions.

Place preference was recorded in a room separate from the colony room. Subjects underwent four CPP treatment phases: habituation, baseline measurement, conditioning, and place preference testing. At 26–27 days of age, all subjects were habituated to the CPP apparatus for 45 min; every subject was placed in each chamb-
for 15 min. At 28 days of age, each subject was placed into the CPP apparatus and given free access of movement throughout the chambers for 15 min. The numbers of seconds spent in each chamber was recorded and served to establish a baseline of side preference per subject. At 29 days of age, place conditioning was initiated. This age was selected since play behavior is observed to peak between 26 and 35 days [16]. Conditioning consisted of four consecutive daily morning (09:00–11:00 h) pairings of 10 min duration. The subject was confined in its non-preferred side with a partner from the same litter. In the afternoon session (13:00–15:00 h), the subject was confined to its preferred side for 15 min. These sessions were repeated daily for 15 days. Each subject was placed in the CPP arena for 15 min to assess place preference. N in each group was between 10 and 12.

3.3. Saccharin preference after stress manipulation

The day before the saccharin preference test, male rats (aged 34–35 days and without CPP experience) were habituated for 1 h to the test cage (polystyrene—38 cm × 21 cm × 18 cm) and to the bottle of saccharin solution (0.02% or 0.004%) that was available and which they will experience on the day of the preference test. In the morning of the test (08:30–09:00), all the animals were exposed to physical stress. Rats were immersed in a Plexiglas cylinder (height = 45.5 cm, diameter = 14.0 cm) filled to 24 cm with fresh tap water in a temperature of 20 °C, for 5 min. After the physical stress the animals were dried and housed in the test cage to which they had been habituated. The animals were not previously deprived of water and food. Each animal was presented with a bottle (250 ml) of one of two concentrations of saccharin (0.004 or 0.02%) and a bottle of tap water. The animals had the opportunity to drink as much of the solutions as they wanted during 24 h. The bottles were weighed before the experiment, after every hour for the first 8 h and after 24 h. The first 8 h were summed as the “day time”, while the other 16 h were summed as the “night time”. Bottles and nipples had been checked for leakage prior to the test. The total consumption of water and saccharin in grams was measured and also was used to calculate the preference ratio as follows: (consumed saccharin × 100)/(consumed water + consumed saccharin). Saccharin (2,3-di-hydro-3-oxonenzisosulfonazole from Sigma) was dissolved in tap water. Food was available throughout the experiment. N in each group and in each concentration was between 10 and 12.

3.4. Freezing behavior

At 28 days of age, each subject was placed into the CPP apparatus and given free access of movement throughout the chambers for 15 min (the CPP baseline measurement). In this phase, we measured a major anxiety symptom—freezing behavior. Freezing behavior was scored if the animal went into one of the chambers, in the beginning of the baseline measurement, and stayed there for the entire 15 min without moving (a pattern that was evident frequently in our initial observations). This all or none criterion, although strict, allowed us to characterize the extreme freezing reaction throughout the entire session in the stressful situation of encountering the novel CPP arena. N in each group was between 10 and 12.

3.5. Open field

The open-field arena (62 cm × 62 cm) was enclosed by walls 30 cm high. The floor was built from blue plexiglass and the walls from green plexiglass (a modified version of that used in [32]). The floor space was divided into nine equal-size squares (3 × 3) by a black marker. At the day of the experiment (postnatal day 34/35) two male rats were transferred to the testing room, and placed separately in two cages (identical in size to the home cages) with a thin layer of bedding. The first animal was removed, weighed and placed gently in the center of the arena. The rat was allowed to explore the open-field freely for 5 min. After 5 min, it was returned to the home cage. The open field arena was cleaned using ethanol and the second rat underwent an identical procedure. Sessions in the open field were filmed using a Sony High-8 video camera placed 130 cm above the cage.

Frequencies and durations of anxiety behaviors were recorded and analyzed. Behaviors examined were: (1) latency to leave the center square; (2) number of squares entered (“crossing”); (3) time spent in the corners of the arena; (4) number of rearing; (5) duration of freezing behavior; (6) number of fecal bolu deposited [based on [33]]. Inter-rater reliabilities on scoring these behaviors ranged from r = 1 to r = 0.926 (p < 0.001). N in each group was between 11 and 15.

3.6. Data analysis

Group differences in saccharin consumption in different concentrations (0.02% or 0.004%) were analyzed by three way ANOVA (water and saccharin consumption, within subjects × saccharin concentration × line) comparing each “depressed” and control line separately (FSL and SD; Wistar and WKY), twice—once for the “day time” and once for the “night time”. Since there were no differences in the consumption of saccharin in the two concentrations, we analyzed by two way ANOVA the differences between the “depressed” and control lines in the different times (collapsing over saccharin concentration). When a difference was found, this was followed by t-tests for independent samples to uncover the source of differences between the two different lines—the consumption of the water or the consumption of the saccharin solution. To evaluate if each line actually exhibited a preference for the saccharin solution, we analyzed by two way ANOVA (saccharin concentration × line) group differences in saccharin preference (0.02 or 0.004%). In addition, the preference ratio was calculated as follows: (consumed saccharin × 100)/consumed water + consumed saccharin) × consumed water, separately in two different times, day and night. Since, again, there were no differences in the consumption of saccharin in the different concentrations, we analyzed by one sample t-tests saccharin preference in the “depressed” and control lines.

CPP was analyzed by independent t-tests comparing the FSL and SD lines and the WKY and Wistar lines, separately. Frequency of freezing behavior in the CPP paradigm was evaluated by chi-square tests, comparing the two “depressed” lines and their controls, separately.

The anxiety behaviors of the groups in the open field was compared by one way multivariate analysis of variance (MANOVA), followed up by one-way univariate ANOVA for each of the six measures of anxiety behavior, for each of the two between-line comparisons.
4. Results

Since there were no differences in consumption of the two concentrations of the saccharin solution (0.02 and 0.004%) in grams, between the different lines, after stress [FSL and SD, $F(1,38) = 0.1666; p > 0.05$; WKY and Wistar, $F(1,39) = 0.446; p > 0.05$], we combined the data from both those concentrations. We also collapsed the two concentrations for analyzing the preference ratio, because there were no differences between them in the different lines in the different times (day or night time) [day time: FSL and SD, $F(1,38) = 0.204; p > 0.05$; WKY and Wistar, $F(1,39) = 0.089; p > 0.05$]. Night time: FSL and SD, $F(1,38) = 1.316; p > 0.05$; WKY and Wistar, $F(1,39) = 0.503; p > 0.05$.

The differences in the amount of saccharin consumption and water consumption in grams, in the day time, between the different lines are presented in Fig. 1 a and b. Two-way ANOVAs did not reveal significant interactions between line and relative consumption of saccharin versus water in the day time [FSL and SD, $F(1,40) = 0.227; p > 0.05$; WKY and Wistar, $F(1,41) = 0.115; p > 0.05$]. But, while calculating the preference ratio (saccharin consumption $\times 100$/saccharin consumption + water consumption) of saccharin in the day time, we can see in Fig. 1 c that the FSL and Wistar lines were “hedonic”, when using the criterion of a preference significantly greater than 50%. Hence, they preferred the saccharin solution over the water solution more than randomly expected (50%) [FSL, $t(1,19) = 2.422; p < 0.05$; Wistar, $t(1,23) = 2.506; p < 0.05$]. In contrast, the SD and WKY lines failed to show such a preference [SD, $t(1,21) = 1.345; p > 0.05$; WKY, $t(1,18) = 0.776; p > 0.05$].

In Fig. 1a and b, we can see the differences in the amount of saccharin consumption and water consumption in grams, in the night time, between the different lines. As evident from the figures, there was a significant difference (“interaction effect” in the ANOVA) between the WKY and Wistar lines in the relative consumption of saccharin versus water ($F(1,41) = 6.662; p < 0.05$). Post-hoc tests showed that WKY rats consumed significantly less saccharin than Wistar controls, $t(1,41) = 5.363; p < 0.05$, but there was no significant difference between these lines in the consumption of water, $t(1,41) = 0.489; p > 0.05$. There was no difference (no “interaction effect”) between the FSL and SD lines in the relative consumption of saccharin versus water in the night time, $F(1,40) = 1.118; p > 0.05$. When calculating the preference ratio of saccharin in the night time, we can see in Fig. 2c that all lines, except the WKY, showed a preference for saccharin, when using the criterion of a preference significantly greater than 50%. Hence, they preferred the saccharin solution over the water solution more than randomly expected (50%) [FSL, $t(1,19) = 5.745; p < 0.01$; SD, $t(1,21) = 2.121; p < 0.05$; Wistar, $t(1,23) = 6.544; p < 0.01$]. In contrast, the WKY line failed to show such a preference [WKY, $t(1,18) = 1.879; p > 0.05$].

In the CPP test, there were no differences between the WKY and Wistar lines in the amount of time that they spent in the nonpreferred side after 4 days of conditioning, $t(1,19) = 0.044; p > 0.05$. There were no differences between the FSL and SD line as well, $t(1,21) = 0.028; p > 0.05$. Comparison of the baseline preferences with post-conditioning preferences showed a significant increase in both of the groups (data are presented in Table 1).

When we measured the freezing behavior in the CPP arena, we found, as evident from Fig. 3a and b, that the WKY line froze significantly more than the Wistar line, $\chi^2 = 9.240, d.f. = 1; p < 0.05$. While there were no differences between the FSL and the SD line, $\chi^2 = 0.833, d.f. = 1; p > 0.05$.
In the open field test, MANOVA revealed that the WKY juveniles demonstrated, overall, significantly higher levels of anxiety behaviors compared to their Wistar controls $F(6,17) = 2.74$; $p < 0.05$ (Fig. 4a and b). One way ANOVA revealed that WKY rats demonstrated higher levels of anxiety-related behaviors on four measures: rearing, $F(1,22) = 12.093$; $p < 0.05$; freezing, $F(1,22) = 4.759$; $p < 0.05$; line crossing, $F(1,22) = 5.952$; $p < 0.05$; latency to leave the start-point (sec, $F(1,22) = 4.744$; $p < 0.05$). In the other two behaviors there were no significant differences: time in the corners, $F(1,22) = 0.356$; $p > 0.05$; number of fecal boli deposited, $F(1,22) = 0.392$; $p > 0.05$. In contrast, MANOVA revealed that the FSL juveniles showed, overall, no significant differences in the levels of anxiety behaviors compared to their SD controls $F(6,19) = 2.581$; $p > 0.05$ (data are presented in Table 2). One-way ANOVA revealed that FSL rats demonstrated lower levels of anxiety in one measure, while in the other measures there were no differences: time in the corners, $F(1,24) = 4.453$; $p < 0.05$; rearing, $F(1,24) = 0.783$; $p > 0.05$; freezing, $F(1,24) = 1.856$; $p > 0.05$; number of fecal boli deposited, $F(1,24) = 1.152$; $p > 0.05$.  

![Fig. 2](image-url)  

(a) Mean night saccharin consumption in grams ($\pm$S.E.M.) of 35 day old WKY prepubertal rats and their controls and FSL prepubertal rats and their control. * $p < 0.05$. (b) Mean night water consumption in grams ($\pm$S.E.M.) of 35 day old WKY prepubertal rats and their controls and FSL prepubertal rats and their control. (c) Mean night calculated saccharin preference ($\pm$S.E.M.) of 35 day old WKY juvenile rats and their controls and FSL juvenile rats and their control. * $p < 0.05$.  

**Table 1**  

<table>
<thead>
<tr>
<th>Line</th>
<th>Time (at baseline) in the non-preferred chamber</th>
<th>Time (after conditioning in the previously non-preferred chamber)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>51.9 ($\pm$27.35)</td>
<td>485.2 ($\pm$138.52)</td>
</tr>
<tr>
<td>FSL</td>
<td>23.7 ($\pm$14.14)</td>
<td>461.8 ($\pm$186.19)</td>
</tr>
<tr>
<td>Wistar</td>
<td>39.8 ($\pm$14.83)</td>
<td>473.4 ($\pm$121.75)</td>
</tr>
<tr>
<td>WKY</td>
<td>0 ($\pm$0)</td>
<td>440.36 ($\pm$102.36)</td>
</tr>
</tbody>
</table>

![Fig. 3](image-url)  

(a) Frequencies of freezing behavior of 27–28 day old WKY and Wistar juvenile rats. ** $p < 0.01$. (b) Frequencies of freezing behavior of 27–28 day old FSL and SD juvenile rats.
Table 2
Mean time duration (s) and frequencies of anxiety behaviors: freezing, time in corners, latency to leave the start-point, rearing, number of fecal boli deposited, crossing (±S.E.M.), of 35 day old FSL juvenile rats and their controls

<table>
<thead>
<tr>
<th>Line</th>
<th>Rearing (number)</th>
<th>Freezing (duration in s)</th>
<th>Time (s) in the corners</th>
<th>Number of fecal boli deposited</th>
<th>Line crossing (number)</th>
<th>Latency to leave the start-point (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>15.92 (±3.52)</td>
<td>62.69 (±8.65)</td>
<td>257.92 (±7.71)</td>
<td>3.84 (±0.67)</td>
<td>41 (±5.06)</td>
<td>2.38 (±0.58)</td>
</tr>
<tr>
<td>FSL</td>
<td>19.84 (±4.35)</td>
<td>65.38 (±15.01)</td>
<td>231 (±10.80)</td>
<td>4.84 (±0.69)</td>
<td>32.15 (±4.94)</td>
<td>3.15 (±1.19)</td>
</tr>
</tbody>
</table>

Fig. 4. (a) Mean frequencies of anxiety behaviors: rearing, number of fecal boli deposited, crossing (±S.E.M.), of 35 day old WKY juvenile rats and their controls. *p < 0.05. (b) Mean time duration (s) of anxiety behaviors: freezing, time in corners, latency to leave the start-point (±S.E.M.), of 35 day old WKY juvenile rats and their controls. *p < 0.05.

5. Discussion

The main purpose of this study was to examine whether prepubertal rats of two different "genetic animal models" of depression in adults and prepubertals as well, have one of the most important criteria for major depressive disorder—anhedonia (DSM-4, American Psychiatric Association) and whether these prepubertal rats exhibit anxiety-related behaviors. We used two different behavioral tests in order to examine hedonia: the saccharin preference test and the CPP test, and two different behavioral tests in order to examine anxiety-related behaviors: open field and freezing behavior.

In the saccharin preference test, which measure the animals' natural preference for sweetened fluids [1], the results suggest that only the WKY prepubertal rats exhibited anhedonia compared to their controls, while the FSL prepubertal rats exhibited hedonic behavior at least to the same extent as their control line, and even more (in the day time). There are some factors that influence the saccharin preference test. One of these is saccharin concentration [1,9]. In this research, we used two different saccharin concentrations: 0.02 and 0.004%. The 0.02% saccharin concentration was chosen because it was the most preferred concentration for adult FSL rats; but the amount of saccharin solution consumed could be submaximal, i.e., less than the intake at the most palatable concentration [1]. We used the 0.004% saccharin solution since it was the preferred concentration for the Wistar rats, for the same reasons [9]. We found no differences between the different concentrations in our young rats of the different lines.

In the CPP paradigm, FSL as well as WKY prepubertal rats found the social interaction rewarding to the same extent that their control lines found it. In this test we isolated the prepubertal rats from the moment they were weaned until the test [8]. In this period, social play behavior mainly occurs [12], while our rats were isolated. As mentioned earlier, we know that social environment (housing conditions) of the subject prior to the treatment examined (social play) is an important factor that determines social behavior. Rearing in a poor social environment early in ontogeny (e.g., social isolation) dramatically changes social behavior [17]. Taking in consideration all of the above, it might be that the social reward provided in the present study, after a prolonged isolation period, was too strong to allow the observation of differences between the “depressed” lines and their controls. Following such a long period of isolation, a play partner might be highly
rewarding, in a way that the prepubertal rats (even if they are “depressed”) always increased the time they spent in their less preferred chamber, regardless of their preference before the procedure.

Freezing behavior is one of the main measures of anxiety [25–27]. Rats tend to explore a novel environment, and when they fail to do so, showing freezing behavior and no exploration, it is evidence for anxiety [28]. As mentioned before, the CPP paradigm has characteristics of a stress test (e.g., open field test), since the animal in this paradigm is being habituated to the apparatus and given free access of movement throughout this novel environment—the apparatus. This kind of habituation and free movement can be stressful for part of the animals, especially if they belong to a genetic animal model for childhood depression [5,48,7,29]. The results suggest that while habituated to the apparatus and given free access through it, WKY prepubertal rats showed more freezing behavior than their controls—Wistar rats, while the FSL juveniles showed less freezing behavior compared to their controls. WKY prepubertal rats showed more freezing behavior than their control line—SD rats. It seems that the WKY prepubertal rats are less active in a novel environment, less explorative, and more anxious. Hence, the WKY prepubertal rats show symptoms corresponding to anxious-depression.

In the open field paradigm, WKY, but not FSL juveniles, exhibited higher levels of anxiety behavior compared to their controls. WKY prepubertal rats showed more freezing behavior, exhibited a longer latency to leave the center of the arena (after being placed there at the beginning of the experiment), showed less incidents of rearing and entered less squares than their controls. There were no significant differences between the WKY prepubertal rats and their controls in the duration they spent in the corners of the arena, and the number of fecal boli deposited. This can be explained by the fact that on the average, the animals in the experiment spent most of their time in the corners (260 s out of 300) and very few fecal boli were deposited (3.5 in average), so it was very hard to find any differences in these measurements. But overall it seems that the WKY showed more anxiety behavior in the open field paradigm than their controls, while the FSL prepubertal rats did not show this pattern. There were no differences between the FSL juveniles and their controls in all of the behaviors measured in the open field, except the duration they spent in the corners of the arena, where the FSL juveniles exhibited less time in the corners compared to their controls, hence according to this single measure they exhibited less anxiety behavior in the open field paradigm, than their controls.

In sum, while the FSL and WKY lines are established models of depression in adult rats, our results, under the conditions mentioned above, suggest that the WKY line, but not the FSL, is a genetic animal model of depression in prepubertal rats, exhibiting one of the most important criteria for major depressive disorder—anhedonia, and showing symptoms of anxiety-related behaviors, observed in many depressed patients as well. These symptoms may reflect some abnormalities in one, or more, of the regions of the brain reward system such as the amygdala, the hippocampus, the Nucleus Accumbens and the ventral tegmental area [30,31,3].

As mentioned earlier, co-occurrence of psychiatric disorders (co-morbidity) is common [36], and in persons with depression or an anxiety disorder, co-morbidity with the other disorder is associated with increased severity [40–42]. Anxiety and depressive symptoms may exert a potentiating effect in co-morbid children and adolescents; depressive symptoms tend to be more severe in depressed youth with co-morbid anxiety compared to non-anxious depressed youth, as was the case in adults [43,44]. This may be the reason why “depressed” prepubertal rats that showed co-morbidity of depression and anxiety (WKY rats), exhibit a severe symptom of depression, anhedonia. In contrast, prepubertal rats that failed to show this kind of co-morbidity (FSL rats), did not exhibit this severe symptom of depression. Further support for this argument comes from the report that there are subgroups of childhood depression. While one subgroup exhibits anhedonia, the other subgroup fails to exhibit this symptom [47]. The anhedonic depressed subgroup is characterized by greater depression severity, alterations in stress cortisol reactivity, increased family history of major depressive disorder, and increased frequency of psychomotor retardation as well as other melancholic symptoms, such as a lack of brightening in response to joyful events [47].

As mentioned earlier, although several behavioral similarities exist between the FSL and the WKY models, they are fundamentally different. They are derived from different lines by a different breeding history, and their patterns of neurochemical abnormalities are separate. Furthermore, as prepubertals, their abnormal patterns of social play and HPA hormone levels are also different. We have found in our lab that prepubertal rats (about 35 days old) from the FSL and the WKY lines exhibit increased immobility in the swim test; abnormal social play as observed after 24-h of isolation, while the FSL juveniles demonstrated much higher levels of social play behaviors compared to their controls, and lower levels of social behavior that is not related to play, WKY juveniles demonstrated much lower levels of social play, and even lower levels of social behavior that is not related to play; and have different abnormality of the hypothalamic-pituitary-adrenal (HPA) axis, compared to their controls. WKY juveniles showed increased levels of CORT and ACTH, while FSL juveniles showed lower levels of CORT and ACTH compared to their controls [22]. Thus, taking in consideration the findings from our lab and the results in this research, it seems that we have two different subgroups of animal models for childhood depression modeling two different styles of depressive behavior, one which is co-morbidity with anxiety and shows severe symptoms of anxiety, and one which is not. While the WKY prepubertal rats showed anxiety behavior in the open field and behavior in the CPP paradigm and exhibited anhedonia in consuming saccharin over water, the FSL prepubertal rats (which have shown symptoms of depression in early experiments in our lab, e.g., increased immobility time in the swim test, com-
pared to their control [22] showed less severe symptoms of depression (failed to exhibit anhedonia) and did not show comorbidity of depression and anxiety. These two subgroups seen in our lab may model the clinical characteristics that resemble the two subgroups found in depressed children [47].

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References

R. psychiatric disorders in United States. Arch Gen Psychiatry 1994;51:8–19.


