Divergent maternal behavioral patterns in two genetic animal models of depression☆

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A B S T R A C T

Maternal behavior was examined in Flinders Sensitive-Line (FSL) and Wistar-Kyoto (WKY) rats, two different genetic animal models of depression. Behavioral patterns were assessed by undisturbed observations in the nest [Post-Partum Days (PPD) 4 and 9] and post-disturbance observations ("retrieval tests") on PPD 10. Litters were randomly allocated to a mild chronic-stress condition (limiting available bedding between PPD 2 and 9) or a standard rearing condition. The findings indicated that FSL dams did not differ from control dams in the undisturbed observations. However, in the post-disturbance observations FSL dams exhibited less pup-directed behaviors, a shorter latency to first pup carrying/retrieval and more self-directed behaviors than controls (the latter effect only in dams’ interaction with whole litter). In contrast, WKY dams performed more pup-directed activities (e.g., nursing and licking) and less self-directed activities in both the undisturbed and post-disturbance observations (in both dams’ interaction with single-pup and with the whole-litter) compared to controls. Accordingly, WKY dams exhibited a shorter latency for first pup-licking bout (in both post-disturbance observations). The early life mild chronic-stress used in the study ("limited-bedding") had a minor effect on the dams’ behavior. Overall, the study investigated for the first time the maternal behavior of WKY dams and suggests that these dams show an almost opposite behavioral pattern to that of FSL dams. The results are discussed with regard to earlier findings in the FSL strain and behavioral patterns documented in depressed human mothers.

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1. Introduction

Depression is one of the most prevalent mental disorders among women with 10–20% experiencing a period of depression in the first year after labor [12]. Depressed women exhibit behavioral deficits and their behavior is characterized as inconsistent, lacking sensitivity, warmth and appropriate responsiveness [3,4]. Possible results of these prolonged states are deficits in physiological and behavioral-emotional development of the offspring [5–8].

Animal models of human depression have been used in attempts to understand the neurobiological basis of this disorder and to predict successful treatment strategies [9–11]. Genetic animal models are a relatively new type of models exhibiting hereditary vulnerability, as found in depressed humans [12]. The current study utilized two genetic models of depression, the Flinders Sensitive-Line (FSL) and Wistar-Kyoto (WKY) rat strains, allowing convergent validation and insights into differences between the models. The FSL model was created by selectively breeding Sprague–Dawley (SD) rats for hypersensitivity to cholinergic agonists, as found in depressed humans [10]. The FSL strain was found to differ from controls in additional central neurochemical and in peripheral hormonal systems associated with depression [e.g., dopaminergic, serotonergic and hypothalamic-pituitary-adrenal (HPA) axis] and to have high face, predictive and construct validity [10,13]. The second animal model, the WKY strain, was inbred from the Wistar strain and used as normotensive controls to the Spontaneously Hypertensive Rat ( SHR) strain [14]. This strain was proposed to be a genetic animal model of depression exhibiting sensitivity to stress alongside characteristic depressive-like behaviors which do not originate in stress [11,15,16]. WKY rats exhibit different neurochemical profiles than controls in several neurochemical systems (e.g., dopaminergic and noradrenergic) as well as in peripheral hormones (e.g., HPA axis and TSH) [17–20]. Previous research in our laboratory indicated behavioral and physiological deficits of FSL pups. For example, young FSL rats exhibited lower body weight, greater immobility in the swim-test (tested around PND35), and differences from controls in anxiety-like behaviors (including neonatal ultrasonic vocalization and prepubertal behavior in the elevated plus-maze) and HPA axis hormones [17,21,22]. In order to
investigate factors associated with the development of the offspring’s emotion-regulation, we observed maternal behaviors of FSL dams [23,24]. These studies focused for the first time on maternal behavior in a genetic animal model of depression and revealed behavioral deficits resembling those of depressed human mothers. The current study is the extension of these earlier studies. The main addition is the inclusion of a second animal model, the WKY strain. Studies in our laboratory indicated that WKY pups show anxiety-like (e.g., increased freezing in tests boxes and remaining in closed arms of an elevated plus-maze) and depression-like (high levels of immobility in the swim test, relative anhedonia in a saccharin ingestion test and less social play and interaction than controls) [18,22,25]. In addition, pups from the WKY and FSL strains differ in their neuro-behavioral profiles and may model different sub-groups of depression. For example, FSL and WKY pups show opposite patterns of social play and HPA axis activity [18]. These differences led us to speculate that dams from the two strains may also differ in maternal behavior.

The present study also utilized an early mild chronic-stress paradigm. This allowed the investigation of maternal behavioral under standard and chronic-stress conditions. The paradigm allows the analysis of the interactive effects of depressive vulnerability and exposure to environmental stress, in accordance to diathesis–stress theories of depression [26]. In addition, the use of different stress-conditions is needed since depressed mothers are more likely to be exposed to stressful conditions than controls (e.g., abusive relationships) [27]. This paradigm is based on the finding that limiting the amount of available bedding material in the cage constitutes a persistent stressor for dam and pups [28]. By creating abnormal cage-bedding conditions [Post-Partum Days (PPD) 2–9] the litter was exposed to an ecologically valid stressor, simulating situations of chronic-stress that do not involve separation of dam and offspring [29]. Both undisturbed observations of the dams with their litters and post-disturbance (“retrieval” or “maternal responsivity”) observations were used in order to acquire comprehensive data on maternal behavior. These two methods often produce different, uncorrelated perspectives on maternal behavior [e.g., 23,24,30]. We hypothesized that the ‘depressed-like’ dams will exhibit a different behavioral profile than control dams. FSL dams were expected to exhibit reduced motivation to reach and care for pups and reduced maternal behaviors (such as licking and non-nutritive contact; [23,24]). Since WKY dams were not tested previously for maternal behavior, no specific hypotheses were made with regard to their maternal behavior. We hypothesized that the dam’s behavior will interact with the early chronic-stress (‘limited-bedding’) since the ‘depressed-like’ strains are considered stress-prone [10,31]. The maternal behavior of ‘depressed-like’ dams was expected to be more affected by the chronic-stress condition compared to the control condition (while control strains were expected to exhibit similar behavior in the two conditions).

2. Materials and methods

2.1. Subjects

Subjects were rat dams belonging to the Wistar, WKY, SD and FSL strains (108 litters, N = 13–14 per strain and growing-condition in the undisturbed observations; 99 litters, N = 12–13 litters per strain and growing-condition in the post-disturbance observations). Rats from all four strains were studied simultaneously. In all the analyses each ‘depressed-like’ strain was compared to its control strain (FSL compared to SD, WKY compared to Wistar). The animals were reared in our colony at the ‘Developmental Psychobiology Laboratory’ in Bar-Ilan University. Cages of pregnant dams (weight between 300 and 350 g; between 3 to 6 month old) were checked daily before noon and parturition day was noted as PPD 0. Dam and pups were maintained in individual solid-bottom polycarbonate cages (26 × 42 × 30 cm), in our colony with temperatures of 22±2 °C and a light–dark cycle of 14:10 (lights on between 5:00 and 19:00). The animals had food and water ad lib. On PPD 2/3 litter size was culled to six (with equal sex distribution when possible) and the litters were divided randomly to two groups, as described in the procedure section. All subjects were naïve and were not exposed to any test before and during the period of observation. 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.

2.2. Procedure

2.2.1. General

Two groups (for each strain) were created on PPD 2 [29]: (1) Mild chronic-stress group: litters and dams were transferred to standard cages equipped with a steel grid that served as the cage floor while allowing the passage of excrements [21 × 34 cm, width × length; hole size was 1 × 6 cm; the grid was situated 2.5 cm above the cage floor]. The only bedding material available were five paper towels (total: 112.5 × 22.5 cm) that the dam shreds and uses to make a nest [29]. The paper towels were replaced at PPD 4. (2) Control group: litters and dams were transferred to standard cages (bedding from wood shavings). Bedding was replaced at PPD 5. Overall, the procedure created eight groups consisting of two independent variables: strain (WKY pups compared to Wistar or FSL compared to SD) and growing-condition (chronic-stress compared to the control, standard housing-condition). At PPD 9 (after the undisturbed observations), all dams and litters were returned to standard growing-conditions.

2.2.2. Undisturbed maternal behavior observations

Observations were performed on PPD 4 and 9. In each day six spot check observations lasting 60 s were conducted (every 10 min for 1 h). This allowed for confirming observation of up to 9 cages within an hour. Observations took place in the afternoon (beginning at 13:00) since previous research showed that differences in maternal behavior (between FSL and SD dams) showed up at the afternoon while morning observations did not reveal significant differences [23]. Various maternal and non-maternal behaviors were recorded in each spot-check on an observation sheet (the score was “1” if the behavior occurred and “0” if it did not occur). Maternal behavior measures were based on previous literature [e.g., 31,32–35] and studies in our laboratory [23]: (1) Nursing posture: The dam is immobile while crouching over her pups, with at least half of the pups attached to the dam’s nipples (combining low and high nursing). Also coded if the dam performed passive nursing, lying on her side or back with pups attached to her nipple [‘supine nursing’; 36,37]; (2) Contact: The dam is hovering over the pups or in contact with at least half of them and engaging in active behaviors directed towards either pups or herself. (3) Licking: The dam performs repetitive tongue and head movements over the pup’s body or anogenital region. (4) Self-grooming: The dam is observed cleaning or scratching her body, fur and face using her tongue or legs. (5) Eating/drinking: The dam is either holding the food in her hands and clearly chewing it or drinking. (6) Resting: The dam is located in a distance from all pups, while lying inactive. (7) Rearing: the dam is standing on her two hind legs. (8) Exploration: the dam is moving from one side of the cage to the other, crossing the middle line. Additional measures (press-posture, digging in wood shavings, playing with tail, pup-carrying/retrieval [the dam picks up the pup and carries it to another location in the cage] and nest-building) were coded but not entered into analyses, because of a very low frequency of occurrence. Note also that several behaviors were not mutually exclusive. For example, a dam may have self-groomed while in contact with the pups (a score of 1 for each behavior).

The two measures of active behaviors that were not directed toward pups (self-grooming and eating/drinking) were combined to one measure, which we called “self-directed behaviors”. In addition, the two measures of motor activity (exploration and rearing) were combined. All other measures were analyzed as single measures [as in;
23]. Two independent observers of 10 lactating dams examined inter-rater reliability. Spearman correlation coefficients were significant and high for all measures \(r > 0.92\), except for \(r = 0.69\) for nest building.

2.2.3. Post-disturbance maternal behavior observations (dam interactions with a single pup and the whole litter)

At PPD 9 all litters and dams were returned to standard growing-conditions for one day until the start of the experiment. At PPD 10 between 7:00 and 9:00, the pups were taken out of the home cage, weighed and immediately placed as a group in a temporary cage (20 × 26 × 22 cm; a small amount of cage bedding was transferred to this cage to maintain original cage scent) in an incubator at 33 °C. At the same time the dam was transferred, in the home cage, to the next room. Next, the isolation–reunion procedure was conducted as follows: (a) First separation from dam: 5 min after initial disturbance of the home cage, a pup was taken out of the incubator and put in the ‘pup separation cage’ (transparent plastic beaker; 12.5 cm diameter, 13.5 cm high) for 5 min [used for ultrasonic vocalization measurement and presented in; 38]. (b) Reunion with dam: the dam was moved to the dams’ cage and the behavior of the dam was continuously video-recorded for 5 min as an expression of regulating strategies by the dam when confronted with the pup. The pup was then removed from the dam’s cage and was placed in the incubator in a separate beaker from the rest of the litter until stage d. (c) Immediately after completing stage b, a second pup underwent an identical procedure (as described in a–b, above). (d) Dam interactions with whole litter: the dam was re-united with the litter. The pups were scattered in the corners of the dam’s cage, and a 20-min interaction was continuously video-recorded. At the end of the experiment the dam and litter were returned to the colony. Only one litter at a time was tested and filmed in this procedure.

2.3. Data analysis

2.3.1. Weight

The mean pup weight for each litter was analyzed using an analysis of variance (ANOVA) with strain and growing-condition as between-subject measures. Undisturbed maternal observations: The six observation spot-check scores (comprising a single observation day) were combined. Thus, behaviors had one score in each observation day (PPD 4 and PPD 9), ranging between 0 and 6. Since repeated-measures ANOVAs revealed no significant differences between the two observation days (PPD 4 and 9), the behavioral indexes of the two days were summed into a single datum (range: 0–12) that presented normal distributions (with minor exceptions of resting and motor activity) [see also; 23]. Analyses were conducted using 2 × 2 ANOVAs with strain and growing-condition as between-subject measures.

2.3.2. Post-disturbance maternal behavior observations

Dam-pup interactions were analyzed, off-line, by research assistants “blind” to the dam’s strain, growing condition and to the study’s hypotheses, using the Observer 5.0, a software package for analysis of observational data (Noldus Information Technology b.v., Wageningen, The Netherlands). Behavioral measures (number and duration) were based on existing literature and previous studies in our laboratory [23,24,39,40]. A separate 2 × 2 ANOVA was conducted for each behavior with strain and growing-condition as between-subject measures. The analysis procedure was conducted twice, for dam interaction with single pup (average of the dam’s interaction with the first and second pups) and dam interaction with whole litter. Bouts (of licking or pup-carrying) were considered completed when the behavior ended, i.e., the dam raised her head and stopped licking, or she put the pup down, ending a pup-carrying episode. Individual measures are presented separately in the results section within the framework of two conceptual behavioral clusters; pup-directed behaviors (contact, licking bouts, and pup-carrying/retrieval) and self-directed behaviors (i.e., self-grooming). Latencies to first bout of licking and first pup carrying/retrieval were also analyzed.

3. Results

3.1. WKY strain compared to Wistar controls (see Table 3. for summary)

3.1.1. Weight

There was a significant strain main-effect for weight comparisons between WKY and Wistar pups; WKY pups weighed less (mean = 18.8, SD = 2.6) than Wistar controls (mean = 26.9 g, SD = 2.6) \(F(1,45) = 125.6, p < 0.001\).

Table 1

<table>
<thead>
<tr>
<th>Maternal behavior</th>
<th>Wistar strain [mean (SEM)]</th>
<th>WKY strain [mean (SEM)]</th>
<th>Strain main-effect (p)</th>
<th>Growing condition main-effect (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=14)</td>
<td>(N=13)</td>
<td>(N=12)</td>
<td>(N=26)</td>
</tr>
<tr>
<td>Nursing posture</td>
<td>6.9 (0.4)</td>
<td>7.8 (0.6)</td>
<td>8.3 (0.7)</td>
<td>8.3 (0.7)</td>
</tr>
<tr>
<td>Non-nutritive contact</td>
<td>9.1 (0.7)</td>
<td>9.7 (0.5)</td>
<td>9.3 (0.6)</td>
<td>9.3 (0.6)</td>
</tr>
<tr>
<td>Licking</td>
<td>1.3 (0.3)</td>
<td>1.3 (0.3)</td>
<td>0.7 (0.3)</td>
<td>0.7 (0.3)</td>
</tr>
<tr>
<td>Active self-directed</td>
<td>3.8 (0.5)</td>
<td>3.7 (0.6)</td>
<td>1.6 (0.4)</td>
<td>1.6 (0.4)</td>
</tr>
<tr>
<td>Resting</td>
<td>1.0 (0.3)</td>
<td>0.9 (0.4)</td>
<td>1.7 (0.6)</td>
<td>1.7 (0.6)</td>
</tr>
<tr>
<td>Motor activity</td>
<td>0.7 (0.3)</td>
<td>0.3 (0.2)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>SD strain [mean (SEM)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=14)</td>
<td>(N=13)</td>
<td>(N=27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursing posture</td>
<td>7.3 (0.5)</td>
<td>9.1 (0.5)</td>
<td>8.0 (0.4)</td>
<td>8.0 (0.4)</td>
</tr>
<tr>
<td>Non-nutritive contact</td>
<td>8.5 (0.7)</td>
<td>10.5 (0.1)</td>
<td>6.8 (0.8)</td>
<td>6.8 (0.8)</td>
</tr>
<tr>
<td>Licking*</td>
<td>1.7 (0.3)</td>
<td>1.2 (0.3)</td>
<td>0.8 (0.3)</td>
<td>0.8 (0.3)</td>
</tr>
<tr>
<td>Active self-directed</td>
<td>2.2 (0.5)</td>
<td>2.3 (0.4)</td>
<td>2.6 (0.5)</td>
<td>2.6 (0.5)</td>
</tr>
<tr>
<td>Resting</td>
<td>2.1 (0.4)</td>
<td>0.3 (0.2)</td>
<td>1.0 (0.5)</td>
<td>1.0 (0.5)</td>
</tr>
<tr>
<td>Motor activity</td>
<td>0.3 (0.2)</td>
<td>0.5 (0.2)</td>
<td>0.6 (0.2)</td>
<td>0.6 (0.2)</td>
</tr>
</tbody>
</table>

Notes:
Active self-directed behaviors = self-grooming + eating/drinking; Motor activity = rearing + exploration.
* = Significant strain × growing-condition interaction \((p < 0.05)\).
There were no significant growing-condition main-effects or strain × growing-condition interactions.

3.1.2. Undisturbed maternal observations (Table 1)

(a) Nursing posture: There was a significant strain main-effect with WKY dams exhibiting more nursing postures than control dams \([F(1,51)=6.2, \ p<.05]\). There was no significant growing-condition main-effect or strain × growing-condition interaction. (b) Contact: There was a significant growing-condition main-effect with dams in the ‘limited-bedding’ condition exhibiting more contact than dams in the standard growing condition \([F(1,51)=4.8, \ p<.05]\). There was no significant strain main-effect or strain × growing-condition interaction. (c) Licking: There were no significant strain and growing condition main-effects or interactions. (d) Active self-directed behaviors: There was a significant strain main-effect with WKY dams exhibiting less self-directed behaviors (self-grooming and eating/drinking) \([F(1,45)=10.8, \ p<.05]\). There was no significant growing-condition main-effect or strain × growing-condition interaction. (e) Resting: There was no significant growing-condition main-effect or strain × growing-condition interaction. (f) Motor-activity: There was no significant strain and growing condition main-effects or interactions (Table 1).

3.1.3. Post-disturbance maternal behavior observations; interaction with a single pup

(a) Pup-directed behaviors: There was a strain main-effect with WKY dams exhibiting more contact with the pup (number and duration), more licking (number of bouts and duration of licking) and less pup-carrying/retrieval (number and duration) \([F(1,45)=9.2, \ p<.01; \ F(1,45)=17.4, \ p<.001; \ F(1,45)=16.2, \ p<.001; \ F(1,45)=8.9, \ p<.01; \ F(1,45)=8.0, \ p<.01; \ F(1,45)=4.8, \ p<.05\]; respectively). In addition, WKY dams exhibited shorter mean latency for first pup-licking \([F(1,45)=21.5, \ p<.001]\). There were no significant main-effects for growing-condition or a strain × growing-condition interaction. (b) Self-directed behavior (self-grooming): There was a strain main-effect with WKY dams performing less self-groomings (number) \([F(1,45)=19.8, \ p<.001]\). There were no significant main-effects for growing-condition or a strain × growing-condition interaction. See Table 2 and Fig. 1A.

### Table 2

<table>
<thead>
<tr>
<th>Measure</th>
<th>Wistar strain (mean [SEM] of raw number)</th>
<th>WKY strain (mean [SEM] of raw number)</th>
<th>Strain main-effect (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam interaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with single-pup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact</td>
<td>0.4 (0.2)</td>
<td>0.6 (0.2)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>Licking</td>
<td>2.2 (0.5)</td>
<td>3.4 (0.7)</td>
<td>2.8 (0.5)</td>
</tr>
<tr>
<td>Carrying/Retrieval</td>
<td>5.0 (1.8)</td>
<td>4.2 (1.2)</td>
<td>4.6 (1.0)</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>2.7 (0.6)</td>
<td>4.3 (0.4)</td>
<td>3.5 (0.4)</td>
</tr>
<tr>
<td>Carrying/Retrieval</td>
<td>3.3 (0.5)</td>
<td>3.5 (0.4)</td>
<td>3.4 (0.3)</td>
</tr>
<tr>
<td>Licking (bouts)</td>
<td>10.0 (1.8)</td>
<td>17.4 (2.2)</td>
<td>13.8 (1.6)</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>13.9 (2.9)</td>
<td>9.6 (2.8)</td>
<td>11.6 (2.0)</td>
</tr>
<tr>
<td>Carrying/Retrieval</td>
<td>8.4 (1.6)</td>
<td>6.8 (1.1)</td>
<td>7.6 (1.0)</td>
</tr>
<tr>
<td>Dam interaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with whole-litter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact</td>
<td>0.5 (0.2)</td>
<td>0.8 (0.3)</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>Licking</td>
<td>8.0 (1.3)</td>
<td>7.3 (1.1)</td>
<td>7.7 (0.8)</td>
</tr>
<tr>
<td>Carrying/Retrieval</td>
<td>1.6 (0.6)</td>
<td>2.9 (1.1)</td>
<td>2.2 (0.6)</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>3.2 (0.4)</td>
<td>2.6 (0.4)</td>
<td>2.9 (0.3)</td>
</tr>
<tr>
<td>Carrying/Retrieval</td>
<td>4.5 (1.0)</td>
<td>5.2 (0.9)</td>
<td>4.8 (0.6)</td>
</tr>
<tr>
<td>Licking (bouts)</td>
<td>28.8 (4.8)</td>
<td>30.6 (2.8)</td>
<td>29.3 (2.8)</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>4.4 (1.7)</td>
<td>5.3 (2.0)</td>
<td>4.8 (1.3)</td>
</tr>
<tr>
<td>Carrying/Retrieval</td>
<td>6.6 (1.4)</td>
<td>7.2 (1.3)</td>
<td>6.9 (0.9)</td>
</tr>
</tbody>
</table>

All measures are raw numbers.

3.1.4. Post-disturbance maternal behavior observations; interaction with the whole litter

(a) Pup-directed behaviors: There was a strain main-effect with WKY dams licking their pups more times (number of bouts) and exhibiting less pup-carrying/retrieval (number and duration) than control dams \([F(1,45)=10.5, \ p<.01; \ F(1,45)=6.6, \ p<.05; \ F(1,45)=5.73, \ p<.05\]; respectively). WKY took less time than Wistar controls to exhibit the first pup licking episode \([F(1,44)=5.5, \ p<.05]\). There was also a strain × growing-condition interaction with WKY dams showing shorter duration of contact episodes and less licking (number of bouts and duration of licking) in the chronic-stress (‘limited-bedding’) condition (compared to the control growing condition), while Wistar dams (controls) showed the opposite pattern \([F(1,45)=5.6, \ p<.05; \ F(1,45)=6.0, \ p<.05; \ F(1,45)=9.8, \ p<.01\]; respectively). WKY dams also took more time to carry/retrieve the first pup in chronic-stress condition compared to the control growing condition, while Wistar dams (controls) showed the opposite pattern \([F(1,44)=8.2, \ p<.01]\). There was no significant growing-condition main-effect. (b) Self-directed behavior (self-grooming): There was a strain main-effect with WKY dams displaying less self-grooming (number and duration) \([F(1,45)=17.0, \ p<.001; \ F(1,45)=13.4, \ p<.001\]; respectively). There was no significant main-effect for growing-condition or strain × growing-condition interaction. See Table 2 and Fig. 1B.

3.2. FSL strain compared to the SD controls (see Table 3 for summary)

3.2.1. Weight

There was a significant strain main-effect for weight comparisons between FSL and SD pups; FSL pups weighed less (mean=26.0 g, SD=2.4) than SD control pups (mean=29.4, SD=4.2) \([F(1,45)=11.3, \ p<.01]\). There were no significant growing-condition main-effects or strain × growing-condition interactions (Table 3).

3.2.2. Undisturbed maternal observations (Table 1)

(a) Nursing posture: There was a significant growing-condition main-effect with dams in the ‘limited-bedding’ growing-condition exhibiting more nursing postures than dams in the control growing-condition \([F(1,48)=11.4, \ p<.01]\). There was no significant strain main-effect or strain × growing-condition interaction. (b) Contact: There was a
significant growing-condition main-effect with dams in the ‘limited-bedding’ condition exhibiting more contact than dams in the standard growing condition \([F(1,49)=17.4, p<.001]\). There was no significant strain main-effect or strain \(\times\) growing condition interaction. (c) Licking: There was no significant strain and growing condition main-effects. However, the strain \(\times\) growing condition interaction was significant \([F(1,49)=4.4, p<.05]\); FSL dams licked their pups more times in the ‘limited-bedding’ growing condition compared to the standard growing-condition, while SD dams exhibited an opposite behavioral profile. (d) Active self-directed behaviors: There was no significant strain and growing condition main-effects or interactions. (e) Resting: There was a significant growing-condition main-effect with dams in the ‘limited-bedding’ condition exhibiting less resting than dams in the standard growing condition \([F(1,49)=29.8, p<.001]\). There was no significant strain main-effect or strain \(\times\) growing condition interaction. (f) Motor-activity: There were no significant strain and growing condition main-effects or interactions.

3.2.3. Post-disturbance maternal behavior observations; interaction with a single pup

(a) Pup-directed behaviors: There was a strain main-effect with FSL dams exhibiting less contact with pups (duration), shorter licking duration and more pup-carrying/retrieval (number and duration) compared to controls \([F(1,45)=4.6, p<.05; F(1,45)=8.6, p<.01; F(1,45)=14.2, p<.001; F(1,45)=11.8, p<.01; \text{respectively}]\). In addition, FSL dams exhibited shorter mean latency for first pup carrying/retrieval \([F(1,45)=6.2, p<.05]\). There was no significant main-effect for growing-condition or strain \(\times\) growing-condition interaction. (b) Self-directed behavior: There was no significant strain main-effect, growing-
condition main-effect or a strain × growing-condition interaction. See Table 2 and Fig. 2A.

3.2.4. Post-disturbance maternal behavior observations; interaction with the whole litter

(a) Pup-directed behaviors: There was a strain main-effect with FSL dams performing less licking (duration) and more pup-carrying/retirement (number and duration), compared to controls [F(1,45) = 6.0, p < .05; F(1,45) = 14.2, p < .001; F(1,45) = 9.7, p < .01, respectively]. FSL dams also showed shorter mean latency for pup carrying/retirement, compared to control dams [F(1,45) = 4.5, p < 0.05]. There was no significant main-effect for growing-condition or strain × growing-condition interaction. (b) Self-directed behavior: There was a significant strain main-effect with FSL dams displaying longer self-grooming episodes and showing a non-significant trend toward a lower number of self-grooming episodes [F(1,45) = 6.3, p < .05; p = .06, respectively]. There was no significant main-effect for growing-condition or strain × condition interaction. See Table 2 and Fig. 2B.

4. Discussion

The current study examined maternal behavior in the FSL and WKY genetic animal models of depression [10,11]. Previous studies found evidence of depressive-like behavior and disturbances in maternal behavior of FSL dams, partially corresponding to those of depressed mothers [23,24]. Furthermore, recent findings suggest that contact with the pups is less rewarding to lactating FSL dams than to SD controls [41]. The present study extends this research, adding a second animal model and a mild chronic-stress paradigm (‘limited-bedding’). Together, a more comprehensive picture may be produced on the maternal behavior of the two animal models and its susceptibility to early mild chronic-stress.

The current study’s findings showed that FSL dams did not differ from control dams in the undisturbed observations. However, in the post-disturbance observations FSL dams exhibited less pup-directed behaviors, a longer latency to first pup-licking and more self-directed behaviors than controls (the latter two effects only in dams’ interaction with whole litter). In contrast, WKY dams performed more pup-directed activities (e.g., nursing and licking) and less self-directed activities in both the undisturbed and post-disturbance observations (in both dams’ interaction with single-pup and with the whole-litter) compared to controls. Accordingly, WKY dams exhibited shorter mean latency for first pup carrying/retirement (in dam-single-pup tests), as well as shorter mean latency for first pup-licking (in both post-disturbance observations). Interestingly, WKY dams took more time to carry/retrieve the first pup in the chronic-stress condition compared to the control growing condition, while Wistar dams showed the opposite pattern. However, all overall the early life mild chronic-stress used in the study (‘limited-bedding’) had a minor effect on the dams’ behavior. Thus this study suggests that WKY dams show an almost opposite behavioral pattern to that of FSL dams.

The findings indicated that in the post-disturbance observations FSL dams performed less pup-directed behaviors, a longer latency to first pup-licking and more self-directed behaviors compared to controls (the latter two only in the interaction with the whole litter). This behavioral profile of FSL dams fits previous studies in our laboratory, indicating reduced motivation to reach and care for pups and reduced tendency to exhibit maternal behaviors [such as licking and non-nutritive contact; 23, 24]. A recent study suggests that this behavioral profile may be linked to the fact that FSL dams are less rewarded by pups, compared to control dams [as assessed by a conditioned place preference paradigm and monoamine measurements in the nucleus accumbens while the dam interacts with the pups; 41]. In contrast to the post-disturbance observations, FSL dams did not differ from controls in the undisturbed observations. This would appear to contrast with an earlier study by our research team, that found FSL-SD differences in undisturbed observations [23]. The contrast may stem from methodological differences between the two studies. More specifically, while the present study focused on postnatal days 4 and 9, Lavi-Avnon et al. [23] utilized four consecutive observation days in each of the first and third post-partum weeks. This may have allowed better detection of differences between the strains. More research is thus needed to establish FSL dams’ behavior in undisturbed “postpartum care” conditions. It should also be noted that Champagne et al. [30] reported that post-disturbance observations (“maternal responsivity”) on postpartum day 1 in mice were not correlated with daily observations of undisturbed “postpartum care”. The authors quote other studies in hamsters and rats that have also demonstrated a lack of intercorrelation between postpartum care and retrieval test performance and they conclude that “it is likely that the latency measures reflect response to novelty and stress reactivity which are triggered by the disruptive nature of this task (p.332)”. Accordingly, the post-disturbance observations in the current study included separation of dam from her pups and human handling and thus may have contained a more significant stress component than the undisturbed observations. It is therefore possible that differences in the ‘depressed-like’ strains’ maternal behavior are better revealed in stress arousing conditions (and less under low-stress conditions). This may indicate a susceptibility of FSL dams to acute stress, strengthening the view of this strain as a model of a genetic pre-disposition to stress-induced depression (and in accordance with diathesis–stress theories of human depression). This suggestion fits earlier findings of more pronounced depressive-like behaviors of FSL rats after exposure to stress. For example, FSL dams expressed less nursing and more carrying of pups, especially after exposure to stress [24].

The current study, as far as we know, is the first investigation of maternal behavior in the WKY strain, with interesting findings. WKY dams exhibited a consistent behavioral profile that was almost opposite that of FSL dams; WKY dams’ performed more pup-directed activities (e.g., nursing and licking) and less self-directed activities, in both the undisturbed and post-disturbance observations (compared to controls). In interpreting these findings one needs to take into account the possibility that this behavioral profile may be
part of a general hypo-activity of WKY dams. Such hypo-activation may lead to a decrease in active self-directed activities (e.g., self-grooming) while increasing their tendency to stay in the vicinity of the pups. Earlier studies indeed found WKY rats to be hypo-active in several behavioral tests [42,43]. Two points argue against this option: First, lactating WKY dams did not differ in the activity measures used in the current study as part of the undisturbed observations. Second, WKY dams were actually more active in several behaviors directed toward their pups, suggesting that reduced activity may not be the single source for the behavioral profile (e.g., they licked their pups more than controls). Third and furthermore, WKY dams exhibited significantly shorter mean latency for first pup carrying/retrieval (in dam-single-pup tests), as well as shorter mean latency for first pup-licking (in both post-disturbance observations), compared to Wistar controls. This seems to contrast with a hypoactive profile. Moreover, even if hypo-activity is at the root of WKY dams’ behavioral profile, such findings do not contradict the findings in humans. Energy depletion and hypo-activity are common in depressed mothers, and in fact are considered symptomatic of depression [1].

Taken together, the two animal models show almost opposing maternal behavioral patterns. These behavioral patterns can be interpreted from the perspective of approaches attempting to
understand the effects of experience on “inborn” patterns of maternal behavior [44]. Maternal experience utilizes a form of perceptual learning, whereby the dam becomes familiar with pup-associated stimuli. This then subsequently reduces her natural “neophobic” tendency to withdraw from novel stimuli. When she encounters pups after previous experience, they no longer induce a fear-withdrawal state [44,45]. In fact, with maternal experience, pups acquire increased reinforcing properties, which further motivate the mother to approach them [44,45]. It is tempting to think of the behavior of our two animal models as representing different profiles of pup reward/withdrawal (see also our discussion above of the rewarding properties of FSL pups for their dams).

An additional question that may be raised concerns the possibility of linking the behavioral profiles of FSL and WKY dams to behavioral profiles exhibited by depressed human mothers. The behavioral profile of FSL dams is somewhat reminiscent of ‘withdrawn motherhood’, an abnormal maternal behavioral profile exhibited by many depressed human mothers [3,46]. This behavioral profile is characterized by a tendency of the mother to divert energy to self, in expense of the infant [7,47]. These mothers under-stimulate their infants and show less involvement in activities, games and imitations with the baby. However, our ability to characterize FSL dams’ behavior as withdrawn is limited by the lack of differences between the behavior of FSL dams’ and controls in the undisturbed observations. At the same time, and as discussed earlier, our team did find such a behavioral profile of the dams in the first and third postnatal weeks using undisturbed observations [23]. Taken together, there is a need for more research to establish FSL dams’ behavior in undisturbed conditions before their behavior profile may be characterized as comparable to the withdrawn profile observed in depressed human mothers. With regard to the second animal model, it is tempting to link WKY dams’ behavior to that of ‘intrinsic motherhood’, an additional maternal behavior profile displayed by depressed human mothers [7,46]. This profile is characterized by focusing attention on the baby in an intrusive interfering way, over-stimulating the baby and exhibiting anger and irritability [48–50]. However, this option is clearly speculative at this point and is presented mainly in order to broaden the horizon with regard to animal models of human psychopathology. The main problem with this regard is the difficulty in finding ethological behaviors in rats that correspond to several intrusive human maternal behaviors (e.g., poking the infant). A similar difficulty is present when trying to find human analogies to the aggressive behavior that some rat dams direct toward their offspring (e.g., infanticide). There is place to address this issue in the future by conducting species-specific ethological analyses of maternal behavior of rats, seeking corresponding behaviors to those exhibited by depressed mothers.

Previous studies showed that several depressive-like behaviors of FSL and WKY rats are more pronounced after exposure to stress, leading to a suggestion to view the strains as models of a genetic predisposition to depression susceptible to stress [10,13,16,51]. Such findings fit diathesis–stress theories, viewing human depression as the interactive result of hereditary sensitivity and exposure to stress [26,52]. The results of the current study indicate that the chronic-stress paradigm (‘limited-bedding’) had a restricted effect. The chronic-stress paradigm interacted with the dams’ strain on their pup-directed behavior: licking behavior of FSL dams and controls (undisturbed observations) and contact behavior of WKY dams and controls (post-disturbance observations with the whole litter). Beside these interactions, the chronic stress increased non-nutritive contact in both WKY dams and their controls. As for the FSL/SD strains; it was associated with more nursing postures, more non-nutritive contact and less resting (compared to the standard rearing condition). Note, however, that these latter behavioral changes in the undisturbed observations may simply be the direct effect of the metal-grid floor (part of the chronic-stress paradigm) increasing the time the dam spent near the pups and the improvised nest. Overall, it is difficult to draw a consistent picture as to the paradigm’s behavioral effects in the context of the current study. It seems therefore that ‘limited-bedding’, the chronic-stress paradigm used in the study, needs more research to explore and perhaps increase its effectiveness.

Future research should also aim to tackle several limitations of the current study (besides the restricted effects of the chronic-stress paradigm), including: (1) Differences between ‘depressed-like’ pups and control pups may, at least partially, affect the behavior of their dam. For example, the ability of adopting a nursing posture is known to be highly mutual and is affected by the pups’ weight [53]. This point is especially noteworthy in light of the findings that both FSL and WKY pups weigh less than control strains (current results and previous studies) and they show additional abnormalities (e.g., ultrasonic vocalizations, motor functioning; 17,18,22,25,54). The standard post-paradigm rat in continual contact with pups is highly responsive to cues from her pups. But whether or not a nursing episode occurs when the dam returns to the nest, as well as the frequency and duration of nursing at a given stage postpartum, depends on the motivation of the pups, the number of pups, and the age/age/weight of the litter mass [55,56]. If pups do not vocalize much, retrieval may be delayed. If pups are deficient in motor functioning, then their ability to root and suckle may be impaired, and with it, the dam’s nursing behavior. In this regard, it should be noted that the chronic-stress paradigm used in the present study was shown, in other studies, to affect several characteristics of pups, including hypothalamic-pituitary-adrenal (HPA) axis activity and lead to a modest loss of weight [28,57]. However, this is not a major issue in the context of the current study, since the paradigm had only a restricted effect on the dams’ behavior. (2) Weight differences between ‘depressed-like’ and control dams were found in earlier studies [e.g.,41] and led us to choose a restricted range of body weights for the dams in the present study (300–350 g). In hindsight this inclusion criteria raises the possibility that dams from the two strains may have differed in other relevant variables (e.g., age). In addition, weight differences may suggest the possibility of inbreeding depression. (3) The failure to find age-related declines in nursing behavior, between postpartum days 4 and 9, may be another imitation of the present study, suggesting that the undisturbed observation method used may not have been sufficiently sensitive. (4) We found strain differences in “pup-carrying/retrieval”. However, at least two possible interpretations can be offered for these differences. One option is that it represents mainly re-arranging or mouthing, which commonly means carrying the pup around in the nest once it is deposited in the nest. Another is that the dam repeatedly picked the pup up and carried it about the cage, putting it down occasionally, in the nest or elsewhere. The latter may indicate a disturbed maternal behavior pattern, which in this case could be attributed largely to the stressful testing situation. We do not have sufficient resolution to decide between these alternatives.

Overall, the current study indicates divergent maternal behaviors in the FSL and WKY strains. The WKY strain in particular presents exciting future research opportunities, in light of findings in the current study and the limited investigation of WKY dams’ maternal behavior in the past. Clearly more research is needed in order to elucidate there behavioral profiles and possibly link them to maternal behavioral profiles exhibited by depressed human mothers. For example, reversal of the maternal behavior phenotype by antidepressants would provide a degree of “predictive” validity. It should be noted, in this regard, that although the ability to infer from an animal-model to human phenomena depends upon the validity of the model, a model can be useful and provide important information even if found to be only partially valid [58,59]. Establishing animal models of depressive behaviors of human mothers is a worthy cause, allowing in the future a more comprehensive investigation of mechanisms underlying intra-generational transmission of depression [60].
References