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A cross-fostering study in a genetic animal model of depression: Maternal behavior and depression-like symptoms

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ABSTRACT

Connections between maternal behavior and childhood depression were examined by using a "genetic animal model"; Flinder Sensitive Line — (FSL) rats, and cross-fostering the offspring with the control strain, Sprague Dawley (SD) rats. The control procedure was "in-fostering", where the foster dam and her pups were from the same strain. Contribution of pups' characteristics/genotype to maternal behavior was examined. After weaning, we measured male offspring's body weight, immobility in the swim test, and basal corticosterone (CORT) and adrenocorticotropin (ACTH) levels at the prepubertal age of 35 days. While maternal behavior (of "depressive-like" dams and their controls) was not altered significantly by the pups' strain, the adoption procedure *per se* appeared to have more adverse effects on "depressive-like" symptoms of the SD prepubertal rats than on the FSL pups. Nevertheless, the combination between abnormal maternal behavior and genetic predisposition affected the hormonal stress responses of the offspring in a more severe manner than genetic predisposition or abnormal maternal behavior *per se*.

Epidemiologic studies show that roughly 40%–50% of the risk for depression is genetic (Sanders et al., 1999; Fava and Kendler, 2000). Yet, the search for specific genes has been frustrating, with no genetic abnormality yet identified with certainty. There are many reasons for this difficulty, including the fact that depression is a complex phenomenon with many genes possibly involved (Nestler et al., 2002). In addition, vulnerability to depression is only partly genetic, with nongenetic factors also being important. Factors such as stress, emotional trauma, viral infections, and even parental behavior have been implicated in the etiology of depression (Akiskal, 2000; Fava and Kendler, 2000; Smith et al., 2004). Therefore, in an attempt to isolate the factors "contributing" to depression, the use of a genetic animal model of depression in a cross-fostering paradigm is appropriate and particularly relevant for the study of the relationship between maternal behavior and depression.

In the present research, the "depressive-like" animals were from a well-validated genetic animal model of depression — the Flinders Sensitive Line (FSL) (Overstreet, 1993, 2005; Yadid et al., 2000). FSL rats were initially selectively bred from Sprague Dawley (SD) rats for hypersensitivity to cholinergic agonists. After this line was established, the selection process was discontinued. Adults from the FSL strain demonstrated depression-like symptoms in several behavioral paradigms in addition to abnormalities in some central neurochemical and peripheral hormonal systems (for review see Overstreet et al., 2005). Recently, we found that prepubertal rats (about 30–35 days old) from the FSL strain exhibited several depression-like symptoms, including abnormalities in central neurochemical and hormonal systems (Malkesman et al., 2006, 2007). Although these pre-weanling FSL pups have not been tested for "depressive-like" behavior, one-day old, newborn FSL pups presented significantly abnormal physiological and behavioral measurements compared to newborn SD pups (Shayit et al., 2003). We recently also reported that FSL dams exhibited abnormal patterns of maternal behavior compared to their SD controls (Lavi-Avnon et al., 2005a,b, 2008).

In the current cross-fostering study, in which "abnormal" FSL dams (Lavi-Avnon et al., 2005a,b, 2007) adopt SD pups, and SD dams adopt "abnormal" FSL pups (Malkesman et al., 2005, 2006, 2007) two effects might occur: an influence of the pups' strains on maternal behavior, and vice versa — an effect of the foster dam on pup behavioral phenotype (control in-fostered groups were also studied: FSL and SD dams fostering pups from the same strain). Therefore we examined changes in maternal behavior as well as changes in the pups' behavior as a result of the cross-fostering effect.

We hypothesized that the pups' strain will differentially alter the maternal behavior of the FSL dams than the behavior of SD dams, and that FSL dams will still exhibit abnormal maternal behavior regardless of the strain of the pups nursed by them, compared to SD dams.

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After the pups were weaned we measured “despair-like” behavior to examine prepubertal rats that had been reared by a foster dam from either the same strain or a different one. The rationale for choosing these measures, explained below, is based on some of the DSM-IV criteria for major depressive disorder and research on the psychobiology of depression. Since it is known that certain prenatal and postnatal manipulations have long term effects on the HPA axis (Maccari et al., 1995) we also measured basal levels of the HPA hormones: corticosterone (CORT) and adrenocorticotropin (ACTH).

Based on earlier studies (Malkesman et al., 2006), we hypothesized that prepubertal rats who had been reared with a “normal” maternal behavior pattern (SD dams) will exhibit less “despair” behavior, which is a well known symptom in depressed children (APA, 2000), compared to prepubertal rats who had been reared under abnormal maternal behavior patterns (FSL dams). Hence, we predicted that prepubertal rats that had been reared under FSL dams, will exhibit more immobility time during the forced swim test. [We note however, that theoretically the pups' differential behavior, if substantial, could influence the pattern of maternal behavior they receive, thus attenuating or otherwise modulating the hypothesized effects of the dam's strain-characteristic patterns of maternal behavior]. We also hypothesized that prepubertal rats raised under abnormal maternal behavior patterns (by FSL dams) will exhibit lower levels of HPA hormones, compared to prepubertal rats that had been reared under normal maternal behavior patterns (SD dams).

1. Methods

1.1. Animals

Nulliparous SD and FSL female rats were mated with males from the same strain in their breeding colonies, in the Developmental Psychobiology lab at Bar-Ilan University, Ramat-Gan, Israel [SD strain, the origin strain of whom FSL were bred from, has been chosen as a control as opposed to the Flinders Resistant Line (FRL), as this strain has been used by our lab previously (Lavi-Avnon et al., 2005a; Malkesman et al., 2006), and does not exhibit hypo-sensitivity to a cholinergic agonist]. Both strains were likely to be inbred because of the relatively small number of original parents. Pregnant rats were housed individually in a clean polypropylene cage (18.5 cm height × 26.5 cm width × 43 cm length), with a stainless steel wire lid and wood shavings as bedding material. Food and water were available *ad libitum*. The colony room was in a temperature controlled vivarium (20–24 °C), under 14-h–10-h light:dark cycle (lights on at 0500). The isolated females were checked daily for parturition. Newborn litters that were found until 12:00 h each day were designated as born on that day (day 0). Due to relatively small FSL litters, on postnatal day 1, litters were culled to 6 pups (Friedman et al., 2006; Lavi-Avnon et al., 2005a). Since it is well known that the sex of the pups affects the pattern of maternal behavior (Moore, 1985, 1986, 1992), sex distribution was kept as equal as possible in each litter. All 6 pups were cross-fostered on postnatal day 1: all the dams received foster pups — FSL dams received 6 SD pups, and SD dams received 6 FSL pups. In the in-fostered control group — FSL dams fostered 6 pups from the same strain (from a different dam), and SD dams fostered 6 SD pups. Hence, the fostering procedure was conducted on the entire litter (fostering dams did not raise their biological pups). All the fostering manipulations were performed at the same time during the day (11:00–12:00). After weaning on day 21, male rats from all the groups were housed in polypropylene cages (38 × 21 × 18 cm), three per cage from the same litter. The animals were studied before sexual maturity, at the age range of 30–35 days [at this age they are able to show immobility in the swim test (Abel, 1993), and their developing HPA system is responsive, as the “stress hyporesponsive period” is behind them (Levine, 2001)]. Different male sibling rats were used for each test (swim test and hormonal assays).

The cross-fostered pups were drawn from 20 litters (10 of each strain), while the in-fostered pups were drawn from 8 litters (4 of each strain). Due to some differences in sex distribution and the need for using at least one male from each litter for the swim test and one for the hormonal assays, group sizes varied from 4 to 14.

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

1.2. Procedure

1.2.1. Maternal behavior

1.2.1.1. Undisturbed observations of maternal behaviors. FSL and SD dams from different cross-fostering groups were observed with their litters in their home cages. All litters observed were completely naive, and were not exposed to any test before and during the period of observation.

Observations took place between 15:00 and 19:00, for 4 days/week during the first and third postpartum weeks and were consistent across all groups. During a 30-min session, each dam was observed in 4 short 60-s “spot checks” (with 9-min intervals between the observations). Various maternal and non-maternal behaviors were recorded in each “spot check”. The score was “1” if the behavior occurred and “0” if it did not occur. This procedure was based on time-interval undisturbed inspections used in previous studies (e.g., Brown et al., 1999; Myers et al., 1989a,b; Priestnall, 1973). Measures were based on previous literature (Rosenblatt, 1969; Caldji et al., 2000; Sharpe, 1975; Cramer et al., 1990) and on pre-tests that were performed in our laboratory. All measures were recorded as frequencies, and defined as follows:

Nursing posture – the dam is immobile/passive while crouching over her pups, with at least half of them under her ventrum. No differentiation was made between low, high and supine nursing.
Milk letdown – milk letdown was coded when dam, while nursing, arches her back and pups are clearly seen changing nipples.
Non-nutritive contact – the dam is hovering over the pups or in near contact with them, huddling with at least half of them and engaging in active behaviors directed towards either pups or herself.
Licking – the dam performs repetitive tongue and head movements over the pup's body or anogenital region.
Nest building – the dam is manipulating the wood shavings near the pups.
Self-grooming – the dam is observed cleaning or scratching her body fur and face using her tongue or legs.
Eating – the dam is holding the food in her hands and clearly chewing it.
Resting – the dam is located in a distance from all pups, while lying inactive.
Activity (rearing + vertical activity) – the dam is standing on her two hind legs; or the dam is moving from one side of the cage to the other, crossing the middle line.

Two independent observers of 10 lactating dams examined inter-rater reliability in the first and third postnatal weeks. Spearman correlation coefficients were significant and high for all measures in the third postnatal week ($r > 0.93$) and for all measures in the first postnatal week ($r > 0.92$, $r = 0.69$ for nest building).

1.2.1.2. Time-limited maternal behavior observations (retrieval test). In their first postpartum week, litters of the different groups were separated from their dam, weighed, and placed in a humid and warm incubator (33 °C). The dams were taken to the experimental room for adaptation. After 40 min, the same dam's pups were scattered in the four corners of their dam's cage, and a 30-min interaction was recorded on videotape and analyzed with the ‘Observer’ system (Noldus Information Technology B.V., Wageningen, The Netherlands). The interactions were analyzed for various maternal and non-maternal behaviors. The experimenter was blind to the rats' experimental group and strain when analyzing the data. Measures were based on previous literature (Pryce et al., 2001). Latencies were measured for first pup-carrying, grouping and adopting a nursing posture. The number of pup-carryings, vertical activities and rearings were also recorded. Nursing postures and all other dam behaviors were measured as durations (and as mentioned earlier). Definitions of additional behavior measurements:

“Carrying” – the dam picks up the pup and carries it to another location in the cage (before grouping) or to the location of the other littermates (after grouping).

“Grouping” – all littermates are relatively inactive, in contact with one another (or close enough to be covered by the dam's ventrum), and located in a distinct place where nursing posture or non-nutritive contact was observed.

In an attempt to focus on the source of fostering differences, should such differences exist, all measures (in both observations) were clustered within three categories, summing the total behaviors (duration or frequencies) observed in each category. The pooling of the data was conducted upon the conceptual hypothesis that some measures represent the same general behavior as other studies have done previously (Lavi-Avnon et al., 2005a): 1. *Motor activity measures* — number of vertical activities and rearings (both are activities of the dams without contact with the pups). 2. *Maternal behaviors directed toward the pups* — frequency (in the non-disturbed observations) or duration (in the retrieval test) of nutritive contact [nursing, milk letdown (only in the non-disturbed observations)], non-nutritive contact, licking and nest building (behaviors having a role in pup nursing). 3. *Non-maternal, self-directed behaviors* — frequency (in the non-disturb observations) or duration (in the retrieval test) of resting, self-grooming and eating (behaviors not known to have any direct role in pup nursing).

Observations were consistent across all groups.

1.2.2. Pups' behavior

1.2.2.1. Forced swim test. In a paradigm designed specially for prepubertal rats (Abel 1993), which has been validated in our lab in several studies using anti-depressant treatments (Malkesman et al., submitted for publication), one male rat from each litter (aged 34–35 days) was randomly selected, weighed, and then placed (after 24 h of isolation), for 5 min in a Plexiglas cylinder (height = 45.5 cm, diameter = 14.0 cm) filled to 24 cm with fresh tap water heated to 34 ±1 °C. No animal was tested more than once. Duration of floating behavior was measured. The criterion for floating was making only the minimal movements necessary to keep the head above water, with no forelimb movements. The water was changed between test animals. All tests were taken during the time of day when the animals are most active, at the beginning of the night cycle (1900–2130 h), in a dark room illuminated by a single 25-W red light bulb, place approximately 50 cm above the cylinder. Inter-rater reliability on the floating measure was 0.948, $p<0.001$ [N in the cross-fostered groups: FSL pups = 13, SD pups = 14; N in the in-fostered control groups: FSL pups = 4, SD pups = 7].

1.2.2.2. Hormonal assays. 34–35-day old male rats from different litters, none of which participated in the swim test, were decapitated after waiting in a separate room (n = 4–6 in each group). Trunk blood was collected into chilled tubes purposed for EDTA determination. After each animal, gloves were changed, and all the equipment was cleaned in order to prevent pre-decapitation stress. Samples were centrifuged for 10 min at 4 °C at 1000 g and plasma was stored at −80 °C until determination. On the day of assay, frozen plasma samples were thawed and plasma ACTH and CORT levels were measured using commercial RIA kits (rat CORT RIA kit – Diagnostic Products Corporation, Los Angeles, CA, USA – intra-assay: CV 4%–12.2%, inter-assay: CV 4.8%–14.9%, sensitivity: 5.7 ng/mL; ACTH RIA kit – Immuno Biological Laboratories, Hamburg, Germany – intra-assay: CV 2%–3.6%, inter-assay: CV 2.3%–3.4%, sensitivity: 3.0 pg/ml). N in the cross-fostered groups: FSL pups = 6, SD pups = 5; N in the in-fostered control groups: FSL pups = 4, SD pups = 4.

1.3. Data analysis

1.3.1. Maternal behavior

1.3.1.1. Undisturbed observations. For each behavior, the four observation scores were summed after each 30-min session, and a summed score was calculated for each week, as done in other studies (Lavi-Avnon et al., 2005b). Thus, behaviors had one score in each week (first week and third week postpartum, separately), presenting normal distributions (with minor exceptions of nest building in the third week postpartum). Statistics were performed on the final scores. Maternal behaviors directed toward the pups; non-maternal self-directed behavioral measures and the activity measure were analyzed using a 2 × 2 × 2 multivariate analyses of variance (MANOVA) with strain (FSL vs. SD) and group condition (in-fostering, cross-fostering) as between-subject measures and weeks as a repeated measure (week 1, week 3). The dependent measures for maternal behaviors directed toward the pups were: nursing posture, milk letdown, non-nutritive contact and licking. Dependent measures for self-directed behaviors were: self-grooming, eating and resting. To uncover the source of significant interaction effects, post-hoc tests were conducted.

1.3.1.2. Time-limited maternal behavior observations (retrieval test). Durations of maternal behaviors directed toward the pups and non-maternal, self-directed behaviors measures were analyzed using 2 × 2 MANOVAs with strain (FSL vs. SD) and group condition (in-fostering, cross-fostering) as the between-subject measures (Lavi-Avnon et al., 2005a). The dependent measures for maternal behaviors directed toward the pups were: nursing posture, licking, non-nutritive contact and pup-carrying. Dependent measures for self-directed behaviors were: self-grooming, eating and resting. To uncover the source of significant interaction effects, post-hoc tests were performed. One-way univariate ANOVAs were performed for the activity and pups' grouping measures.

1.4. Pups' behavior

Two-way univariate analyses of variance (ANOVA) were performed, separately, for the body weight of the pups, their CORT basal levels, ACTH basal levels and their floating/immobility duration, with strain (FSL vs. SD) and group condition (in-fostered, cross-fostered) as the independent variables. To uncover the source of significant interaction effects, Scheffe post-hoc tests were performed.

All results are expressed as mean ± SEM.

2. Results

2.1. Maternal behaviors

2.1.1. Undisturbed observations

2.1.1.1. Maternal behaviors directed towards pups. Repeated measures MANOVA revealed significant effects for week $F(4,19) = 4.94$, $p<0.01$, and strain $F(4,19) = 3.248$, $p<0.05$. The interaction (which is the major effect of interest in a cross-fostering study) was not significant. A significant difference between the weeks was found in non-nutritive behavior $F(1,22)=16.51$, $p<0.01$, showing that in the first week, dams exhibited more non-nutritive behavior compared to the third week. A significant difference between the strains was found in licking behavior $F(1,22)=6.196$, $p<0.05$, revealing, that overall, FSL dams exhibited less episodes (mean: 0.88±0.18) of licking behavior than SD dams (mean: 1.53±0.186; Table 1).

2.1.1.2. Non-maternal, self-directed behaviors. Repeated measures MANOVA on self-directed behaviors revealed significant effects only for week $F(3,21)=7.65$, $p<0.01$. Further examination of this effect revealed specific differences in each of the three measures included. Significant differences between the weeks were found in eating behavior, $F(1,23)=6.701$, $p<0.05$; resting behavior, $F(1,23)=7.3$, $p<0.05$; and self-grooming behavior, $F(1,23)=5.199$, $p<0.05$. Dams exhibited less frequent eating and resting behaviors in the first week compared to the third week. Self-grooming behavior was exhibited more frequently in the first week compared to the third postpartum week (Table 1).

2.1.1.3. Motor activity measures. Repeated measures ANOVA on the motor activity measures revealed no significant effects (Table 1).

2.1.2. Time-limited maternal behavior observations

2.1.2.1. Maternal behaviors directed towards pups. MANOVA revealed significant effects only for strain $F(4,21)=3.215$; $p<0.05$ (Table 2). A significant difference between the strains in the time spent carrying the pups was found [$F(1,24)=6.36$; $p<0.05$]: FSL dams (mean: 17.32±2.39) exhibited a longer duration of carrying their pups compared to SD dams (mean: 8.77±2.39).

Table 1
Indices of maternal behaviors (mean+SEM) in non-disturbed observations of cross-fostering and in-fostering FSL and SD dams in the first and third postpartum weeks (*N* in each group between 4 and 10)

Behavior	Week	Strain	In-fostered	Cross-fostered
Nursing	Week 1	SD	3.5±2.51	2.75±1.035
		FSL	4.25±2.5	4±2.05
	Week 3	SD	4±4.08	2.12±1.12
		FSL	3.25±2.21	2.7±1.56
Milk letdown	Week 1	SD	1.75±1.25	1.375±1.3
		FSL	1±0.81	1.6±0.84
	Week 3	SD	1.75±1.7	0.62±0.74
		FSL	1±0.81	0.6±0.69
Non-nutritive behavior	Week 1[a]	SD	2.75±0.95	2.12±1.24
	(2.38±0.34)	FSL	2.25±1.25	2.4±2.01
	Week 3	SD	0.5±0.57	0.875±0.99
	(0.61±0.17)	FSL	0.25±0.5	0.8±0.78
Licking	Week 1	SD	2±1.82	1.75±1.16
		FSL	0.75±0.5	1.5±1.26
	Week 3	SD	1.25±1.5	1.125±1.35
		FSL	0.5±1	0.8±0.78
Eating	Week 1[a]	SD	1.75±0.89	1.77±0.59
	(1.78±0.38)	FSL	1.5±0.89	2.1±0.56
	Week 3	SD	2.5±0.91	2.33±0.61
	(2.95±0.39)	FSL	3.75±0.91	3.2±0.58
Resting	Week 1[a]	SD	3.25±0.93	2±0.62
	(2.48±0.39)	FSL	3.25±0.93	1.4±0.58
	Week 3[a]	SD	3±0.97	5±0.65
	(3.97±0.41)	FSL	3.5±0.97	4.4±0.61
Self-grooming behavior	Week 1[a]	SD	3.25±1.2	5.44±0.801
	(4.27±0.51)	FSL	3.5±1.2	4.9±0.76
	Week 3[a]	SD	3.5±0.83	3.11±0.55
	(3.01±0.35)	FSL	2.25±0.83	3.2±0.52
Activity	Week 1	SD	3.5±1.43	2.44±0.95
		FSL	2±1.43	5.9±0.90
	Week 3	SD	4±1.49	4.44±0.99
		FSL	3.75±1.49	4.8±9.4

[a] $p<0.05$ week 1 vs. week 3.

Table 2
Indices of maternal behaviors (mean+SEM) in time-limited observations (retrieval test) of cross-fostering and in-fostering FSL and SD dams (*N* in each group between 4 and 10)

Behavior	Strain	In-fostered	Cross-fostered
Nursing	SD	537.95±166.002	488.77±104.98
	FSL	261.98±166.002	412.63±104.98
Non-nutritive behavior	SD	815.95±139.89	669.11±88.47
	FSL	733.510±139.89	847.55±88.47
Licking	SD	492.38±96.75	378.83±61.19
	FSL	255.68±96.75	427.93±61.19
Pup-carrying	SD	8.75±4.05	8.8±2.56
	FSL	16.25±4.05	18.4±2.56
Self-grooming	SD	74.175±53.804	152.22±34.03
	FSL	154.77±53.804	115.7±34.03
Eating	SD	0±0	0±0
	FSL	0±0	0±0
Resting	SD	5.047±0.99	0.430±0.62
	FSL	0±0.99	0.402±0.62
Activity	SD	45.5±9.19	44.2±5.81
	FSL	45±9.19	39.3±5.81

2.1.2.2. Non-maternal, self-directed behaviors. MANOVA revealed significant effects for strain $F(2,23)=4.91$; $p<0.05$ and for the group condition×strain interaction $F(2,23)=5.84$; $p<0.01$. The strain effect revealed a significant difference in resting behavior [$F(1,24)=9.36$; $p<0.01$]: FSL dams exhibited shorter duration of resting than SD dams. The interaction for resting $F(1,24)=9.131$; $p<0.01$, revealed that this SD–FSL difference was significant only in the in-fostering groups, while there were no significant differences between the SD dams and FSL dams in the cross-fostering groups (Table 2).

2.1.2.3. Motor activity measures. Two-way ANOVA revealed no significant differences between the FSL and SD dams and the two different group conditions (Table 2).

2.1.2.4. 'Pup-grouping' measure. Two-way ANOVA revealed no significant differences between the FSL and SD dams and the two different group conditions (Table 2).

2.2. Pups' behavior

2.2.1. Weight differences

Two-way ANOVA revealed significant effect only for strain $F(1,32)=6.547$; $p<0.05$ [fostering condition $F(1,32)=1.167$; NS, strain×fostering condition interaction $F(1,32)=0.044$; NS — Table 3] showing that FSL prepubertal rats weighed less than SD rats.

2.2.2. Forced swim test

Significant effect in immobility duration was found, by two-way ANOVA, for strain $F(1,34)=5.41$; $p<0.05$ (Fig. 1): SD prepubertal rats exhibited longer immobility duration compared to FSL prepubertal rats. No significant differences were found for fostering condition [$F(1,34)=0.33$; NS] nor for strain×fostering condition interaction [$F(1,34)=0.181$; NS], [due to the complexity of the cross-fostering paradigm (the need to match on postnatal day 1 two different litters), in some of the experiments we had small number of animals which might effect the power of these experiments. For the given effect size, SD, sample size and alpha

Table 3
Mean weight in grams (±SEM) of 34–35-day old in-fostered and cross-fostered SD and FSL rats (*N* in each group between 4 and 14)

Line	Weight
SD fostered by FSL dam	141.73±(4.61)
FSL fostered by SD dam	129.00±(2.94)
SD fostered by SD dam	136.5±(14.47)
FSL fostered by FSL dam	113.4±(6.94)

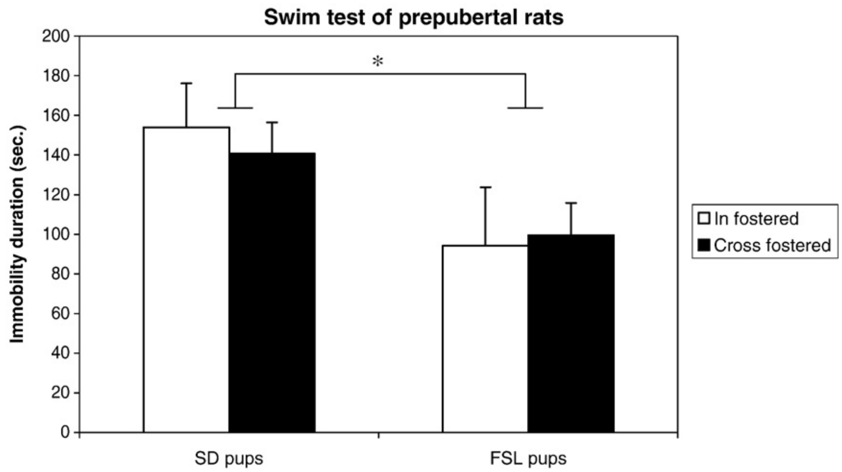

Fig. 1. Mean immobility duration in seconds (±SEM) of 30–35-day old in-fostered and cross-fostered SD and FSL rats (*N* in each group between 4 and 14). $*p<0.05$.

(0.05; 2tailed), power for stain = 1; power for foster <0.2 and power for the interaction <0.1].

2.2.3. HPA hormones

2.2.3.1. CORT. Two-way ANOVA, revealed significant effects for strain $F(1,15)=8.87$; $p<0.01$, for fostering condition $F(1,15)=9.85$; $p<0.01$, and for condition × strain interaction $F(1,15)=12.93$; $p<0.01$. FSL prepubertal rats in the in-fostered group exhibited higher CORT levels than of all the other groups (Fig. 2A).

2.2.3.2. ACTH. Two-way ANOVA showed no significant effects [strain $F(1,12)=0.12$; NS, fostered condition $F(1,12)=1.49$; NS, strain × fostered condition $F(1,12)=3.18$; NS (Fig. 2B)].

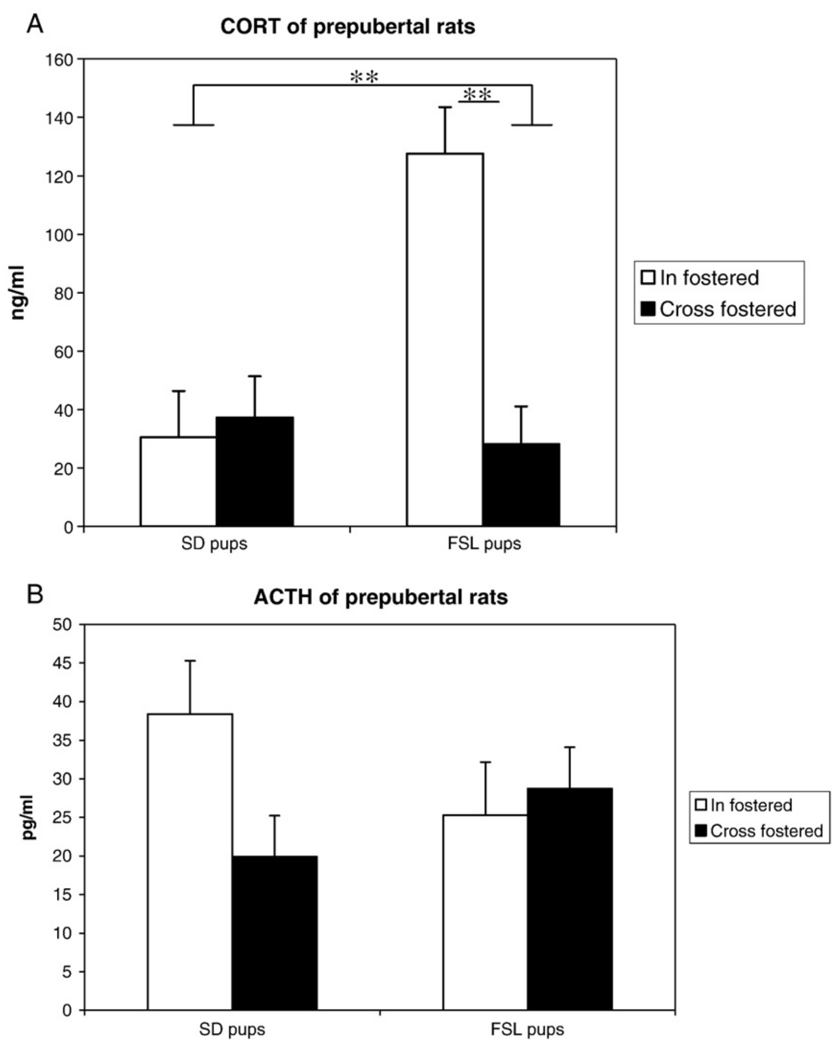

Fig. 2. A, Mean basal plasma levels of CORT (±SEM) of 30–35-day old in-fostered and cross-fostered SD and FSL rats (*N* in each group between 4 and 6). $**p<0.01$. B, Mean basal plasma levels of ACTH (±SEM) of 30–35-day in-fostered and cross-fostered SD and FSL rats (*N* in each group between 4 and 6).

3. Discussion

In an attempt to study the contribution of abnormal maternal behavior to "childhood depression" in an animal model, a cross-fostering paradigm was utilized. The influence of different patterns of maternal behavior on depression-like symptoms in "depressed" prepubertal rats (Malkesman et al., 2006) and their controls were studied. In addition, maternal behavior was analyzed, in order to explore the alteration of the pups' strain ("depressed-like", control) on maternal behavior.

We examined prepubertal rats of the "depressive-like" strain and their controls, raised by the different dams (cross-fostered; in-fostered) on three different measures: body weight, immobility in the swim test, and HPA hormones.

Depressed children often have weight disorders (APA, 2000). Earlier studies in our lab showed weight differences between FSL rats and their SD controls as early as day 1 (Shayit et al., 2003). These differences were found also at PND 35 (Malkesman et al., 2006) and adulthood (Overstreet et al., 2005). The different rearing conditions in the current study (different dams) had no significant effect on the body weight of the offspring. SD rats weighed significantly more than the FSL prepubertal rats in all the rearing conditions, as observed in earlier studies (Shayit et al., 2003; Malkesman et al., 2006; Overstreet et al., 2005). The lack of affect of maternal behavior on the weight of the prepubertal rats (in contrast to our hypothesis) indicates a genetic and/or prenatal factor.

Though there are several measures for depressive-like behavior in rats (i.e. Malkesman et al., 2005) and using only one measure is a limitation of the current study, the forced swim test developed by Porsolt et al. (1977) has become a widely used paradigm for studying stress responses and screening antidepressant drugs (Abel, 1993; but see Kawashima et al., 1986). Prolonged duration of immobility in this test is often regarded as behavioral despair, an animal analogue of human depression. Abel (1993) has demonstrated that the vertical immobility response in the forced swim test has a sudden onset, beginning at 21 days of age and quickly stabilizing at 26 days of age. Overstreet (1993) and Yadid et al. (2000) have shown longer immobility durations in adult FSL male rats than their controls, using the modified one session procedure. Similarly, we found that both lactating female and prepubertal male FSL rats demonstrated longer immobility duration than their SD controls (Lavi-Avnon et al., 2005b; Malkesman et al., 2006). We have also managed to show that the longer immobility duration in the forced swim test exhibited by the FSL compared to their SD controls is not due to the weight differences between these strains (Malkesman et al., 2006). In the current study, surprisingly, SD prepubertal rats exhibited *longer* immobility duration than FSL rats, regardless of their foster dam's strain. There were no significant differences between the FSL prepubertal rats raised by different dams, or between the SD prepubertal rats in the different fostered groups (in- and cross-fostered groups). From the results it seems that the fostering procedure *per se* had a negative effect on the depressive-like symptoms of the SD prepubertal rats, but not on the FSL rats. This negative effect, whether provided by dams from the same strain or from different strains, affected only the control prepubertal rats, while it had no effect on the "depressive-like" strain — they kept showing the same duration of immobility regardless of the maternal behavior they had experienced in childhood.

Glucocorticoids exert profound effects on general metabolism and also dramatically affect behavior via direct actions on numerous brain regions (McEwen, 2000a; Brown et al., 1999). The HPA axis can be altered by abnormal maternal behavior patterns, subsequently affecting the stress responses of the offspring (Weaver et al., 2004). In addition, there is considerable evidence demonstrating that early environment modifies the development of the HPA axis, together with subsequent brain functions and behavior (e.g., Francis et al., 2002; Levine, 1994; Plotsky and Meaney 1993; McEwen, 2000b; Weaver et al., 2004). ACTH (but not corticosterone) levels were found to be lower in adult male FSL compared to control Flinders Resistant Line rats (Owens et al., 1991), and previous studies in our lab found that prepubertal FSL rats have lower levels of corticosterone and ACTH compared to their SD controls (Malkesman et al., 2006).

Examining the hormones of the HPA axis by comparing FSL pups and their SD controls raised by different dams, revealed interesting patterns. Fostered FSL prepubertal rats exhibited higher levels of CORT compared to fostered SD rats. Close examination of the FSL CORT level results, showed that the in-fostered FSL prepubertal rats exhibited higher levels compared to cross-fostered FSL rats, and it seems that this group is the main reason for the significant differences between the strains in the current study. Nevertheless, the rearing conditions and the fostering paradigm had no significant influence on the ACTH levels of both the strains. Several studies showed changes only in CORT and not ACTH levels as a response to chronic stress — implying the involvement of other molecules on this axis (for review see Swaab et al., 2005).

The results of the current study imply that the combination between abnormal maternal behavior (FSL fostered dams) and genetic predisposition (FSL strain) affects the hormonal stress responses of the offspring in a more severe manner than genetic predisposition (FSL pups fostered by SD dams) or abnormal maternal behavior (SD pups fostered by FSL dams) *per se*.

In an attempt to explore alterations of the pups' strain ("depressed-like", control) on maternal behavior, we examined patterns of maternal behavior in undisturbed observations in the first and the third postpartum week (early to late postpartum) and time-limited maternal behavior observations (retrieval test) only in the first postpartum week (when the pups are less active and thus have relatively low contribution to the maternal interaction).

We observed developmental pattern in maternal behavior, with a change occurring between first and third postpartum weeks, corresponding to previous observations in the literature and in our lab (Cramer et al., 1990; Lavi-Avnon et al., 2005b). Dams from both of the strains tended to show more behaviors directed toward the pups (non-nutritive behavior) and less self-directed behaviors (eating, resting) in the first postpartum week, compared to the third postpartum week. The decrease in self-grooming behavior on the third postpartum week might be explained by the increase in all other self-directed behaviors — the dams in this period exhibited a wider variety of self-directed behaviors compared to the first postpartum week.

In previous studies in our lab, FSL dams exhibited maternal depression-like symptoms — lower frequency of behaviors directed toward the pups, compared to SD dams (Lavi-Avnon et al., 2005a,b). Similarly, in the current study FSL dams exhibited less episodes of licking behavior (maternal behavior directed toward the pups) beyond the strain of the foster pups they were raising. However both of the strains in the fostering procedure showed a tendency to extensive behavior directed toward the dam. This pattern of behavior is similar to avoidant motherhood — a behavioral profile characterized by a tendency to divert energy to one's self, at the expense of the infant (Field 1994; Field et al., 1990). These mothers are described as under-stimulating their infants, withdrawn, and spending the majority of the time looking away from the infant (Field 1994). They also show less involvement in activities, games, and imitations with the baby (Field et al., 1990).

Though Maccari (1995) found that cross-fostering paradigm does influence maternal behavior and alters adult behavior and stress physiology, in the major analysis of our cross-fostering study, there were no significant interactions in pup-directed maternal behavior between the dam's strain (FSL and SD) and the group conditions (in-fostering and cross-fostering). The same general pattern occurred in our retrieval tests, with one exception: FSL dams spent more time than

SD controls carrying pups around (possibly reflecting anxiety or a less efficient retrieval pattern). Our results resemble the pattern of another, recently published study (Friedman et al., 2006), in which FSL and FRL (Flinders Resistant Line) pups were cross-fostered onto multiparous FSL and FRL dams. The researchers assessed nest quality and pup retrieval on postnatal days 5 and 8. There were no strain differences in nest quality, or in the latency to begin retrieving pups. Overall, it took a longer time for the FSL dams to complete pup retrieval, and they dropped the pups more than FRL dams. This strain difference was only statistically significant on day 5, but not on day 8. Most importantly, as in the current study, no significant interactions were reported on maternal behavior between the dam's strain and the group condition (in-fostering vs. cross-fostering) (Friedman et al., 2006).

In the time-limited observations (retrieval test), there were less consistent patterns than in the non-disturbed observations. There were significant differences between FSL dams and SD dams raising fostered pups in resting (non-maternal) behavior. In addition, SD fostering dams also exhibited longer duration of resting compared to FSL fostering dams, but only in the in-fostering group. However, both of these behaviors were very rare, and it seems that it had no significant effect on the total behaviors observed (no significant differences were found between the strains on any other behaviors).

The differences between the retrieval test and the undisturbed observations may be related to a differential stress component in the two types of observations. The retrieval test includes separation of dam from pups and human handling, and may therefore contain a more significant stress component than the undisturbed observations. It seems that only in the non-disturbed observations the strain-difference in maternal behavior between fostering dams and non-fostering dams can be clearly noticed, while in the retrieval test, the additional stress may have affected the maternal behaviors of the FSL dams as well as the SD dams.

In sum, while maternal behavior (of “depressive-like” dams and their controls) was not altered significantly by the pups' strain, the adoption procedure *per se* had more adverse effects on the “depression-like” symptoms of the SD prepubertal rats than on the FSL pups. The pattern of the fostering dams behaviors (in-fostered and cross-fostered) showed similarities to avoidant motherhood. This “avoidant-like” maternal behavior apparently influenced “depression-like symptoms” of the FSL pups in a less severe manner than their controls. It is well known that different stressors influence different strains of rodents in different manner (Stöhr et al., 1998; Gomez-Serrano et al., 2001). There are even studies showing strain-dependent antidepressant-like effects (Crowley et al., 2005). Therefore, it is not surprising that the “avoidant-like” maternal behavior influenced only one strain (SD) in the forced swim test. “Avoidant-like” dams managed to contribute to a more “depressive-like profile” in the SD prepubertal rats. Nevertheless, the combination between abnormal maternal behavior (FSL fostered dams) and genetic predisposition (FSL strain) affected the hormonal stress responses of the offspring in a more severe manner than genetic predisposition (FSL pups fostered by SD dams) or abnormal maternal behavior (SD pups fostered by FSL dams) *per se*.

Overall, it seems that “avoidant-like” maternal behavior has the potential to contribute to the risk for childhood depression. In addition, if a genetic predisposition to depression is present, as seen in the FSL strain (Overstreet et al., 1993, 2002; Yadid et al., 2000), one may also be likely to find offspring with a childhood depression-like profile and excessive reactivity to stress.

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