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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