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Cross-Fostering Differentially Affects ADHD-Related Behaviors in Spontaneously Hypertensive Rats

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Abstract

Although both genetic and non-genetic factors are known to contribute to the occurrence of Attention-Deficit Hyperactivity/Disorder (ADHD), little is known about how they impact specific symptoms. We used a cross-fostering approach with an established animal model of ADHD, the Spontaneously Hypertensive Rat strain (SHR), to test the influence of genotype and maternal behavior on ADHD-related behaviors. SHRs and their normo-active genetic relative, Wistar Kyoto rats (WKY), were cross-fostered to an unfamiliar dam of either the same or different strain. Behavioral testing took place when the rats reached adulthood. Locomotor hyperactivity was completely dependent on the strain of the offspring. In contrast, social behavior was primarily determined by the strain of the mother, while attentional orienting behavior was influenced by both the strain of the offspring and the strain of the dam. Anxiety-related behavior was influenced by an interaction between offspring and dam strain.

Keywords

SHR; attention; social behavior; locomotor activity; Attention-Deficit/Hyperactivity Disorder

Attention-Deficit/Hyperactivity Disorder (ADHD) is one of the most common childhood psychological disorders, affecting ~5–11% of school-age children (American Psychiatric Association, 2013; Visser et al., 2014) and persisting into adulthood for the majority of those who are diagnosed (American Psychiatric Association, 2000; Mannuzza & Klein, 2000; Wender, Wolf, & Wasserstein, 2001). ADHD is characterized by core symptoms of inattention, hyperactivity, and impulsivity, which have a variety of cognitive, social, and behavioral consequences (Barkley, 1990, 1991; Barkley, DuPaul, & McMurray, 1990; Mannuzza & Klein, 2000).

Prior research has established that genetic factors contribute significantly to ADHD; indeed, the results of twin studies have revealed heritability estimates of 0.76 to 0.88 (Faraone et al., 2005; Langer, Grabe, Banaschewski, & Mikolajczyk, 2013; Larsson, Chang, D'Onofrio, & Lichtenstein, 2013). However, various non-genetic factors are also known to increase the risk of developing ADHD, including complications during pregnancy and birth, stressful childhood environments, smoking and alcohol use during pregnancy, and low birth weight

(Biederman & Faraone, 2005; O'Conner, Heron, Golding, Beveridge, & Glover, 2002). Moreover, heritability estimates are often influenced not only by genetic background, but also by gene-environment interactions (Purcell, 2002; Thapar, Cooper, Eyre, & Langley, 2013). Yet, despite these established contributions of both genetic and environmental factors to ADHD, little is currently known about how the *individual* cognitive and behavioral symptoms of ADHD are influenced by nature and/or nurture (Franke et al., 2012). Of particular interest is the influence of maternal behavior (e.g., the frequency and nature of interaction between mother and child) on ADHD-related behavior. Indeed, it has been shown that parents of children with ADHD are 2 to 8 times more likely to have ADHD themselves (Biederman & Faraone, 2005; Faraone, 2004); yet, it remains unclear if and how differences in maternal behavior influence the occurrence of specific ADHD symptoms in the offspring.

Animal models of ADHD may be particularly useful for addressing these issues. One such model is the Spontaneously Hypertensive Rat strain (SHR; Davids, Zhang, Tarazi, & Baldessarini, 2003; Sagvolden, 2000; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). SHRs exhibit the behavioral and cognitive impairments typically associated with the disorder, including hyperactivity, impulsivity, and inattention compared to control strains (Hopkins, Sharma, Evans, & Bucci, 2009; Kantak et al., 2008; Robinson, Hopkins, & Bucci, 2011; Robinson, Eggleston, & Bucci, 2012; Russell, 2007; Sagvolden et al., 2005; Thanos et al., 2010). SHRs also exhibit alterations in dopamine and norepinephrine neurotransmission that are reminiscent of the neurochemical dysfunction thought to underlie ADHD (Heal, Smith, Kulkarni, & Rowley, 2008; Russell, 2000; 2002; Solanto & Conners, 1982). A particularly important feature of the SHR model is that it was originally derived from the normo-active Wistar-Kyoto strain (WKY; Okamoto & Aoki, 1963). Thus, a cross-fostering approach can be used with SHR and WKY rats to determine how the behavioral characteristics that are unique to SHRs are influenced by biological factors such as strain, and non-genetic factors such as differences in maternal behavior. Indeed, earlier studies have revealed differences in maternal behavior in that SHR dams interact more with their offspring than WKY dams (Cierpial, Murphy, & McCarty, 1990). Moreover, when SHR and WKY pups were cross-fostered, mothers of both strains shifted their frequency of maternal behavior, defined by licking and nursing, towards the strain of their cross-fostered pups (Cierpial et al., 1990). Cross fostering SHR and WKY pups has been shown to affect both behavioral and physiological characteristics of the offspring (Cierpial et al., 1989).

The present study used a cross-fostering approach with SHR and WKY rats to determine how attention, social behavior, and locomotor activity are influenced by genetic factors versus being raised by an SHR or WKY mother. Attentional function was assessed by observing orienting responses to repeated presentations of a non-reinforced visual stimulus. Orienting is defined as rearing up on the hind legs towards the stimulus (Holland, 1977, 1984) and is an often-used measure of attentional processing (Gallagher, Graham, & Holland, 1990; Kaye & Pearce, 1984; Lang, Simons, & Balaban, 1997). In normal rats, rearing behavior rapidly decreases when the cue is not followed by reinforcement, reflecting an adaptive decrease in attention to a behaviorally-irrelevant stimulus (Gallagher et al., 1990; Holland, 1977; Kaye & Pearce, 1984). We have shown previously that SHRs exhibit

hyper-orienting behavior compared to normo-active control strains such as WKYs (Hopkins et al., 2009; Robinson et al., 2011, 2012), indicating that they are more prone to respond to distracting, irrelevant stimuli. Social interaction was assessed using a procedure adapted from File and colleagues (File, 1980; File & Seth, 2003) and used previously to demonstrate that SHRs exhibit hyper-social behavior. Indeed, compared to normo-active control rats, SHRs initiate more interactions with an unfamiliar rat (Hopkins et al. 2009; Robinson et al., 2012). Importantly, locomotor activity was assayed at the same time as social behavior, providing a means to differentiate genetic and non-genetic influences on different aspects of behavior within the same apparatus and testing session. Lastly, rats were tested in an elevated plus-maze to determine if differences in anxiety-related behavior could account for any of the observed differences in attention, social behavior, or locomotor activity.

Materials and Methods

Subjects

Eight-week old male and female Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SHR) rats were obtained from Harlan Laboratories (Indianapolis, IN, USA). Upon arriving in the vivarium, rats were allowed 7 days to acclimate before being allowed to mate (two females and one male per cage) over an 8-day period. A total of 13 WKY and 11 SHR females became pregnant and gave birth to the pups used in the study. The pregnant rats were checked for birth daily, and the day pups were first observed was designated postnatal day 0 (PND 0). The following day (PND 1), each litter was culled to 3 or 4 male rats, which were then removed and placed in a new cage with an unfamiliar dam of either the same or different strain (WKY or SHR). Littermates were placed with different dams to reduce the potential influence of any litter-specific differences (Lazic & Essioux, 2013). We used 3–4 rats per litter because that is the maximum number of males we obtained from SHR dams since SHR dams have a tendency to cannibalize pups. Thus, four different groups were obtained: WKY offspring that were raised by a foster WKY dam (WKY_o:WKY_d group), WKYs that were raised by a foster SHR dam (WKY_o:SHR_d group), SHRs raised by a foster WKY dam (SHR_o:WKY_d group), and SHRs that were raised by a foster SHR dam (SHR_o:SHR_d group). The pups were raised by their foster mothers until they were weaned on PND 25, after which they were group-housed according to sex (3 or 4 per cage) with their siblings. Only the male offspring were used in the behavioral studies, which began when they reached 106 days of age. The rats were handled for 2–3 minutes each day for three days before behavioral testing began.

The sample sizes of the offspring and dams in the different groups are reported in Tables 1 and 2. All rats had free access to food (Purina standard rat chow, Nestle Purina, St. Louis, MO, USA) and water and were maintained on a 14:10 hr light-dark cycle throughout the study. All procedures were conducted in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines and the Dartmouth College Institutional Animal Care and Use Committee.

Apparatus

Orienting behavior—Unconditioned orienting behavior was assessed in a standard conditioning chamber (24 cm × 30.5 cm × 29 cm; Med Associates, Inc.) connected to a computer and enclosed in a sound-attenuating cubicle (62 cm × 56 cm × 56 cm) equipped with an exhaust fan to provide airflow and background noise (~68dB). The chambers consisted of aluminum front and back walls with clear acrylic side walls and ceiling, and a grid floor. A food cup (not used in this study) was recessed in the center of one wall at a height of 5 cm. The stimulus light was a 2.8-W bulb located on the center of the chamber wall opposite the food cup, 1 cm from the ceiling. A red house light (2.8W) was located on the ceiling of the sound-attenuating cubicle to provide background lighting. Three pairs of photobeam sensors were mounted in the chamber and used to detect rearing behavior. The sensors were placed 15 cm above the grid floor and were evenly spaced along the wall so that a rearing response produced anywhere in the chamber would be detected by one of the sensors.

Social interaction—The social interaction procedure was conducted in a white plastic tub measuring 119.4 cm × 59.7 cm × 59.7 cm. In the center of the tub was a clear plexiglass cylinder (27.9 cm long × 7.6 cm diameter) containing an unfamiliar rat of the same strain and gender ('target rat'). There were five holes on each side of the cylinder (1.9 cm diameter), two holes on top (1.9 cm diameter), and one hole on each end (3.2 cm and 1.9 cm). A camera was mounted directly above the center of the tub and behavior was recorded on a DVD recorder.

Elevated plus maze—Testing was conducted in a small dimly-lit room using a black Plexiglas platform with two open and two closed arms extending out from the center (10 X 75 cm). The maze arms were 45 cm above the floor, and the walls of the closed arms were 39 cm tall. A video camera was mounted above the maze and behavior was recorded on a DVD recorder.

Behavioral Procedures

The offspring underwent the following behavioral procedures in the order in which they are presented below. One procedure took place each day with 24 hours between procedures.

Orienting behavior—During a single 32-min session, rats received 12 non-reinforced presentations of the stimulus light (10 sec in duration). During the 10-sec presentation period, the red house light was extinguished and the stimulus light flashed on/off at a frequency of 1Hz. The average inter-stimulus interval was 2.75 min.

Social interaction—The day following completion of the orienting procedure rats were exposed to the social interaction apparatus. At the beginning of the session, a target rat (unfamiliar conspecific – same strain and gender) was placed in the restrainer in the center of the tub. Each rat was then placed in the corner of the tub and was allowed to explore for 10 min. After the session all surfaces were cleaned with 70% ethanol followed by Quatricide disinfectant to remove any odors before the next rat was placed in the tub.

Elevated plus maze—The day after the social interaction test, rats were tested in the elevated plus maze to assess anxiety-like behavior. Rats were individually placed in the center of the maze at which point the investigator left the room and the rat was allowed to explore freely for 5 min. The amount of time spent in the open arms was recorded as the dependent measure. Increased open arm exploration is thought to reflect lower levels of anxiety-like behavior (Carobrez & Bertoglio, 2005). The maze was cleaned with ethanol and Quatricide between sessions as described above.

Behavioral Measures and Data Analyses

Orienting behavior—During the unconditioned orienting session, breaks in the photobeams mounted on the walls of the chamber were monitored by a computer and used to measure orienting behavior during non-reinforced presentations of the light. Orienting was defined as rearing on the hind legs with both forepaws off the ground (Holland, 1977). The number of breaks in the three photobeams used to detect rearing behavior was summed for each trial because previous studies indicate that it is unlikely that a rearing response will simultaneously break more than one photobeam (Keene & Bucci, 2007). The number of beam breaks was then summed across trials.

Social interaction—The primary measure of social interaction was the number of times the experimental rat approached and sniffed inside one of the holes in the cylinder that contained the target rat (scored by an observer who was blind to experimental condition). In addition, walking around the cylinder with continuous sniffing was counted as an interaction. Exploration of other parts of the cylinder (i.e., areas without holes) was not counted as an interaction. Interactions initiated by the target rat when it poked its noses out of the front hole were not scored unless the experimental rat reciprocated the interaction.

Locomotor activity—The recording of the social interaction session was also scored (by an observer blind to condition) to assess the levels of general locomotor activity in each group of rats. To measure locomotion, two lines were drawn perpendicular to the long side of the tub on the video screen, thus dividing the tub into three equal areas. A line crossing was counted when all four paws crossed over onto the other side of the line.

Elevated plus maze—To measure anxiety-like behavior, the amount of time spent in the open arms of the maze was recorded using a stopwatch by an observer who was blind to experimental condition. To count as an entry into an open arm, the rat needed to have all four paws in the open arm.

Statistics—For each of the four behavioral measures above, a two-way ANOVA was used to assess group differences using Offspring Strain (WKY_o, SHR_o) and Dam Strain (WKY_d, SHR_d) as between-subjects variables. Significant interactions were followed up with pairwise comparisons using corrected t-tests. All statistical analyses were conducted using an alpha level of 0.05.

Results

Orienting Behavior

The amount of orienting behavior displayed by each group of rats is shown in Figure 1. A two-way ANOVA revealed a significant main effect of Offspring Strain, $F(1,43)=4.8$, $p<0.03$, in that SHRs oriented to the visual stimulus more than WKYs regardless of whether they were raised by WKY or SHR dams. The Offspring Strain X Dam Strain interaction was also statistically significant, $F(1,43)=5.4$, $p<0.03$, and post-hoc comparisons indicated that the WKY_o:WKY_d group reared less than each of the other three groups (SHR_o:WKY_d, $p<0.004$; WKY_o:SHR_d, $p<0.02$; SHR_o:SHR_d, $p<0.05$), which was responsible for the significant interaction. No other pairwise comparisons were significant ($ps>0.3$). There was no main effect of Dam Strain ($p>0.8$).

Social Behavior

Figure 2A illustrates the average number of social interactions made by rats in each group. A two-way ANOVA revealed a main effect of Dam Strain, $F(1,43)=8.1$, $p<0.007$, indicating that rats raised by SHR dams made more social contacts with an unfamiliar rat compared to rats raised by WKY dams, regardless of offspring strain. The main effect of Offspring Strain and the Offspring Strain X Dam Strain interaction were not statistically significant ($ps > 0.7$).

Locomotor Activity

Locomotor activity exhibited during the social interaction session is shown in Figure 2B. SHR rats, regardless of whether they were raised by SHR or WKY dams, crossed more lines during the session than WKY rats, as evidenced by a significant main effect of Offspring Strain, $F(1,43)=41.7$, $p<0.0001$. There was no significant main effect of Dam Strain ($p>0.6$) and no significant Offspring Strain X Dam Strain interaction ($p>0.7$).

Anxiety-like Behavior

Data collected during the exploration of the elevated plus maze are shown in Figure 3. Although the main effects of Offspring Strain and Dam Strain were not statistically significant ($ps>0.2$), there was a significant Offspring Strain X Dam Strain interaction, $F(1,43)=7.7$, $p<0.009$. Subsequent pairwise comparisons revealed that the WKY_o:WKY_d group spent less time in the open arms of the plus maze compared to the WKY_o:SHR_d group ($p<0.03$) and to the SHR_o:WKY_d group ($p<0.001$). No other pairwise comparisons were statistically significant ($ps>0.03$).

Discussion

The present study used an animal model of ADHD, the SHR strain, to compare the effects of biological (strain) and environmental factors on the occurrence of ADHD-related behaviors. In this case, the environmental variable of interest was being raised by either a normo-active mother or a mother that exhibits behavioral and neurobiological phenotypes that are associated with ADHD. To that end, SHRs and their closest genetic normo-active relatives (WKY rats) were cross-fostered shortly after birth to either a foster SHR mother or a foster

WKY mother (i.e., a 2 X 2 design). Thus, the design served as an approximation of the differential effects of being raised by a mother with ADHD versus a mother without ADHD on the behavior of the offspring. As adults, the offspring were tested on a battery of procedures that measured ADHD-related behaviors.

As summarized in Table 3, a different pattern of results was obtained for each of the four behaviors that were measured. First, we found that hyper-social behavior that is typically exhibited by SHR rats (Hopkins et al., 2009; Robinson et al., 2012) was influenced exclusively by the strain of the mother. Specifically, SHR and WKY rats that were raised by SHR dams interacted more with an unfamiliar rat than SHR or WKY rats raised by WKY dams. This suggests that reported differences in maternal behavior exhibited by SHR dams (Cierpial et al., 1990) may have a significant impact on the social behavior of the offspring, consistent with reports that social behavior in children with ADHD can be affected by maternal warmth or criticism (Richards et al., 2014). Although hyper-social behavior is not considered a core deficit in ADHD, it is often associated with the disorder and dramatically affects interpersonal relationships and communication, resulting in negative peer relationships and social ostracism (Pelham, Fabiano, & Massetti, 2005; Whalen & Henker, 1992). Indeed, although the specific constellation of symptoms arising from ADHD may vary considerably among individuals, the social problems resulting from the disorder are less varied and often have the greatest impact on those who suffer from ADHD (Pelham et al., 2005). Importantly, these deficits in social behavior are also notoriously resistant to interventions (Swanson et al., 2001), highlighting the need to understand their basis. The present findings suggest that interventions that focus on maternal behavior may be useful in ameliorating impairments in social behavior. However, additional research is needed on the nature of social dysfunction in ADHD, which is likely a complex phenomenon with characteristics that are not fully realized in the rat model used here.

Interestingly, despite being evaluated in the same apparatus and at the same time as social behavior, we found that locomotor behavior was impacted differently than social behavior. Specifically, locomotor activity was completely dependent on the strain of the offspring in that SHRs were hyperactive compared to WKYs, regardless of the strain of the mother that raised them. This is consistent with a multitude of studies that have demonstrated hyperactivity in SHRs (see Sagvolden et al., 2005 for review) and suggests that the hyperactive phenotype associated with ADHD, in contrast to hyper-social behavior, may be best treated by focusing interventions on the individual diagnosed with the disorder. Indeed, the hyperactive phenotype has also been linked primarily to genetic causes in persons with ADHD (Nicolas & Burt, 2010).

The effects of genotype and cross-fostering were more complex with regard to the amount of orienting behavior (rearing) elicited by repeated presentations of an uninformative visual stimulus. In normal rats, rearing behavior rapidly decreases when the cue is not followed by reinforcement, reflecting an adaptive decrease in attention to a behaviorally-irrelevant stimulus (Gallagher et al., 1990; Holland, 1977; Kaye & Pearce, 1984). As shown previously, SHRs typically orient to the stimulus more than normo-active control strains (Hopkins et al., 2009; Robinson & Bucci, in press; Robinson et al., 2011, 2012), indicating that they are more prone to respond to distracting, irrelevant stimuli. Here we found that

orienting behavior was dependent on the strain of the offspring in that SHRs exhibited hyper-orienting behavior regardless of whether they were raised by an SHR or WKY mother. This is consistent with the results of a study in humans that found that attentional impairments were highly heritable (heritability estimate of 0.73; Nicolas & Burt, 2010). In contrast, we found no main effect of the strain of the mother on orienting behavior. However, there was an interaction between the strain of the mother and the strain of the offspring, indicating that cross-fostering to a mother of the opposite strain also contributed to higher levels of orienting behavior. This is particularly interesting given evidence that adopted children have a higher rate of ADHD compared to the general population (Keyes, Sharma, Elkins, Iacono, & McGue, 2008).

Finally, we found that anxiety-like behavior, as measured in the elevated plus maze, exhibited a profile that was different still from any of the other behaviors. There were no main effects of either the strain of the offspring or the strain of the mother, but there was a significant interaction in that rats raised by a mother of a different strain exhibited less anxiety-related behavior than rats raised by a mother of the same strain. This is in agreement with substantial research indicating that mild stressors during development can lead to anxiety-resilience later in adulthood (Levine & Mody, 2003; Macri, Zoratto, & Laviola, 2011).

Together, the present findings indicate that specific ADHD-related behaviors in SHRs are differentially influenced by strain and experience, and expand upon earlier cross-fostering studies that focused only on locomotor behavior. Indeed, to date, only a few other studies have investigated the effects of cross-fostering on the behavior of SHR rats. For example, consistent with the present findings, the strain of the mother was found to have no effect on locomotor hyperactivity of the offspring, with SHRs exhibiting more open field activity than WKY rats regardless of foster mother strain (Howells, Bindewald, and Russell, 2009; Cierpial et al., 1989). Importantly, the current study extends this work by showing that other ADHD-related behaviors, including distractibility and hyper-social behavior, are modulated differently by strain and by environmental variables. Thus, it will be valuable in future studies to evaluate the effects of cross fostering on additional measures, such as impulsivity and working memory, to gain a complete understanding of how each of the behavioral symptoms of ADHD are influenced by biological versus environmental factors.

The approach used in the current study was also designed to address some potential shortcomings of prior work. For example, in the studies by Howells et al. (2009) and Cierpial et al. (1989), the controls for cross-fostering SHRs to WKY dams (and vice versa) were groups that remained with their birthmothers. Thus, the observed effects of cross-fostering could have been simply due to being raised by a foster mother, regardless of whether she was an SHR or WKY. Here, we controlled for this by fostering all of the pups in the study, including the control groups. In other words, our controls consisted of WKYs that were fostered to another WKY mother, and SHRs that were fostered to another SHR mother. Thus, the effects we observed on social behavior, locomotor activity, orienting, and anxiety-like behavior are not likely attributable simply to being raised by a non-birth mother. Indeed, this difference may explain why Howells et al. (2009) found different effects in the elevated plus maze. In particular, they found that SHRs spent more time in the

open arms of the plus maze compared to WKYs, regardless of the strain of the mother that raised them. Instead, we found an interaction between offspring strain and dam strain indicating that rats raised by a mother of a different strain exhibited less anxiety-related behavior than rats raised by a mother of the same strain.

Another difference between our study and prior cross-fostering experiments with SHR is that we purposefully chose to focus on the behavior of the offspring when they were *adults*. Indeed, despite growing evidence that a substantial number of children diagnosed with ADHD continue to meet the criterion for the diagnosis in adulthood (Kessler et al., 2005; Mao, Babcock, & Brams, 2011), adults with ADHD have received far less research attention compared to children or adolescents. Similarly, few studies of SHR or other animal models of ADHD have considered the behavior of adults. Such animal studies may be particularly valuable in elucidating the biological factors that determine whether ADHD will continue into adulthood, especially since few risk factors have yet to be identified (Franke et al., 2012; Kessler et al., 2005). For instance, recent studies in laboratory animals have established that early life experiences, including alterations in maternal behavior, can result in epigenetic changes that influence the behavior of the offspring much later in life (Weaver et al., 2004). This epigenetic sensitivity to experiences in early life has now been documented in humans as well as rodents (Suderman et al., 2012). Thus, future experiments might build on the results of the present study to examine how epigenetic changes (as well as other mechanisms) may underlie the effects of early life experience on the manifestation of ADHD-related symptoms in adulthood. For example, recent studies have shown that the level of maternal care can regulate the development and function of the dopaminergic pathways that are thought to be dysfunctional in ADHD (Peña, Neugut, Calarco, & Champagne, 2014) and that epigenetic changes resulting from altered maternal behavior can be reversed via cross-fostering (Weaver et al., 2004).

An additional avenue for future research involves systematically characterizing the differences in early life experience that are associated with alterations in maternal behavior. In cross-fostering SHR and WKY rats, Cierpial and colleagues (1990) characterized critical factors such as differences in arched-back nursing, contact with the litter, nest building, licking and sniffing pups, and pup carrying and retrieval. However, other aspects of maternal behavior await future research, and it will also be important to standardize the times of day and frequency with which maternal behavior is observed. In addition, Cierpial et al. (1990) did not include control groups in which the offspring were raised by a non-birth mother of the same strain. Furthermore, prior studies indicate that other factors could influence the behavioral results obtained here and in prior studies (Cierpial et al., 1989, Howells et al., 2009). For instance, there is evidence that milk uptake differs in SHR and WKY rats, with SHR dams producing less milk (Gouldsborough, Black, Johnson, & Ashton, 1998). Additionally, maternal separation has been shown to differentially affect norepinephrine release (Sterley et al., 2013) and dopamine transporter function in SHRs and WKYs (Womersley, Hsieh, Kellaway, Gerhardt, & Russell, 2011), which may be particularly significant since alterations in dopamine and norepinephrine levels are present in SHRs and are associated with ADHD (Heal et al., 2008; Russell, 2000; 2002; Solanto & Conners, 1982). These issues are especially important to resolve since a substantial number

of studies have revealed corresponding differences in humans. For example, children with ADHD are known to be breastfed less than non-ADHD children (Mimouni-Bloch et al., 2013; Sabuncuoglu, Orengul, Bikmazer, & Yilmaz Kaynar, 2014). In addition, associations between maternal warmth and ADHD have been reported (Richards et al., 2014; Tully, Arseneault, Caspi, Moffitt, & Morgan, 2004) as well as range of alterations in parental behavior in those rearing a child with ADHD (Modesto-Lowe, Danforth, & Brooks, 2008; Murray & Johnston, 2006; Weinstein, Apfel, & Weinstein, 1997). At the same time, other studies have argued that parental behavior does not influence the occurrence of ADHD-related behavior in children (Barkley & Cunningham, 1997), underscoring the need for additional research in this area.

As with other developmental and cross-fostering studies, there is a risk that litter-specific differences may influence the findings (Lazic & Essioux, 2013). In this study, littermates were placed with different dams to limit the potential of obtaining spurious results due to litter differences. In addition, we conducted two supplemental statistical analyses. First, we averaged the data from littermates so that the group sizes were equal to the number of litters in each of the conditions. For each of the four behaviors that were examined, the resulting group means produced the same pattern of findings obtained with the individual subject data presented in the Results and illustrated in Figures 1–3. Indeed, with these reduced sample sizes, the group difference in orienting behavior and locomotor activity were still statistically significant ($p < 0.05$) and the differences in social behavior and anxiety-like behavior exhibited a trend towards significance ($p < 0.09$). In the second supplemental analysis we limited the number of subjects in each condition to 7, which was the lowest sample size obtained (in SHR:SHR group). Again, the same pattern of results emerged for each of the behavioral measures. The findings for locomotor activity and anxiety-like behavior were still statistically significant ($p < 0.05$) and the differences in orienting behavior and social behavior exhibited strong trends ($p < 0.08$). Thus, it is unlikely that treating each of the offspring as an individual subject or having a relatively small number of litters in some of the conditions produced inaccurate findings.

Certainly there are limitations in translating the findings of cross-fostering studies with SHR rats to understanding the basis for ADHD in humans. For one, the SHR model cannot completely reflect all of the symptoms and underlying factors that contribute to this disorder. At the same time, animal models allow us to carry out experimental manipulations that would be unethical in humans (i.e., cross-fostering), that may yield findings that could be used to guide research with humans. For instance, the present findings suggest that studying social behavior in individuals with ADHD who were raised by non-ADHD parents versus parents diagnosed with ADHD may provide insight into new interventions. Conversely, children with siblings who have ADHD often report feelings of jealousy over the lack of attention they receive, a finding that could inform studies in rats in which SHR and WKY pups are raised together by an SHR or WKY dam to determine if there are differences in maternal behavior, and if so, what the underlying causes may be. Thus, future studies that strive to translate findings from rats to humans, as well as from humans to rats, will likely prove valuable in furthering our understanding of the relationship between genetic and experiential factors on ADHD-related behavior.

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Orienting Behavior

Figure 1.

Orienting behavior (rearing) exhibited by SHR and WKY rats that were cross-fostered to a dam of the same or opposite strain. The amount of rearing behavior was dependent on the strain of the offspring and also influenced by the interaction between offspring strain and dam strain. Data are means $\pm$ SEM.

Figure 2.

(A) The number of social interactions exhibited by SHR and WKY rats when there were exposed to an unfamiliar conspecific rat. Social behavior was dependent entirely on the strain of the mother. (B) Locomotor activity as defined as the number of line crossings during the social interaction session. Activity was dependent solely on the strain of the offspring. Data are means $\pm$ SEM.

Anxiety-like Behavior

Figure 3.

Time spent in the open arms of the elevated plus maze. There was an interaction between offspring strain and dam strain on the amount of time spent in the open arms. Data are means $\pm$ SEM.

Table 1

Sample sizes for each group of dams

Group	n
WKY dams fostering WKYs	8
WKY dams fostering SHRs	5
SHR dams fostering WKYs	7
SHR dams fostering SHRs	4

Table 2

Sample sizes for each group of male offspring

Group	n
WKY _o :WKY _d	20
WKY _o :SHR _d	7
SHR _o :WKY _d	13
SHR _o :SHR _d	7

[Subscripts indicate the strain of the offspring (o) and strain of the dams (d)]

Table 3

Summary of the effects of offspring strain and dam strain on behavior

	Orienting Behavior	Social Interaction	Locomotor Activity	Anxiety-like Behavior
Offspring Strain	yes	no	yes	no
Dam Strain	no	yes	no	no
Offspring X Dam Interaction	yes	no	no	yes