Published in final edited form as:

Horm Behav. 2007 May ; 51(5): 655–664.

Individual differences in novelty-seeking and emotional reactivity correlate with variation in maternal behavior

Sarah M. Clinton^{1,†}, Delia M. Vázquez^{1,†}, Mohammed Kabbaj², Marie-Helen Kabbaj², Stanley J. Watson¹, and Huda Akil¹

¹Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI

²Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL.

Abstract

Numerous studies have demonstrated that Sprague-Dawley rats exhibit a wide range of locomotor reactivity when placed in a novel environment. High Responder (HR) rats show exaggerated locomotor response to novelty, enhanced neuroendocrine stress reactivity, decreased anxiety-like behavior, and propensity to self-administer psychostimulants, compared to the less active Low Responder (LR) animals. Few studies have explored the early environmental factors which may underlie the HR-LR differences in emotional reactivity. Considering the enormous impact of maternal care on rodent neurodevelopment, we sought to examine maternal behavior in HR-LR dams to determine whether they exhibit differences which could contribute to their offspring's differential temperaments. Females, like males, can be classified as HR vs. LR, showing marked differences in novelty-induced locomotor activity and anxiety-like behavior. HR-LR mothers behaved differently with their pups during the first two postpartum weeks. LR dams spent greater time licking and nursing their pups compared to HR dams, with the most prominent differences occurring during the second postpartum week. By contrast, when non-lactating HR-LR females were presented with orphaned pups, the pattern of maternal response was reversed. HR females were more responsive and showed greater maternal care of the novel pups compared to LR females, which were probably inhibited due to fear of the unfamiliar pups. This underscores the critical interplay between the female's emotional phenotype, her hormonal status and her familiarity with the pup as key factors in determining maternal behavior. Future work should explore neural and hormonal mechanisms which drive these HR-LR differences in maternal behavior and their impact on the development of the offspring.

Keywords

high-responder; low-responder; stress; Light-Dark Test; anxiety

INTRODUCTION

Outbred Sprague Dawley rats display a variety of behavioral responses when placed in a novel situation, with some rats (High Responders (HR)) actively exploring the new environment, and others (Low Responders (LR)) showing a blunted locomotor response. Numerous studies have demonstrated a high correlation between novelty-induced locomotor activity, drug-taking

Address all correspondence to: Sarah M. Clinton, Ph.D. University of Michigan Molecular and Behavioral Neuroscience Institute 205 Zina Pitcher Place Ann Arbor, MI 48109-0720 Email: clintons@umich.edu Telephone: 734-936-2034 Fax: 734-647-3140

[†]These authors contributed equally to this research.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and other risk-taking behaviors, as well as neuroendocrine stress-reactivity (Piazza et al., 1989, Hooks et al., 1991, Piazza et al., 1991a, Kabbaj et al., 2000). HR rats exhibit exaggerated stress-induced corticosterone secretion (Piazza et al., 1991a, Kabbaj et al., 2000), increased behavioral reactivity to psychostimulants (Piazza et al., 1989, Hooks et al., 1991, Piazza et al., 1991a), diminished fear and anxiety-like behavior (Kabbaj et al., 2000, Stead et al., 2006a), and increased aggressive behavior (Abraham et al., 2006) compared to their LR counterparts. Neurochemical and neural gene expression differences appearing to contribute, at least in part, to these observed HR-LR behavioral phenotypes (Piazza et al., 1991b, Hooks et al., 1994a, Hooks et al., 1994b, Kabbaj et al., 2000, Kabbaj, 2004). Many of these features are homologous to novelty-seeking and impulsive behavioral traits shown in humans to predispose to propensity for drug abuse (Zuckerman and Neeb, 1979, Cloninger, 1987). Thus, the HR-LR trait may tap into the broad dimension of behavioral disinhibition vs. behavioral control—a dimension that has been implicated in the vulnerability versus resilience to numerous psychiatric and addictive disorders (Ball et al., 2005).

While numerous studies have evaluated behavioral and biological differences between adult HR-LR animals, there is a paucity of information regarding what heritable or non-genetic environmental factors may contribute to the development of the HR-LR phenotypes. A vast literature clearly illustrates the profound impact of early life experience on emotional temperament and neuroendocrine reactivity (Levine et al., 1957, Levine, 1962, Denenberg et al., 1967, Levine et al., 1967, Zarrow et al., 1972, Hofer, 1973, Russell, 1973, Ladd et al., 2000, Sanchez et al., 2001, Arnold and Sivi, 2002). While we recently showed that the HR-LR trait appears to be heritable (Stead et al., 2006a), we also wanted to ascertain whether early maternal influences could also modulate the phenotype. Given the broad behavioral differences between HR-LR animals, we hypothesized that HR-LR mothers may differentially interact with their young offspring, and that this differential treatment may in turn contribute to emergence of the well-characterized adult HR-LR behavioral, neuroendocrine, and neuronal phenotypes.

In the present study we screened a group of male and female rats using exploration in a novel environment as an index of the HR-LR trait. We also ascertained anxiety-like behavior in the HR-LR groups to confirm that both male and female HR-LR rats show similar behavioral phenotypes. Next, we mated HR male-female pairs and LR male-female pairs and evaluated maternal behavior of HR-LR lactating dams as they cared for their litters during the first 2 postpartum weeks. In a follow-up study we examined maternal response of non-lactating HR-LR females when presented with novel orphaned pups, the so-called “maternal sensitivity test” initially designed and described by (Fleming and Sarker, 1990). Maternal behavior is largely driven by hormonal changes that occur during late pregnancy and parturition. Non-lactating female rats are generally fearful of novel pups (Fleming and Rosenblatt, 1974a, Fleming and Rosenblatt, 1974b, Fleming et al., 1979), but their aversion can be mitigated with a hormonal regimen that mimics the changes in estrogen and progesterone typical of pregnancy and partition (Fleming et al., 1989). Alternatively, non-lactating females can eventually exhibit maternal behavior in the absence of hormonal priming if they are able to habituate to the novel pups through frequent exposure to them (Fleming and Sarker, 1990, Rosenblatt et al., 1994, Bridges et al., 1996).

Our results show that HR-LR mothers behave differently with their litters during the first two postpartum weeks, with LR mothers being significantly more attentive to their pups than HR mothers. However, maternally-experienced HR-LR females presented with orphaned pups beyond the lactation period have a pattern of maternal response that is reversed, with HR females showing greater maternal care of the novel pups than LR females. The HR females' increased willingness to approach the novel pups is likely related to their general novelty-

seeking and exploratory tendencies. The pattern of maternal behaviors is consistent with the individual differences in the HR-LR behavior phenotypes originally described in male rats.

MATERIALS AND METHODS

Animals

Forty two male and sixty female Sprague-Dawley rats (Charles River Wilmington, MA, USA) were housed in separate rooms in groups of three per $43 \times 21.5 \times 25.5$ -cm polycarbonate cage (Nalgene, $24 \times 45 \times 20$). The rooms were kept under constant temperature ($25 \pm 2^\circ\text{C}$) and lighting conditions. Males were kept in a 12 h light:12 h dark cycle. Females were housed in a room with a 14 h light:10 h dark cycle (lights on 6:00am-8:00pm) to promote reliable reproductive cycles. Rats were provided with rat chow and tap water *ab libitum*, and maintained in accordance with the *NIH Guidelines for the Care and Use of Laboratory Animals*. The University of Michigan Animal Use Committee approved all animal protocols utilized.

HR/LR Classification: Screening for locomotor response to novelty

Prior to mating, male and female rats were screened for locomotor response to novelty. Animals were acclimated to housing conditions for a minimum of 7 days. Starting on the eighth day, female rats were subjected to daily vaginal smears to monitor estrous cycle for 1 wk. Locomotor testing was performed during the D1 phase of the estrous cycle. Horizontal and rearing activity was monitored by computer in 5 min intervals over 60 min by placing animals into clear acrylic $43 \times 21.5 \times 25.5$ cm (high) cages equipped with infrared photocell emitters mounted 2.3 and 6.5 cm above the grid floor. Male and female rats were tested separately, and all testing was performed between 8:00-11:30 am. Total locomotion scores for each rat were calculated by adding the total number of horizontal and rearing movements. Rats that exhibited locomotor scores in the highest third of the sample population were classified as high responders (HR), whereas animals with scores in the lowest third of the population were classified as low responders (LR). Animals whose scores fell in the middle third of the population were classified as intermediate responders (IR) and were not used for subsequent studies.

Light-Dark test

One week after locomotor testing, the HR-LR males and females ($N=8$ per group) were subjected to the Light-Dark test to assess anxiety-like behavior. The test apparatus was a $30 \times 60 \times 30$ cm Plexiglas shuttle-box divided into two equal-sized compartments by a wall with a 12-cm-wide open door. One compartment was painted white and brightly illuminated (100 lux), and the other compartment was painted black with very dim light. Rows of five photocells located 2.5 cm above the stainless steel grid floor monitored the rats' locomotor activity and time spent in each compartment. A microprocessor recorded the number of photocell beams interrupted, and the time spent in each compartment during the 5 min test. Rats were initially placed in the dark compartment at the beginning of the test. Male and female rats were tested on different days after careful cleansing of the apparatus to devoid it from odors. Females were tested during the D1 phase of the estrous cycle. All testing was performed between 8:00-11:30 am.

Mating HR-LR animals

One week after light-dark testing, HR-LR females were paired for 10 days with HR-LR males, respectively. Conception was verified by the presence of a vaginal plug. Pregnant females were individually housed on the calculated eighteenth day of gestation, and litters were culled to 8 healthy pups (4 males, 4 females) shortly after birth. After the initial handling of cages at birth, the mothers and litters were not disturbed in order to minimize disruption of mother-pup

interactions, except for weekly cage change. Pups were weaned on postnatal day 21 and grouped 4 animals per cage according to sex, with water and food available *ab libitum*.

Monitoring maternal behavior during lactation

HR dams and litters (N=6) and LR dams and litters (N=6) were videotaped to monitor maternal behavior from postnatal days 1 to 14, alternating the recording days such that an equal number of HR-LR mothers were recorded on odd and even days. Thus, 3 HR and 3 LR litters were taped for a 24 h period (e.g. postnatal day 1), and then the other 3 HR and 3 LR litters were videotaped for the following 24 h period (e.g. postnatal day 2). This alternation continued for the entire 14-d observation period so that each female was observed for a total of seven 24-h periods (e.g. postnatal days 1, 3, 5, 7, 9, 11, 13). Videotape recording was accomplished with cameras equipped with infrared light (Panasonic, Model WV-GP650) and a time lapse recorder (Panasonic, Model AG-6730). The lactating mothers' behavior was scored with a computerized program (Noldus Observer 5.0, Leesburg, VA), which recorded the duration and frequency of events from the videotape. The person scoring the tapes was blind to the experimental treatments, and trained to observe the following behaviors: passive nursing, arched-back nursing, licking of pups, transport of pups, contact without maternal care, self-directed behaviors (eating, drinking, grooming), horizontal movement, and resting.

Maternal responsivity testing in non-lactating females

HR-LR dams (N=10 per group) were weaned from their own pups at 21 days post-partum and housed two per cage, with one HR and one LR rat housed together. A month later, the females were subjected to a pup sensitization paradigm based on the protocol described by (Fleming and Sarker, 1990). Every day for 14 days females were placed in a test cage and exposed for 2 hours to **a**) four freshly nourished pups (2 male, 2 female) aged 1-14 days with their age corresponding to the day of testing followed by **b**) four inanimate control objects (small pieces of plastic, 1 inch x 1 inch). The novel pups' age increased with each day of testing, and the litters of "donor pups" were rotated over the 14-day test period so that each of the HR-LR females encountered completely novel pups each day. The order of presentation (pups versus inanimate object) was counter-balanced between the groups of animals tested each day and across testing days. Behavior was recorded and scored as described above. Females were considered maternal if they retrieved the novel pups and exhibited maternal behavior for 2 consecutive days. Testing was conducted between 9:00 am and 12:00 pm.

Statistical Analyses

Locomotion scores and light-dark box behaviors of HR-LR male and female rats were compared using 2-way-ANOVAs (HR-LR phenotype $\times$ gender). For the maternal behavior studies, repeated measures ANOVAs were used to assess HR-LR dam behavior across the first 14 postpartum days. For these analyses, ANOVAs were followed by Fisher's post hoc comparisons. For the maternal sensitivity test, we used a Chi Square test to compare the proportion of HR-LR females that were considered "maternal" or "non-maternal" on each test day. Once non-lactating HR-LR females were considered "maternal", we used Mann-Whitney U tests to compare the latency to retrieve pups and time spent performing maternal behavior on each test day. Data were analyzed using Statview 5.0.1 for Windows, and for all tests $\alpha=0.05$.

RESULTS

Locomotor response to novelty in male and female rats

Groups of male and female rats were tested separately for their locomotor activity in a novel environment, then subdivided into HR (most active one-third of the group), IR (intermediate group), and LR (least one-third active of the group). The 8 most extreme HR males and females

and 8 most extreme LR males and females were selected for subsequent breeding. There was a main effect for gender ($F=114.85$, df 1,25, $p<0.0001$), with females being generally more active than males. There was also a strong main effect for HR-LR phenotype ($F=246.81$, df 1,25, $p<0.0001$), and a significant gender $\times$ phenotype interaction ($F=41.07$, df 1,25, $p<0.0001$) (Figure 1A). Both male and female HR groups were significantly more active than their LR counterparts.

Light-Dark Anxiety test

The Light-Dark test was used to assess anxiety-like behavior in HR-LR males and females. There was a main effect for gender, with female rats generally spending more time in the light compared to males ($F=7.15$, df 1,25, $p<0.05$). There was also a main effect for HR-LR phenotype ($F=14.16$, df 1,25, $p<0.001$), but no gender $\times$ phenotype interaction (Figure 1B). Female HR rats spent significantly more time in the anxiogenic light compartment compared to LR females ($F=9.62$, df 1,25, $p<0.01$), and similarly, HR males spent significantly more time in the light than LR males ($F=5.24$, df 1,25, $p<0.05$). Thus, as previously reported in males, females also show an association between measures of exploration in a novel environment and tests of spontaneous anxiety.

Behavior of HR-LR dams during the first postpartum two weeks

HR-LR dams' behavior was monitored from postnatal days 1 to 14, alternating the recording days such that an equal number of HR-LR mothers were recorded on odd and even days. Consequently, data in Figure 2 and Table 1 are presented for each 2 day interval (days 1-2, days 3-4, etc.). LR mothers were generally more attentive to their pups compared to HR dams, since repeated measures ANOVA revealed a main effect of HR-LR phenotype on licking ($F=15.61$, df 1,24, $p<0.01$; Figure 2A-C), arched-back nursing ($F=27.34$, df 1,24, $p<0.001$; Figure 2D-F), and nesting ($F=13.74$, df 1,24, $p<0.01$; Figure 2G-I). There was also a main effect of the light-dark period on all three behaviors. Over the 14-day observation period, dams spent significantly greater time licking pups ($F=32.86$, df 1,24, $p<0.0001$; Figure 2A) and nesting ($F=101.90$, df 1,24, $p<0.0001$; Figure 2C) during the dark period compared to the light period. Dams, however, spent greater time performing arched-back nursing during the light phase compared to the dark phase ($F=102.55$, df 1,24, $p<0.0001$; Figure 2B). All dams spent progressively less time performing maternal behaviors over the 14-day observation period, which was evident by a main effect of postpartum day on nesting ($F=21.01$, df 1,6, $p<0.0001$), licking ($F=16.38$, df 1,6, $p<0.0001$), and nursing ($F=12.85$, df 1,6, $p<0.0001$). While there were no significant phenotype $\times$ day interactions for any of the maternal behaviors, inspection of the data shows that the most prominent HR-LR differences appear at the end of first week of life, and especially around the transition between the first and second weeks (postnatal days 5-9), a period during which the HR dams show a considerable drop in nesting and licking, while the LR dams show relative persistence of these behaviors (Figure 2C and 2I).

Table 1 summarizes the time that HR-LR dams spent performing non-maternal behaviors across the first two weeks of life. There was a main effect of HR-LR phenotype of self-directed behaviors (combination of grooming, eating, and drinking, ($F=11.67$, df 1,12, $p<0.01$), and rearing ($F=5.6$, df 1,12, $p<0.05$), with HR mothers displaying more of these behaviors across the 14-day test period compared to LR dams. There was a main effect of day on self-directed behaviors ($F=23.52$, df 1,6, $p<0.0001$) and resting ($F=43.19$, df 1,6, $p<0.0001$), with all mothers spending progressively more time performing these behaviors over the observation period. There was no effect of day or phenotype on the amount of time spent in non-maternal contact with pups, or horizontal movement (data not shown), and there were no significant day $\times$ phenotype interactions for any of these behaviors.

Maternal responsivity testing in non-lactating females

Non-lactating HR-LR females with previous maternal experience were exposed to foreign pups 2 hours daily for 14 days to assess maternal responsivity. As a control, females were also exposed to novel inanimate objects for 2 hours. On the first day of testing, two of the LR females cannibalized the foreign pups and were therefore eliminated from remaining test sessions. The remaining females (N=10 HR, N=8 LR) generally showed progressively increasing maternal response towards the pups over the 14-day test period, although, surprisingly, nonlactating HR females were much more responsive to the pups than LR females (Figures 3 and 4). Chi square analysis was used to compare the proportion of HR-LR females that were considered “maternal” or “non-maternal” on each test day. A similarly low proportion of HR-LR females (<50%) exhibited maternal behavior towards the novel pups over the first 4 test days. By test days 5 and 6, however, a significantly greater proportion of HR females retrieved and cared for the pups compared to LR females ($\chi^2 = 8.01$, $p < 0.01$). Similarly, a greater proportion of HR females exhibited maternal behavior compared to LR females on test days 7 and 8 ($\chi^2 = 84.26$, $p < 0.001$), 9 and 10 ($\chi^2 = 44.81$, $p < 0.001$), and 11 and 12 ($\chi^2 = 8.87$, $p < 0.01$). On test days 13 and 14, all HR-LR females were considered maternal (Figure 3A).

Once HR-LR females were considered “maternal” (displaying maternal behavior for 2 consecutive days), we compared the average latency for them to retrieve the orphaned pups (Figure 3B) as well as the average time spent performing maternal behaviors (retrieving and licking pups, crouched in a nursing position, or building a nest) on each test day (Figure 3C). For simplicity, data were collapsed into 2-day bins, with an animal's score averaged across two days (test days 1-2, test days 3-4, etc). The average score of all HR females considered “maternal” on a given day were compared to the average score of “maternal” LR animals using a Mann-Whitney U test. HR females showing significantly shorter latency to initially retrieve orphaned pups compared to LR females on test days 7-8 (Mann-Whitney U test, $p < 0.05$), test days 9-10 (Mann-Whitney U test, $p < 0.01$), and test days 11-12 (Mann-Whitney U test, $p < 0.05$) (Figure 3B). HR females also spent significantly more time performing maternal behaviors from test day 3 through 12 (Mann-Whitney U test, $p < 0.05$), but by test days 13 and 14, HR-LR females spent a similar length of time tending to the pups (Figure 3C).

We used an ANOVA to assess the willingness of HR-LR females to approach and contact the novel pups versus inanimate novel objects (Figure 4). HR females made significantly more contact with pups than the LR females, evident by a main effect of HR-LR phenotype on the number of contacts (including sniffing and non-maternal contacts) made with pups ($F = 11.62$, $df = 1, 78$, $p < 0.01$) (Figure 4A). There was no main effect of test day, but there was a significant day $\times$ phenotype interaction ($F = 2.52$, $df = 1, 78$, $p < 0.05$). Post hoc analysis showed that HR females made more contacts with pups compared to LR females on test days 3-10, and on days 13-14 (Figure 4A). Interestingly, HR-LR females made similarly low number of contacts with the inanimate objects placed in the test cage (Figure 4B); there was no main effect for phenotype, test day, and no phenotype $\times$ test day interactions for contacts with the inanimate objects. Analysis of other non-maternal behaviors showed a main effect of HR-LR phenotype on running and rearing when the animals were in the cage with the pups ($F = 10.95$, $df = 1, 13$, $p < 0.01$), with HR females showing greater activity than LR females. There was no effect of phenotype on activity, though, when rats were placed in the cage with the inanimate objects (data not shown). Finally, HR-LR females showed no differences in self-directed behaviors (grooming, eating, and drinking) during any of the test sessions (data not shown).

DISCUSSION

Numerous studies have demonstrated the marked differences in novelty-induced locomotor activity, anxiety-like behavior, drug-taking, and neuroendocrine stress-reactivity in HR-LR animals (Piazza et al., 1989; Piazza et al., 1991a; Dellu et al., 1996; Kabbaj et al., 2000; Kabbaj

and Akil, 2001, Klebaur et al., 2001, Kabbaj, 2004, Kabbaj et al., 2004), however, few if any studies have explored the early environmental factors which may underlie the HR-LR differences in emotional reactivity. Considering the enormous impact of maternal care on the development of the rodent brain and HPA stress axis, we sought to examine maternal behavior in HR-LR dams to determine whether they exhibit differences which could contribute to their offspring's differential temperaments. Our initial behavioral screen showed that HR-LR females, like HR-LR males, exhibit marked differences in novelty-induced locomotor activity and anxiety-like behavior (Figure 1), which is consistent with two earlier reports of HR-LR traits in female rats (Klebaur et al., 2001, Sell et al., 2005). Further, our analysis of maternal behavior during the first two postpartum weeks revealed that HR-LR mothers behave differently with their litters, with LR mothers being significantly more attentive to their pups than HR mothers (Figure 2). LR dams spent greater time licking and nursing their pups, and spent great time tending their nest compared to HR dams, with the most prominent differences occurring during the transition time between the first and second week postpartum (Figure 2). Interestingly, in a follow-up experiment where maternally-experienced HR-LR females were presented with orphaned pups, the pattern of maternal response was reversed, with HR females being more responsive and showing greater maternal care of the novel pups than LR females (Figures 3 and 4). We believe that this is yet another dimension of the HR-LR phenotype. Unlike LR females, HR rats exhibiting maternal behavior do not avoid novelty, but rather approach that which is unfamiliar.

Overlapping neural circuits govern maternal behavior, reward, and emotionality

A considerable body of work has identified neuroanatomical circuits governing the expression of rodent maternal behavior. This “maternal behavior circuit” encompasses several brain areas, including the medial preoptic area, lateral septum, nucleus accumbens, and amygdala, and is activated just prior to giving birth and throughout the postpartum period (Numan, 1974, Numan et al., 1985, Fleming and Anderson, 1987, Numan, 1988, Fleming et al., 1994, Fleming and Walsh, 1994, Fleming and Korsmit, 1996). Hormonal changes during late pregnancy and parturition initially activate the neural maternal behavior circuit (Rosenblatt et al., 1994, Bridges et al., 1996), but the behavior of the pups themselves and various pup-related stimuli (e.g. odor, sounds, and tactile sensations) powerfully motivate dams to maintain maternal care, even as hormonal levels dwindle (Fleming et al., 1999). Moreover, as dams experience motherhood, pups become a potent reinforcing stimulus (Lee et al., 1999, Mattson et al., 2001, Mattson et al., 2003, Ferris et al., 2005, Mattson and Morrell, 2005). Interestingly the reinforcing properties of pups depends in part on the postpartum period, such that dams in early stages of motherhood (before postpartum day 8) prefer access to their pups over access to cocaine, but dams in later postpartum stages (postpartum day 16) gravitate to cocaine-associated cues (Mattson et al., 2001).

There are several possibilities which may contribute to the observed HR-LR maternal behavior differences. First, HR-LR maternal behavior differences may be related to differential activation of maternal brain circuits. Meaney and colleagues have elegantly demonstrated differences in oxytocin receptor binding and estrogen receptor α expression in the medial preoptic area, lateral septum, and the central nucleus of the amygdala in dams exhibiting natural differences in licking-grooming and arched back nursing (LG-ABN) of their pups (Francis et al., 2000, Champagne et al., 2001, Champagne et al., 2003). Their findings with the high- and low-LG-ABN dams suggest that the natural variations in maternal care are related to differences in oxytocin and estrogen receptor levels (Francis et al., 2000), thus, future work will explore whether similar neural changes underlie the behavior of HR-LR mothers.

Alternatively, the HR-LR maternal behavior differences may derive from their differential responsiveness to rewards, which may be driven by HR-LR differences in dopaminergic reward

circuits (Piazza et al., 1991b, Hooks et al., 1994a, Hooks et al., 1994b, Dietz et al., 2005). Pups are powerful reinforcing stimuli (Lee et al., 1999, Mattson et al., 2001, Mattson et al., 2003, Ferris et al., 2005), but HR-LR dams may find them to be differentially reinforcing, which may, in turn, lead them to be differentially motivated to care for their pups. It is particularly interesting that the most prominent HR-LR maternal behavior differences occur during postpartum days 5-10 (Figure 2), which corresponds to the 'maintenance phase' of maternal behavior when hormones wane, and maternal care is primarily driven by the pups (Rosenblatt, 1969, Fleming and Sarker, 1990, Rosenblatt et al., 1994). LR mothers may find pups to be especially reinforcing, and therefore spend a greater time engaged in maternal behaviors. HR mothers, on the other hand, may not find pups and the maternal experience as reinforcing, or perhaps over time become distracted by other factors and thus become less attentive to their pups. Future behavioral studies may examine whether HR-LR dams are differentially motivated to care for their pups, whether pups have different reinforcing properties for HR-LR dams, and perhaps whether the pups themselves act differently (e.g. are more or less demanding), which may elicit different responses from their mothers.

While LR mothers showed robust interest in their own pups during the first 2 postpartum weeks, maternally-experienced non-lactating LR females largely avoided orphaned pups in the maternal sensitivity test. HR females, on the other hand, which were less attentive to their own offspring compared to LR, showed intense interest in the novel pups by initially approaching the pups more quickly, and also expressing more maternal behavior towards the pups compared to LR (Figures 3 and 4). Non-lactating females typically avoid pups (Fleming and Rosenblatt, 1974a, Fleming and Rosenblatt, 1974b, Fleming et al., 1979), and this pup aversion, like other fear-mediated behaviors, appears to be mediated at least in part via the amygdala (Fleming et al., 1980). LR rats in the present study and others show increased fear- and anxiety-like behavior in the Light-Dark Anxiety test compared to HR animals (Figure 1 and (Kabbaj et al., 2000, Stead et al., 2006a)). LR females were probably fearful of the novel pups in the maternal sensitivity test, which largely inhibited expression of maternal behavior over the 14-day test period. By contrast, HR rats are generally drawn to novelty, and this may have led them to approach the novel pups, which may have in turn induced the maternal behavior that unfolded over the 2-week testing period.

Contrasting HR-LR offspring and High/Low LG-ABN Offspring

At first glance the observed HR-LR maternal behavior differences are somewhat unexpected since they seem to counter previous findings with the High- and Low-LG-ABN model. Work by Meaney and co-workers established that offspring of high-LG-ABN mothers show decreased anxiety-like behavior and stress reactivity compared to offspring of low-LG-ABN mothers (Meaney, 2001). In the present study, however, the more attentive LR mothers, which spend greater time licking, grooming, arched-back nursing, and nesting compared to HR mothers, raise offspring that grow up to exhibit greater behavioral inhibition and anxiety-like behavior. Our disparate findings may be related to a host of factors that include the developmental window of increased maternal behavior, and genetic factors inherent to the rat strain. First, the maternal behavior differences between high/low LG-ABN mothers occur primarily during the first week of life, while the differences between HR-LR mothers are most prominent during the transition into the second week of life. The first two weeks postpartum represent a critical developmental period for rodent neural systems, evident in radical changes in behavior (e.g. (Smart and Dobbing, 1972, Altman and Sudarshan, 1975, Eilam and Golani, 1988, Wiedenmayer and Barr, 1998), neuroendocrine response (Sapolsky and Meaney, 1986), synaptic connectivity (e.g. (Rinaman et al., 2000, Card et al., 2005))), and global neural gene expression (Stead et al., 2006b). Environmental perturbations (e.g. handling, maternal separation) and/or naturally occurring differences in the quality or quantity of maternal care may differentially impact developing offspring depending upon the specific timeframe when

these differences occur. Secondly, it is important to note that variations in maternal care appear to exert similar effects on the neuroendocrine stress response in both the HR-LR and high/low LG-ABN models. For example, offspring of high-LG-ABN mothers show reduced stress-evoked ACTH and corticosterone release, increased glucocorticoid receptor (GR) mRNA expression in the hippocampus, and decreased CRH mRNA expression in the paraventricular nucleus (PVN) of the hypothalamus, compared to low-LG-ABN offspring (Liu et al., 1997). We previously reported that LR rats (offspring of the more attentive LR mothers), show blunted corticosterone levels in response to mild novelty stress, and also show increased GR mRNA expression in the hippocampus, and decreased CRH mRNA expression in the PVN, compared to HR rats (Kabbaj et al., 2000).

A final important distinction between the offspring of High/Low-LG-ABN mothers and HR/LR mothers is the effect of cross-fostering. The neuroendocrine and behavioral traits of Low-LG-ABN offspring can be completely reversed by cross-fostering those pups to the more attentive High-LG-ABN mothers (Francis et al., 1999). We have begun to evaluate the impact of cross-fostering on the HR/LR behavioral phenotypes (Stead et al., 2006a). HR-LR litters from our Selectively Bred HR-LR lines were cross-fostered to LR and HR mothers, respectively, and their novelty-induced locomotor activity and anxiety-like behavior was compared to that of HRLR animals raised by their birth mothers. Surprisingly, cross-fostering had no detectable impact on the locomotor response to novelty of adult offspring, and moderately impacted anxiety-like behavior (Stead et al., 2006a). It should be noted that since these studies were conducted in selectively bred lines, the HR-LR phenotypes were more extreme, and it is conceivable that maternal behavior could play some greater role in shaping behavior of more intermediate animals. In fact, preliminary work in our laboratory shows that cross-breeding the selectively bred HR-LR lines produces animals with an intermediate behavioral phenotype that is influenced by maternal care (unpublished observations). Future studies will evaluate the impact of cross-fostering on neuroendocrine stress reactivity in outbred and Selectively Bred HR-LR animals. Other maternal behavior studies may also examine whether cross-fostering affects maternal care styles of HR-LR offspring when they grow up and tend to their own pups.

Conclusions

Our present findings demonstrate that individual differences in novelty-seeking correlate with variations in maternal care. LR females, which exhibit reduced novelty-induced locomotor reactivity and increased anxiety-like behavior, are significantly more attentive to their pups during the first two postpartum weeks compared to HR dams. Consistent with their apparent fear of novelty, non-lactating LR females are hesitant to approach novel pups in the maternal sensitivity test, while HRs readily approach the pups and express maternal behavior towards them. Future studies will explore neural and hormonal mechanisms which may drive these HR-LR maternal behavior differences, and further explore how these variations in maternal care ultimately influence behavioral, neuroendocrine, and neurobiological mechanisms which contribute to differences in emotional reactivity in later life.

ACKNOWLEDGEMENTS

We are extremely grateful to Sue Miller, Antony Abraham, and Tracy Bedrosian for excellent technical assistance. This study was funded by the Office of Naval Research, grant N00014-02-1-0879 to HA, NIDA RO1 DA13386 to HA and NIMH PO1 MH42251 to SJW.

REFERENCES

- Abraham, A.; Clinton, SM.; Watson, SJ.; Akil, H. Individual Differences in Novelty-Seeking Correlate with Differences in Aggressive Behavior; Proceedings of the Society for Neuroscience 36th Annual Meeting; Atlanta, GA. 2006.

- Altman J, Sudarshan K. Postnatal development of locomotion in the laboratory rat. *Animal Behavior* 1975;23:896–920.
- Arnold JL, Siviyy SM. Effects of neonatal handling and maternal separation on rough-and-tumble play in the rat. *Developmental Psychobiology* 2002;41:205–215. [PubMed: 12325135]
- Ball SA, Cobb-Richardson P, Connolly AJ, Bujosa CT, O'Neill T, W. Substance abuse and personality disorders in homeless drop-in center clients: symptom severity and psychotherapy retention in a randomized clinical trial. *Compr Psychiatry* 2005;46:371–379. [PubMed: 16122538]
- Bridges RS, Robertson MC, Shiu RP, Friesen HG, Stuer AM, Mann PE. Endocrine communication between conceptus and mother: placental lactogen stimulation of maternal behavior. *Neuroendocrinology* 1996;64:57–64. [PubMed: 8811667]
- Card JP, Levitt P, Gluhovsky M, Rinaman L. Early experience modifies the postnatal assembly of autonomic emotional motor circuits in rats. *Journal of Neuroscience* 2005;25:9102–9111. [PubMed: 16207869]
- Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences of the USA* 2001;98:12736–12741. [PubMed: 11606726]
- Champagne FA, Weaver IC, Diorio J, Sharma S, Meaney MJ. Natural variations in maternal care are associated with estrogen receptor alpha expression and estrogen sensitivity in the medial preoptic area. *Endocrinology* 2003;144:4720–4724. [PubMed: 12959970]
- Cloninger CR. A systematic method for clinical description and classification of personality variants. A proposal. *Archives of General Psychiatry* 1987;44:573–588. [PubMed: 3579504]
- Dellu F, Piazza PV, Mayo W, Le Moal M, Simon H. Novelty-seeking in rats--biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. *Neuropsychobiology* 1996;34:136–145. [PubMed: 8916071]
- Denenberg VH, Brumaghim JT, Haltmeyer GC, Zarrow MX. Increased adrenocortical activity in the neonatal rat following handling. *Endocrinology* 1967;81:1047–1052. [PubMed: 6052935]
- Dietz DM, Tapocik J, Gaval-Cruz M, Kabbaj M. Dopamine transporter, but not tyrosine hydroxylase, may be implicated in determining individual differences in behavioral sensitization to amphetamine. *Physiology and Behavior* 2005;86:347–355. [PubMed: 16126238]
- Eilam D, Golani I. The ontogeny of exploratory behavior in the house rat (*Rattus rattus*): the mobility gradient. *Developmental Psychobiology* 1988;21:679–710. [PubMed: 3234601]
- Ferris CF, Kulkarni P, Sullivan JM Jr, Harder JA, Messenger TL, Febo M. Pup suckling is more rewarding than cocaine: evidence from functional magnetic resonance imaging and three-dimensional computational analysis. *Journal of Neuroscience* 2005;25:149–156. [PubMed: 15634776]
- Fleming A, Vaccarino F, Tambosso L, Chee P. Vomeronasal and olfactory system modulation of maternal behavior in the rat. *Science* 1979;203:372–374. [PubMed: 760196]
- Fleming AS, Anderson V. Affect and nurturance: mechanisms mediating maternal behavior in two female mammals. *Progress in Neuropsychopharmacology and Biological Psychiatry* 1987;11:121–127.
- Fleming AS, Cheung U, Myhal N, Kessler Z. Effects of maternal hormones on 'timidity' and attraction to pup-related odors in female rats. *Physiology and Behavior* 1989;46:449–453. [PubMed: 2623069]
- Fleming AS, Korsmit M. Plasticity in the maternal circuit: effects of maternal experience on Fos-Lir in hypothalamic, limbic, and cortical structures in the postpartum rat. *Behavioral Neuroscience* 1996;110:567–582. [PubMed: 8889002]
- Fleming AS, O'Day DH, Kraemer GW. Neurobiology of mother-infant interactions: experience and central nervous system plasticity across development and generations. *Neuroscience and Biobehavioral Reviews* 1999;23:673–685. [PubMed: 10392659]
- Fleming AS, Rosenblatt JS. Olfactory regulation of maternal behavior in rats. I. Effects of olfactory bulb removal in experienced and inexperienced lactating and cycling females. *Journal of Comparative and Physiological Psychology* 1974a;86:221–232. [PubMed: 4811553]
- Fleming AS, Rosenblatt JS. Olfactory regulation of maternal behavior in rats. II. Effects of peripherally induced anosmia and lesions of the lateral olfactory tract in pup-induced virgins. *Journal of Comparative and Physiological Psychology* 1974b;86:233–246. [PubMed: 4590492]
- Fleming AS, Sarker J. Experience-hormone interactions and maternal behavior in rats. *Physiology and Behavior* 1990;47:1165–1173. [PubMed: 2395921]

- Fleming AS, Suh EJ, Korsmit M, Rusak B. Activation of Fos-like immunoreactivity in the medial preoptic area and limbic structures by maternal and social interactions in rats. *Behavioral Neuroscience* 1994;108:724–734. [PubMed: 7986366]
- Fleming AS, Vaccarino F, Luebke C. Amygdaloid inhibition of maternal behavior in the nulliparous female rat. *Physiology and Behavior* 1980;25:731–743. [PubMed: 7443835]
- Fleming AS, Walsh C. Neuropsychology of maternal behavior in the rat: c-fos expression during mother-litter interactions. *Psychoneuroendocrinology* 1994;19:429–443. [PubMed: 7938344]
- Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* 1999;286:1155–1158. [PubMed: 10550053]
- Francis DD, Champagne FC, Meaney MJ. Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. *Journal of Neuroendocrinology* 2000;12:1145–1148. [PubMed: 11106970]
- Hofer MA. Maternal separation affects infant rats' behavior. *Behavioral Biology* 1973;9:629–633. [PubMed: 4761069]
- Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr. Individual differences in locomotor activity and sensitization. *Pharmacology Biochemistry and Behavior* 1991;38:467–470.
- Hooks MS, Juncos JL, Justice JB Jr, Meiergerd SM, Povlock SL, Schenk JO, Kalivas PW. Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. *Journal of Neuroscience* 1994a;14:6144–6152. [PubMed: 7931568]
- Hooks MS, Sorg BA, Kalivas PW. The relationship between mRNA levels and the locomotor response to novelty. *Brain Research* 1994b;663:312–316. [PubMed: 7874516]
- Kabbaj M. Neurobiological bases of individual differences in emotional and stress responsiveness: high responders-low responders model. *Archives of Neurology* 2004;61:1009–1012. [PubMed: 15262729]
- Kabbaj M, Akil H. Individual differences in novelty-seeking behavior in rats: a c-fos study. *Neuroscience* 2001;106:535–545. [PubMed: 11591454]
- Kabbaj M, Devine DP, Savage VR, Akil H. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. *Journal of Neuroscience* 2000;20:6983–6988. [PubMed: 10995843]
- Kabbaj M, Evans S, Watson SJ, Akil H. The search for the neurobiological basis of vulnerability to drug abuse: using microarrays to investigate the role of stress and individual differences. *Neuropharmacology* 2004;47(Suppl 1):111–122. [PubMed: 15464130]
- Klebaur JE, Bevins RA, Segar TM, Bardo MT. Individual differences in behavioral responses to novelty and amphetamine self-administration in male and female rats. *Behavioral Pharmacology* 2001;12:267–275.
- Ladd CO, Huot RL, Thirivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Progress in Brain Research* 2000;122:81–103. [PubMed: 10737052]
- Lee A, Clancy S, Fleming AS. Mother rats bar-press for pups: effects of lesions of the mpoa and limbic sites on maternal behavior and operant responding for pup-reinforcement. *Behavioral Brain Research* 1999;100:15–31.
- Levine S. Plasma-free corticosteroid response to electric shock in rats stimulated in infancy. *Science* 1962;135:795–796. [PubMed: 14464660]
- Levine S, Alpert M, Lewis GW. Infantile experience and the maturation of the pituitary adrenal axis. *Science* 1957;126:1347. [PubMed: 13495470]
- Levine S, Haltmeyer GC, Karas GG, Denenberg VH. Physiological and Behavioral Effects of Infantile Stimulation. *Physiology and Behavior* 1967;2:55–59.
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 1997;277:1659–1662. [PubMed: 9287218]
- Mattson BJ, Morrell JI. Preference for cocaine- versus pup-associated cues differentially activates neurons expressing either Fos or cocaine- and amphetamine-regulated transcript in lactating, maternal rodents. *Neuroscience* 2005;135:315–328. [PubMed: 16112474]

- Mattson BJ, Williams S, Rosenblatt JS, Morrell JI. Comparison of two positive reinforcing stimuli: pups and cocaine throughout the postpartum period. *Behavioral Neuroscience* 2001;115:683–694. [PubMed: 11439457]
- Mattson BJ, Williams SE, Rosenblatt JS, Morrell JI. Preferences for cocaine- or pup-associated chambers differentiates otherwise behaviorally identical postpartum maternal rats. *Psychopharmacology (Berl)* 2003;167:1–8. [PubMed: 12607073]
- Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience* 2001;24:1161–1192.
- Numan M. Medial preoptic area and maternal behavior in the female rat. *Journal of Comparative and Physiological Psychology* 1974;87:746–759. [PubMed: 4426995]
- Numan M. Neural basis of maternal behavior in the rat. *Psychoneuroendocrinology* 1988;13:47–62. [PubMed: 2897700]
- Numan M, Morrell JI, Pfaff DW. Anatomical identification of neurons in selected brain regions associated with maternal behavior deficits induced by knife cuts of the lateral hypothalamus in rats. *Journal of Comparative Neurology* 1985;237:552–564. [PubMed: 4044898]
- Piazza PV, Deminiere JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. *Science* 1989;245:1511–1513. [PubMed: 2781295]
- Piazza PV, Maccari S, Deminiere JM, Le Moal M, Mormede P, Simon H. Corticosterone levels determine individual vulnerability to amphetamine self-administration. *Proceedings of the National Academy of Sciences of the USA* 1991a;88:2088–2092. [PubMed: 2006148]
- Piazza PV, Rouge-Pont F, Deminiere JM, Kharoubi M, Le Moal M, Simon H. Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. *Brain Research* 1991b;567:169–174. [PubMed: 1726140]
- Rinaman L, Levitt P, Card JP. Progressive postnatal assembly of limbic-autonomic circuits revealed by central transneuronal transport of pseudorabies virus. *Journal of Neuroscience* 2000;20:2731–2741. [PubMed: 10729354]
- Rosenblatt JS. The development of maternal responsiveness in the rat. *American Journal of Orthopsychiatry* 1969;39:36–56. [PubMed: 5776164]
- Rosenblatt JS, Wagner CK, Morrell JI. Hormonal priming and triggering of maternal behavior in the rat with special reference to the relations between estrogen receptor binding and ER mRNA in specific brain regions. *Psychoneuroendocrinology* 1994;19:543–552. [PubMed: 7938353]
- Russell PA. Effects of maternal separation and maternal disturbance on offspring growth and behavior in rats. *Journal of General Psychology* 1973;88:127–133. [PubMed: 4689148]
- Sanchez MM, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Developmental Psychopathology* 2001;13:419–449.
- Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Research* 1986;396:64–76. [PubMed: 3011218]
- Sell SL, Dillon AM, Cunningham KA, Thomas ML. Estrous cycle influence on individual differences in the response to novelty and cocaine in female rats. *Behavioral Brain Research* 2005;161:69–74.
- Smart JL, Dobbing J. Vulnerability of developing brain. IV. Passive avoidance behavior in young rats following maternal undernutrition. *Developmental Psychobiology* 1972;5:129–135. [PubMed: 4671405]
- Stead JD, Clinton SM, Neal C, Schneider J, Jama A, Miller S, Vazquez DM, Watson SJ, Akil H. Selective Breeding for Divergence in Novelty-seeking Traits: Heritability and Enrichment in Spontaneous Anxiety-related Behaviors. *Behavioral Genetics*. 2006aIn Press
- Stead JD, Neal C, Meng F, Wang Y, Evans S, Vazquez DM, Watson SJ, Akil H. Transcriptional profiling of the developing rat brain reveals that the most dramatic regional differentiation in gene expression occurs postpartum. *Journal of Neuroscience* 2006b;26:345–353. [PubMed: 16399705]
- Wiedenmayer CP, Barr GA. Ontogeny of defensive behavior and analgesia in rat pups exposed to an adult male rat. *Physiology and Behavior* 1998;63:261–269. [PubMed: 9423968]

- Zarrow MX, Campbell PS, Denenberg VH. Handling in infancy: increased levels of the hypothalamic corticotropin releasing factor (CRF) following exposure to a novel situation. *Proc Soc Exp Biol Med* 1972;141:356–358. [PubMed: 4538827]
- Zuckerman M, Neeb M. Sensation seeking and psychopathology. *Psychiatry Research* 1979;1:255–264. [PubMed: 298353]

Figure 1.

Behavioral characterization of male and female Higher Responder (HR) and Low Responder (LR) rats. When placed in a novel cage, male and female HR rats showed exaggerated locomotor reactivity compared to their LR counterparts (A). HR male and female rats also showed reduced anxiety in the Light-Dark test, compared to LR animals, which spent significantly less time in the anxiogenic light compartment than HRs (B). N=8 for all experimental groups. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.0001$

Figure 2.

Maternal behavior of HR (N=6) and LR (N=6) dams during the first two postpartum weeks. Bar graphs in the left column show the average time spent licking (A), arched-back nursing (D), and nesting (G) during the light or dark phase over the entire 14-day postpartum period. Graphs in the middle column display the average number of minutes HR-LR dams spent licking pups (B), nursing (E), or building their nests (H) during the lights-on period each day, while graphs in the right column (C, F, I) show corresponding data for the dark phase. LR mothers spent significantly more time licking their pups (A-C), arched-back nursing (D-F), and nesting (G-I) compared to HR dams over the 14-day observation period. Dams, on average, spent greater time licking pups (A) and nesting (G) during the dark phase compared to the light phase, but spent greater time performing arched-back nursing during the light phase, compared to the dark phase (D). *indicates $p < 0.05$; **indicates $p < 0.01$, ***indicated $p < 0.0001$.

Figure 3.

Maternal responsivity in non-lactating HR (N=10) and LR (N=8) females. HR-LR females reacted differently when presented with novel pups 2 h daily for 14 days. HR females responded more quickly to the novel pups compared to LRs, with a majority of HR females exhibiting maternal behavior by test day 5, while most LR females did not become maternal until test day 11 (A). When HR-LR females did exhibit maternal behavior, HRs showed a reduced latency to retrieve the pups and initiate maternal care (B). HR females spent significantly greater time performing maternal behaviors (retrieving and licking pups, crouched nursing posture, building a nest) compared to LRs on test days 3 through 12 (C). *indicates $p < 0.05$; **indicates $p < 0.01$, ***indicated $p < 0.0001$.

Figure 4.

Contact with novel pups versus novel inanimate objects. In a control experiment, non-lactating HR-LR females were exposed to pups for 2 h, and then exposed to novel inanimate objects for another 2 hour period. HR females made significantly more contact with pups compare to LRs, but the two groups did not differ in the number of contacts with inanimate objects. * indicates $p < 0.05$; **indicates $p < 0.01$

Table 1

Time that HR and LR dams spent performing non-maternal behaviors during postpartum days 1-14.

		Days 1-2		Days 3-4		Days 5-6		Days 7-8		Days 9-10		Days 11-12		Days 13-14	
		<i>t me spent (min)</i>	SEM	<i>t me spent (min)</i>	SEM	<i>t me spent (min)</i>	SEM	<i>t me spent (min)</i>	SEM	<i>t me spent (min)</i>	SEM	<i>t me spent (min)</i>	SEM	<i>t me spent (min)</i>	SEM
<i>Self-directed behavior</i> ^{**†}	HR	266	38	444	44	533	46	519	60	545	42	515	49	535	22
	LR	185	36	253	28	393	26	428	44	526	10	420	38	541	38
<i>Rearing</i> [*]	HR	73	17	87	20	86	19	111	43	138	54	96	31	85	27
	LR	55	13	57	13	57	14	41	10	45	12	29	6	67	24
<i>Resting</i> [‡]	HR	18	6	60	12	203	28	358	99	375	96	473	34	530	24
	LR	24	6	75	31	138	45	240	35	324	44	483	45	472	66

^{**} Repeated measures ANOVA revealed a main effect of HR-LR phenotype of self-directed behaviors (indicates $p < 0.01$)

^{*} Repeated measures ANOVA revealed a main effect of HR-LR phenotype of rearing (indicates $p < 0.05$).

[‡] There was also a main effect of day on self-directed behaviors and resting (indicates $p < 0.0001$).