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Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses

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ABSTRACT

Social isolation of rodents during development is thought to be a relevant model of early-life chronic stress. We investigated the effects of early-life social isolation on later adult fear and anxiety behavior, and on corticosterone stress responses, in male rats. On postnatal day 21, male rats were either housed in isolation or in groups of 3 for a 3 week period, after which, all rats were group-reared for an additional 2 weeks. After the 5-week treatment, adult rats were examined for conditioned fear, open field anxiety-like behavior, social interaction behavior and corticosterone responses to restraint stress. Isolates exhibited increased anxiety-like behaviors in a brightly-lit open field during the first 10 min of the test period compared to group-reared rats. Isolation-reared rats also showed increased fear behavior and reduced social contact in a social interaction test, and a transient increase in fear behavior to a conditioned stimulus that predicted foot-shock. Isolation-reared rats showed similar restraint-induced increases in plasma corticosterone as group-reared controls, but plasma corticosterone levels 2 h after restraint were significantly lower than pre-stress levels in isolates. Overall, this study shows that isolation restricted to an early part of development increases anxiety-like and fear behaviors in adulthood, and also results in depressed levels of plasma corticosterone following restraint stress.

Introduction

Social isolation of rats during development is a common model used to study the effects of early-life stress on later behavior and brain activity (Hall, 1998; Robbins et al., 1996). Specifically, the most potent effects of isolation-rearing of rats occur during a critical phase from weaning to early adulthood (Arakawa, 2003, 2005; Einon and Morgan, 1977; Ferdman et al., 2007; Leng et al., 2004; Weiss et al., 2004). Although a number of studies have investigated the effects of early-life social isolation on behavioral measures in rodents, findings have been inconsistent. For example, locomotor hyperactivity in a novel open field has been observed following isolation-rearing (Sahakian and Robbins, 1977; Wright et al., 1991). In contrast, other studies have found that isolates exhibited decreased locomotion or no change in locomotor behavior in a novel open field when compared to group-reared rats (Archer, 1969; Dalrympie-Alford and Benton, 1981; Gardner et al., 1975; Holson, 1986). Although not confirmed by all studies, isolates appear to be more sensitive to novel environments (Hall, 1998), and the observed hyper- or hypo-locomotion depends on the context or type of novel environment. For instance, isolates exhibit less locomotion compared to group-reared rats in an aversive novel environment (Hall et al., 1997), which could be indicative of greater-anxiety-like behavior.

When anxiety-like behavior has been studied directly, isolation-rearing increased latency to approach a novel object, and emergence into an unfamiliar environment (Einon and Morgan, 1977). Furthermore, increased anxiety-like behaviors following isolation rearing have been observed using the elevated plus maze, and bright-light two compartment shuttle-box tests (Einon and Morgan, 1977; Stanford et al., 1988; Wright et al., 1991). Again, these findings are not always replicated, with isolates showing similar or decreased levels of anxiety-like behaviors compared to group-reared rats in an open field, during a social interaction test, or within an elevated plus maze (Gentsch et al., 1981; Hall, 1998; Rex et al., 2004; Thorsell et al., 2006). These inconsistencies could be due to the fact that many studies measuring anxiety-like behavior do not allow for the possibility that isolation rearing can induce alterations in locomotion within a novel environment, which can confound the interpretation of results in behavioral tests of anxiety.

The effects of isolation-rearing on neuroendocrine or hypothalamic–pituitary–adrenal (HPA) axis responses to stress have also been inconsistent. Isolation-rearing increases basal plasma corticosterone levels when measured in adulthood (Gamallo et al., 1986; Sanchez

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et al., 1995), but other studies show that isolation-rearing results in decreased basal plasma corticosterone (Miachon et al., 1993) or no changes at all (Holson et al., 1991; Jones et al., 1989; Weiss et al., 2004). These inconsistencies may be due to differences in timing and methods of social isolation. To illustrate, the total duration of isolation varies between studies from 2 weeks to 11 weeks after weaning, and many studies do not re-socialize animals prior to testing. Overall, the variability in isolation procedures does not allow findings from previous studies to apply to all models of early-life social isolation. This is particularly problematic because the sensitivity of the HPA axis to stress, and the development of neural stress systems, vary depending on developmental stage (Andrews and Matthews, 2004; Bremne and Vermetten, 2001; Spear, 2000; Spear et al., 1985).

Because of the inconsistencies described above, we further examined whether adult rats subjected to an early-life isolation/re-socialization procedure show heightened fear and anxiety-like behaviors in adulthood. Importantly, rats were acclimated to the testing environments for several days to avoid the potential confound of increased novelty-induced locomotion observed in isolates by other investigators (Sahakian and Robbins, 1977; Sahakian et al., 1975; Wright et al., 1991). Corticosterone responses to and recovery from restraint stress were also evaluated to further elucidate HPA axis function of isolation-reared rats that were re-socialized prior to stress-testing in adulthood.

Materials and methods

Animals and social isolation protocol

One-hundred and twenty-four male Sprague–Dawley rats (University of South Dakota Laboratory Animal Services, Vermillion, SD, USA), were obtained at postnatal day (P) 21, housed at a constant room temperature (22 °C, 60% relative humidity) and with a reverse 12 h light: 12 h dark cycle (lights off at 10:00 am). Food and water were available *ad libitum*. The following procedures were approved by the Institutional Animal Care and Use Committee of the University of South Dakota, and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

On P21 (weaning age corresponding to pre-adolescence), male rats derived from 8 litters were randomly assigned to the group-reared and isolation-reared conditions (n=62 per condition). This involved housing rats either individually or in groups of 3 for a period of 3 weeks during pre-adolescent to mid-adolescent development (P21–P42; (Andersen, 2003; Bowling and Bardo, 1994; Hall, 1998; Spear, 2000). After 3 weeks of isolation, rats were weighed and housed in groups of 3 for a re-socialization period of 2 weeks (McCormick et al., 2004; Nunes Mamede Rosa et al., 2005; Pascual et al., 2006). At this time, group-reared rats were also randomly reassigned to a new group of three rats to ensure that any observed differences between isolates and group-reared rats were attributable to isolation-rearing and not the re-socialization procedure. For both isolation- and group-reared conditions, rats were randomly assigned cage-mates across litters (within rearing condition) when re-socialized. The re-socialization period allowed rats to complete their development from mid-adolescence to early adulthood. Furthermore, re-socialization ensured that any observed behavioral or endocrine changes were due to social isolation during the pre-adolescent critical period instead of at any other point during early-life. At the end of the 5-week isolation/re-socialization procedure, rats reached early adulthood (P56) and were used in the following experiments. Behavioral testing was performed during the dark light cycle from 11:00 am to 5:00 pm. In each of the three experiments described below, separate groups of rats were used to reduce carry-over effects of multiple testing.

Experiment 1: Effects of early-life social isolation on anxiety-like behavior in adulthood

Habituation to a novel dark open field

After the 5-week isolation/re-socialization procedure, twenty-four male (n=12 per rearing condition) rats were placed in a dark open field for 30 min for 3 consecutive days. The open field consisted of an oblong arena (97.8 cm×70.17 cm×31.8 cm) made of black plastic, which was located in a dark experimental room illuminated by red light. A video camera was fixed above the arena that was relayed to a computer monitor and a video observation system (Ethovision 3.1, Noldus Information Technology, Wageningen, The Netherlands). Total distance moved (cm) was recorded for 30 min over the three day acclimation period.

Bright-field anxiety-like behavior in a familiar open field

After 3 days of acclimation to the dark open field, rats were then tested in the same open field illuminated by bright room-lighting at 8240 lx (normal room lighting in the holding room was 4234 lx) for 30 min. To assess anxiety-like behavior, a virtual center circle was defined in each open field. Number of center entries, time spent in the center (seconds) and the total distance moved (cm) were measured by behavioral software (Ethovision 3.1), and were analyzed in 10 min time bins over the 30 min testing period.

Social interaction test

The following day, rats underwent a social interaction test where they were exposed to a weight-matched unfamiliar male conspecific (±20 g) for 30 min in the same open field within a dark room illuminated by red lighting. The social interaction test consisted of placing an unfamiliar pair-housed conspecific rat at one end of the open field and a group- or isolation-reared rat in the other end of the open field. Behavior was scored by an observer blind to treatment using Observer XT (Noldus Information Technology). The behaviors scored were the latency to first approach the unfamiliar conspecific (seconds), total duration (seconds) and number of social contacts (sniffing other animal, chasing, crawling over, grooming other animal), and the total duration of freezing behavior (seconds) (Spiga et al., 2006).

Experiment 2: Effects of early-life social isolation on unconditioned and conditioned fear behavior in adulthood

Unconditioned and conditioned fear behavior

After the 5-week isolation/re-socialization procedure, twenty male rats (n=10 for both group- and isolation-reared rats; different rats from experiment 1) were tested for unconditioned and conditioned fear behavior in a foot-shock chamber (30 cm×30 cm; Noldus Information Technology). Foot-shock chambers had a speaker that delivered the tone (Ethovision Phenotyper 3000 v1.1 with shock grid) and a video camera in the lid of the chamber that relayed information to a computer and video observation system (Ethovision 3.1). Chambers also had a grid floor that delivered the foot-shock, which was controlled by Ethovision 3.1. The chamber was enclosed in a dark sound-attenuating chamber (Med Associates, Inc, St. Albans, VT). Rats were allowed to acclimate to the foot-shock chamber for 30 min each day over 3 days. Distance moved (cm) over each of the acclimation days was recorded by Ethovision 3.1.

On the tone+foot-shock pairing day (fourth day), rats were placed in the foot-shock chamber for 10 min prior to being exposed to 10 acoustic tones (56 dB, 5 s duration, presented every min over 10 min) paired with a mild foot-shock (0.5 mA, 0.5 s duration) (Goldstein et al., 1996; Liu and Weiss, 2002; Shaham et al., 2000; Wang et al., 2002), where the mild foot-shock overlapped with the last 0.5 s of the 5 s tone. Rats remained in the foot-shock chamber for 10 min following the tone+foot-shock pairings. Twenty-four hours later, rats were