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Social isolation in adolescence alters behaviors in the forced swim and sucrose preference tests in female but not in male rats

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ABSTRACT

Social interactions in rodents are rewarding and motivating and social isolation is aversive. Accumulating evidence suggests that disruption of the social environment in adolescence has long-term effects on social interactions, on anxiety-like behavior and on stress reactivity. In previous work we showed that adolescent isolation produced increased reactivity to acute and to repeated stress in female rats, whereas lower corticosterone responses to acute stress and decreased anxiety-related behavior were noted in isolated males. These results indicate a sex specific impact on the effects of social stress in adolescence. However, little is known about whether social isolation impacts behaviors related to affect and whether it does so differently in male and female rats. The present study investigated the impact of adolescent social isolation from day 30–50 of age in male and female Sprague Dawley rats on behavior in the forced swim test at the end of adolescence and in adulthood and on behavior in the sucrose preference test in adulthood. Adult female rats that were isolated in adolescence exhibited increased climbing on the first and second day of the forced swim test and showed an increased preference for sucrose compared to adult females that were group-housed in adolescence. There were no effects in male rats. The results indicate that social isolation in adolescence produces a stable and active behavioral phenotype in adult female rats.

1. Introduction

Adolescence is a period of life characterized by maturation of cognitive, reproductive and social skills and capacities in all mammals [1,2]. These maturational processes are based in robust and widespread changes in neuronal structure and function [3]. In adolescence, peer relationships are the primary source of life stressors in boys and girls though there are striking sex differences [4]. Adolescent girls report higher levels of stress associated with their friendships, report more negative life events and experience more distress when such negative life events occur [4]. Understanding the impact of stress in adolescence is important because of the strong link between stress and affective and anxiety disorders [4–6]. Furthermore, the stress-responsive hypothalamic–pituitary–adrenal axis becomes sexually differentiated in adolescence suggesting that the impact of stress during adolescence may differ between males and females.

Rodent models of adolescent social stress have validity for understanding the impact of social stress in adolescent humans because adolescent rodents live in groups and exhibit higher levels of social behavior than either younger or older animals [7]. Social behavior in adolescence, including play and social grooming, is thought to facilitate normal cognitive and social development [8]. Social interactions in rodents are rewarding and motivating whereas social isolation is aversive [9,10]. Accumulating evidence suggests that disruption of the social environment in adolescence has long-term effects on social interactions [11–13] and on anxiety-like behavior [14,15] though evidence for effects on anxiety-like behavior is conflicting [16]. In previous work we showed that adolescent isolation produced increased reactivity to acute and to repeated stress in female rats, whereas lower corticosterone responses to acute stress and decreased anxiety-related behavior were observed in isolated males [15]. These results indicate a sex specific impact on the effects of social stress in adolescence, consistent with other findings [17]. However, little is known about whether social isolation impacts behaviors in the forced swim and sucrose preference tests and whether it does so differently in male and female rats. These tests examine important components of affective disorders, including behavioral despair and anhedonia, respectively, and have been extensively validated with anti-depressant drugs [18–21]. In studies in which anti-depressants are not administered, behavior in forced swim test provides indications of coping strategies. On the first day of the forced swim test, animals are typically more proactive (more time spent climbing and swimming than in immobility) and these active behaviors shift toward a more passive phenotype as indicated by increased time spent in immobility on the second day of the test [22]. Thus, the present study investigated the impact of adolescent social isolation on behavior in the forced swim test at the end of adolescence and in adulthood and on behavior in the sucrose preference test in adulthood.

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2. Materials and methods

2.1. Animals

Sprague–Dawley rats were purchased from Charles River Laboratory (Wilmington, MA). Twenty female and male rats arrived at postnatal day 23, (P23) and were housed in same-sex groups of 3 to 4 per cage. Water was provided through one bag per cage and the capacity of each bag was approximately 450 ml. Half of the males and females were assigned to the control group and the other half was assigned to the socially isolated, experimental group. On P30, the control rats were re-housed in groups of 2–3 and the isolated rats were re-housed one per cage. On P49–P50, all rats were tested in the two-day Porsolt forced swim test (FST). On P51, the rats in the control group were re-housed in new same-sex, same-treatment groups of 2–3 rats per cage. The isolated rats were housed in same-sex, same-treatment groups of 2–3 rats per cage. On P70 and 71, the FST was re-administered. Then at least 2 weeks later, all rats were singly housed in order to conduct the sucrose preference test. Rats were housed in larger cages for this test to allow fluid provisions through two bags. These studies were approved by the Children's Hospital of Philadelphia Research Institute's Institutional Animal Care and Use Committee. The experimental design is depicted in Fig. 1.

We chose to isolate rats from day 30 to 50 of age for the following reasons. Social isolation between day 25–45 of life, but not before day 25 or after day 45, delays emergence into an open field and slows the declines in novel object contact in adulthood at day 90 [23,24]. Similarly, isolation during day 26–40 of age in males increases anxiety-related behavior in adulthood, whereas equivalent periods of isolation at later ages (day 65 or 130) have little effect in male rats [25,26]. Thus, isolation from day 30 to 50 encompasses the period when the enduring effects of isolation have been observed. In addition, rats in the wild typically leave their burrows around 28 days of age and after this time, adolescent-typical neurobehavioral characteristics appear, vaginal opening occurs and increases in mature spermatids in seminiferous tubules are observed [27]. Based on such evidence, it is now increasingly accepted that day 28–50 of age encompasses the full extent of adolescence [17]. Finally, this study is based on our previous study in which rats were isolated from day 30 to 50 of age [15].

2.2. Forced swim test (FST)

The forced swim test (FST) test was adapted from the original procedures of Porsolt et al. [28] with modifications of Detke, Rickels and Lucki [20]. During adolescence, at P49, each of the 40 animals was subjected to 15 min of pre-exposure to the test environment — a plastic cylinder filled to 37 cm with 25 °C water. Animals could neither escape the water-filled cylinder nor support themselves by touching the bottom of the cylinder. Twenty-four hours later, on day 2, each animal was subjected to 5-minute swim in the same cylinder. Stressed rats remained isolated during the FST on day 49 and 50. Three behaviors were scored: immobility, swimming and climbing. Immobility was defined as no active movement besides minor efforts to keep the head afloat. Swimming was identified when the animal pedaled around the cylinder and moved more than ¼ of the circumference with all four paws immersed under water. Climbing was defined as the animal floating upright and actively attempting to climb out with their front paws extending above the water. These behaviors were coded every fifth second. The frequency of the occurrence of each behavior was converted to total time spent engaged in each behavior. The same 15-minute habituation and 5-minute tests were performed in adulthood, on P70 (day 1) and P71 (day 2), respectively, in all rats. Videorecordings were made on day 2 of the test in adolescence (day 50) and adulthood (day 71) and on day 1 of the test in adulthood (day 70). In forced swim test, at each age 10 control male rats, 10 male rats isolated during adolescence, 10 control female rats and 10 female rats isolated during adolescence were tested.

2.3. Sucrose preference test (SPT)

Based on Bechtholt et al. [19], a two-bottle choice paradigm was used to test for differences between the adult group-housed control rats and adult rats socially isolated during adolescence for their relative preference for sucrose over water. At P93, male rats were singly housed in new cages that allowed placement of two water bags. Animals were individually housed in order to assess fluid consumption for each individual rat. Animals were left to habituate to their new housing and drinking conditions for 3 days. Then, water consumption at baseline and body weights were taken for the next 48 h, twice a day at 1100 h and 1700 h. For assessment of daily 24 h consumption of water and of sucrose, the consumptions at 110 h and 1700 h were summed. Liquid consumption was conducted by weighing the water bags on a standard laboratory scale. The positions of the water bags were reversed after each measurement to control for any side preference throughout the study. Water baseline measurement was followed by the replacement of all water with sucrose solutions and 4 days of habituation to a 1% sucrose solution [19]. Again, consumptions were measured twice a day (1100 h, 1700 h) and side preference was controlled. After the sucrose habituation period, all sucrose solutions were replaced by water. For the following 3 days, animals were subjected to an acute stress of 30-min restraint once a day at 1000 h. Restraints were performed in rat decapitation cones. This was done to reinforce a stronger level of stress to induce a differential preference for sucrose between the control and isolated groups. Rats were permitted free access to water through the two water bags and chow throughout the restraint period. The restraint procedure was followed by an overnight, 20-hour deprivation of all fluids (chow was still available). The next morning (P107) at 1000 h, one bag of water and one bag of 1% sucrose were made available randomizing to control side preference. Consumption measurements were taken after the first and second hour. All of the same procedures were performed on females with the habituation period starting at P117. Females were run separately due to limited availability of cages that could accommodate two water bags. Sucrose preference was calculated

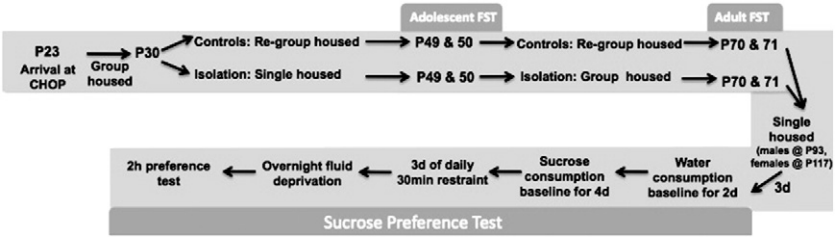

Fig. 1. The timeline and experimental designs for the current studies.