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Research report

A novel escapable social interaction test reveals that social behavior and mPFC activation during an escapable social encounter are altered by post-weaning social isolation and are dependent on the aggressiveness of the stimulus rat

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HIGHLIGHTS

- Male rats were exposed to post-weaning social isolation or control conditions.
- A novel escapable social interaction test was used to assess social behavior or escape.
- Stimulus rats were either aggressive or non-aggressive.
- Characteristics of both the experimental and stimulus rats determined social and escape behavior.
- Characteristics of both the experimental and stimulus rats determined protein expression in the medial prefrontal cortex.

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ABSTRACT

Post-weaning social isolation (PSI) has been shown to increase aggressive behavior and alter medial prefrontal cortex (mPFC) function in social species such as rats. Here we developed a novel escapable social interaction test (ESIT) allowing for the quantification of escape and social behaviors in addition to mPFC activation in response to an aggressive or nonaggressive stimulus rat. Male rats were exposed to 3 weeks of PSI (ISO) or group (GRP) housing, and exposed to 3 trials, with either no trial, all trials, or the last trial only with a stimulus rat. Analysis of social behaviors indicated that ISO rats spent less time in the escape chamber and more time engaged in social interaction, aggressive grooming, and boxing than did GRP rats. Interestingly, during the third trial all rats engaged in more of the quantified social behaviors and spent less time escaping in response to aggressive but not nonaggressive stimulus rats. Rats exposed to nonaggressive stimulus rats on the third trial had greater c-fos and ARC immunoreactivity in the mPFC than those exposed to an aggressive stimulus rat. Conversely, a social encounter produced an increase in large PSD-95 punctae in the mPFC independently of trial number, but only in ISO rats exposed to an aggressive stimulus rat. The results presented here demonstrate that PSI increases interaction time and aggressive behaviors during escapable social interaction, and that the aggressiveness of the stimulus rat in a social encounter is an important component of behavioral and neural outcomes for both isolation and group-reared rats.

1. Introduction

Social interaction during adolescence is critical for the development of competent and developmentally appropriate social behavior during adulthood. Social behavior is dependent upon a number of factors including the social history of the individual [1,2], the testing environment [3], and characteristics of social

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partners [4–6]. Post-weaning social isolation (PSI, also known as isolation rearing) in rats has been shown to produce altered social behavior that includes both increased social interaction as well as increased aggression [7,8]. This suggests that even though the motivation for social interaction is increased after PSI, the social interactions themselves may be unpleasant for individuals that have been deprived of normal social interaction during the adolescent period. Conversely, social conflict may be less aversive, or even reinforcing, for individuals that have been subjected to PSI and this social conflict may drive the PSI-induced increase in social interaction. One potential consequence of this is that isolates may be more likely to remain in abusive or maladaptive social situations.

PSI consists of depriving adolescent rats of social experience by housing them individually (ISO), as compared to housing in same-sex groups [GRP] for a period of 4–8 weeks after weaning. PSI of male rats produced increases in aggression [9], especially when testing occurred in an unfamiliar environment [3]. We have observed increases in social interaction after 4 weeks of PSI, and this increase was almost completely accounted for by increases in time spent engaging in aggressive grooming [7]. In spite of the finding that ISO rats spend more time in social interaction with a novel conspecific than GRP rats, we have not observed an increase in the reinforcing property of social interaction after PSI [10]. Conditioned place preference (CPP) studies in our laboratory have indicated that males, but not females, rapidly develop CPP to a context associated with a novel, same-sex, conspecific regardless of the experimental rat's rearing condition. Thus, even though isolation rearing dramatically increased the time spent interacting with a novel conspecific, it did not increase preference for a conspecific-associated context [10], consistent with the results of Douglas et al. [11].

The medial prefrontal cortex (mPFC) undergoes significant developmental fine-tuning during adolescence [12] and is crucial for the regulation of emotion [13] as well as for executive function [14]. In young men, executive function is negatively correlated with high frequency of physical aggression [15], thus changes in mPFC function produced by PSI may be directly related to the aggression observed in rats exposed to PSI. PSI leads to abnormalities in mPFC structure and function including alterations in dendritic spine morphology [16] as well as decreased expression of immediate early genes [7,17,18] and synaptic-associated proteins including PSD-95 [19]. PSD-95 expression was decreased in the mPFC after social isolation when assessed by Western blot [19]. However, reorganization of PSD-95 into large clusters (punctae) is associated with plasticity [20] independently of protein expression *per se,* and PSD-95 can be quantified by assessing the numbers of small or large punctae using immunohistochemistry [21].

Here we developed a novel procedure, the escapable social interaction test (ESIT), to quantify social preference and escape behavior in PSI-exposed rats in response to a novel stimulus rat. The ESIT provides the first method to assess social interaction in rats that allows for social preference to be examined with experimental rat-induced social interactions similar to the social approach task (SA) [22,23] while simultaneously monitoring social behaviors as can be done with standard social interaction tasks or CPP. The SA is limited in this regard as the stimulus animal is confined to a cage, and while CPP allows for behavioral monitoring during training, the later testing phase prohibits harvesting tissue within relevant time frames to examine immediate early gene expression in response to the social behavior, which occurs within a timeframe between 1 and 2 h [24]. Additionally, the use of "escapable" social interaction may provide a direct measure of social interaction-seeking behavior reflective of motivational drive in the absence of a learned association with a specific environment as in CPP, which may be confounding in ISO rats as PSI induces learning deficits [25]. Furthermore, the ESIT allows for the manipulation of the behavioral phenotype of the stimulus rat. Here, we manipulated the aggressiveness of the stimulus rat by exposure to either group or PSI rearing. We hypothesized that because of their social incompetence and increased motivation for social interaction, PSI rats would be more likely to spend time interacting with an aggressive stimulus rat than GRP rats would. We assessed escape behavior as well as social and aggressive behavior after either 1 or 3 trials with either a nonaggressive or an aggressive stimulus rat. Finally, we assessed the immediate early genes c-Fos and Arc, as well as PSD-95 in the anterior cingulate (AC), prelimbic (PL), and infralimbic (IL) subdivisions of the mPFC.

2. Materials and methods

2.1. Animals

Male (n = 88) Sprague-Dawley rats (Harlan; Indianapolis, IN) were purchased at postnatal day (P) 28 and were housed in standard Plexiglas cages either individually or in groups of 4 with food and water freely available in a 12:12 light:dark cycle. Isolated rats were exposed to the sight, sound, and smell of other rats in the colony room but were deprived of physical contact. Rats were weighed weekly but were not otherwise handled. Experimentation took place after 3 weeks of either isolation (ISO) or group (GRP) rearing, between days P49 and P52, a period corresponding to late adolescence [26]). Rats were run in squads of 16 per day; all groups were run in a counterbalanced design. Experiments occurred at the same time each day between 10:00 a.m. and 1:00 p.m. All experiments were carried out in accordance with the NIH best practices for animal use and approved by the University of Colorado Denver Institutional Animal Care and Use Committee and by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

2.2. Apparatus

The apparatus was in a testing room separate from the animal colony, under dim lighting, and maintained at 23 °C. It consisted of two rooms, the interaction room and the escape room. The interaction room was 30 cm × 30 cm square with 35 cm high silver Plexiglas walls, standard black bar type flooring, (5 mm diameter spaced 1.5 cm apart) with an 8 cm × 8 cm doorway in the lower rear corner leading into the escape room. The escape room was 12 cm × 30 cm × 35 cm high with a black Plexiglas floor and black walls. A tether was mounted via swivels to the top corner of the experimental room opposite to the escape door via an Irwin Quick grip 4″ clamp (Irwin). Cable ties (Jansco Products) were placed directly posterior to the front limbs of the stimulus rat, but caudal to the top of the rib cage, at the narrowest point roughly above the shoulder blades. The tie was loose enough to allow for normal breathing and full range of motion as well as allowing normal movement of the stimulus rat throughout the experimental room of the apparatus, but not the escape room. The experimental rat had freedom to roam throughout both rooms. The tether prevented access to the escape room by the stimulus rat, and thus the experimental rat had the option to escape social interaction by entering the escape room.

2.3. Stimulus rats

Stimulus rats were either isolation or group-housed for 4–5 weeks; stimulus rats that were slightly older and larger than the experimental rats were used to simulate bullying. Because not all isolation-reared stimulus rats appeared to be more aggressive than group-reared rats, the behavior of the stimulus rats during the social encounters was assessed for aggressive grooming (aggressive grooming by the stimulus rat of the experimental rat) by a blinded experimenter; stimulus rats were then designated as Aggressive