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Adolescent social defeat disturbs adult aggression-related impulsivity in wild-type rats

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ABSTRACT

Adolescence is generally considered as a developmental period during which adverse social experiences may have lasting consequences in terms of an increased vulnerability to affective disorders. This study aimed at determining the individual susceptibility to adolescent social stress using a rat model.

We used rats of the Wild-type Groningen strain, which are characterized by a broad variation in adult levels of aggression and impulsivity. We hypothesized that experience of social defeat in adolescence results in heightened aggression and impulsivity levels in adulthood.

In contrast to our expectation, adolescent social defeat did not lead to a difference in the average adult level of aggression and impulsivity, but the significant correlation between offensive aggression and impulsivity found in control animals was not present in animals defeated during adolescence.

1. Introduction

Adolescence is generally considered as a developmental period that is essential for the development of adult social skills. Several studies indicate that adverse social experiences in this phase of life may have lasting consequences in terms of an increased vulnerability to affective disorders (Arseneault et al., 2010; Gladstone et al., 2006; Van Os and Jones, 1999).

In human adolescents, an important source of social stress is bullying. Although bullying may have serious and lasting consequences for some adolescents, others do not seem to suffer from any negative consequences after experiencing bullying (Ortega et al., 2009; Rudolph et al., 2011). This clearly indicates that certain individuals are more vulnerable to bullying whereas others seem to be resilient. This is consistent with the well-recognized phenomenon that individual differences in sensitivity to stress are a critical factor in the development of psychopathologies both in humans and in animals (Kotov et al., 2010; Schmidt et al., 2008). However, the behavioral characteristics of individuals that are susceptible to bullying and to its consequences are poorly understood.

This study aims at an experimental approach of the individual susceptibility to adolescent social stress using a rat model. In adult male rats, coping style is an important determinant of individual stress vulnerability. Coping styles are defined as suits of correlated behaviors that are consistent over time and across situations (Koolhaas et al., 1999). High levels of aggressive behavior and impulsivity are characteristics of a proactive coping style (Coppens et al., 2014). Delville and colleagues suggested that individual differences in aggressive and impulsive behavior may be related to individual variation in prefrontal cortex functioning (Cervantes and Delville, 2007). The prefrontal cortex in particular undergoes major changes during adolescence (Andersen, 2003; Giedd et al., 1999; Spear, 2000). Furthermore, the prefrontal cortex has been shown to be involved in the development of adult behavioral skills by facilitating juvenile play (Pellis et al., 1992). Hence, the present study will focus on prefrontal cortex related behavioral tasks.

We used rats of the wild-type Groningen (WTG) strain to study the consequences of adolescent social stress on adult behavior. Rats of the WTG strain show a broad variation in the level of offensive aggressive behavior toward an unfamiliar intruder, ranging from no aggression at all to very high levels of aggressive behavior (De Boer et al., 2003). Furthermore, high-aggressive animals are inefficient in an unpredictable operant conditioning paradigm (variable-interval 15 s) for food reinforcement compared to low-aggressive animals (Coppens et al., 2014). Since the capacity to learn an operant conditioning paradigm is largely independent of age, the performance in a variable interval schedule can be used as an early indicator of individual variation in juveniles and a predictor of adult coping style.

To induce social stress in the adolescent male rats, we used the social defeat paradigm (Koolhaas et al., 2013). In adult male rats, social defeat induces long lasting behavioral and neurochemical

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changes (Bjorkqvist, 2001; Koolhaas et al., 1997). For that reason, Björkvist suggested that the social defeat paradigm may be an ecological valid animal model to study the consequences of bullying during adolescence (Bjorkqvist, 2001).

We hypothesize that adolescent social stress leads to heightened levels of adult offensive aggressive behavior and impulsivity and that this might be due to altered prefrontal cortex functioning.

2. Methods

2.1. Animals

Male wild-type Groningen (WTG) rats (*Rattus norvegicus*), originally wild-trapped animals and consequently bred under laboratory conditions for over 50 generations in our own facilities, were used as experimental subjects. They were weaned at pnd 21 and housed in non-sibling (randomly mixed) groups of three animals in standard macrolon cages (type 4).

Cages with the animals were placed in temperature-controlled rooms (21 ± 2 °C) under a 12 h light-dark cycle (lights off at 12 am). Water was available ad libitum throughout the experiment. Food was restricted to maintain animals at ~90% of normal bodyweight during operant conditioning tests, but otherwise lab chow was available ad libitum.

2.2. Experimental design

Animals were divided in a control ($n = 21$) and a defeat group ($n = 21$), subjects in the defeat group were defeated during late adolescence (pnd 45–57). At an age of 4 months, all subjects were tested for their level of offensive aggression using a resident-intruder paradigm. One day after the resident-intruder tests, operant conditioning for food reinforcement started to test the level of adult impulsivity.

2.3. Adolescent social defeat

The resident-intruder paradigm was used to induce social defeat. A similar procedure has been used in previous experiments in our laboratory (Vidal et al., 2007; Koolhaas et al., 2013). Resident rats of the WTG strain were housed with a tubally-ligated female in large observation cages (80 × 55 × 50 cm) to facilitate territorial aggression. Shortly (10 min) before the conflict, females were removed from the cage of the residents. Residents were trained to attack naïve intruders and only those with attack latencies shorter than 2 min were used for the experiment. By using animals with a more or less similar readiness to attack and aggression level, we tried to avoid variation in attack intensity.

Adolescent rats were subjected to social defeat at pnd 45 and 48 with direct physical contact with the aggressive resident for 10 min, thereafter animals were placed in a wire mesh cage (31 × 15 × 15 cm) for an additional 50 min in the cage of the resident. In this way, animals were protected from further attacks and injury, but remained in full visual, auditory and olfactory contact with the resident. This period of psychosocial stress is known to be highly stressful (Tornatzky and Miczek, 1994). On pnd 51, 54 and 57 animals were only psychosocially stressed by placing them in the residents' cage for 15 min. Defeats took place during the first half of the dark phase at approximately the same time each trial. Defeated animals were solitary housed after the first defeat for the rest of the experiment.

Control animals were placed in a clean cage at corresponding days and times compared to defeat animals. Control animals were housed in groups of three animals until the start of the offensive aggression screening.

2.4. Aggression screening

Experimental subjects were tested for aggressive behavior at ~pnd 120 using the resident-intruder paradigm as described by Koolhaas et al. (2013). Animals were housed in large observation cages (80 × 55 × 50 cm) with a tubally-ligated WTG female to avoid social isolation and facilitate territorial behavior.

After one week, the level of aggressive behavior was tested daily in the resident-intruder test on four consecutive days. Females were removed from the test cage prior to testing. During the first three tests an unfamiliar male con-specific (intruder) was introduced into the cage and the attack latency (time between introduction of the intruder and first attack) was scored. The intruder was removed after the first attack. If no attack occurred within 10 min the intruder was removed.

During the fourth test the full range of behaviors was scored during 10 min. The frequency and duration of behavioral elements were scored. All behavioral acts and postures were scored and grouped in 6 behavioral categories: (1) *offense* (lateral threat, clinching, keep down, chasing, upright posture); (2) *social exploration* (moving toward, nosing, investigating opponent, ano-genital sniffing, crawl over, attempted mount, social grooming); (3) *social interaction* (sum of total offense and social exploration); (4) *non-social exploration* (ambulation, rearing); (5) *immobility* (sitting, lying, immobile, freezing); (6) *grooming*. The behavioral data of the last test and the four attack latencies were used to classify the offensive behavior of animals. For correlations with operant conditioning, we used the percentage of time spent on offensive behaviors.

2.5. Operant conditioning

Skinner-box equipment (Med Associates Inc., St. Albans, VT) was installed in clear perspex cages (45 × 30 × 50 cm) with sawdust as bedding material. One retractable lever was located next to a food receptacle; the position of the lever was counterbalanced between cages. Food receptacle entrances were detected with an infrared detector located inside the food receptacle. A food dispenser distributed 45 mg food pellets (Dustless Precision Pellets, Product# F0165; Bioserv, Frenchtown, NJ, USA). The training schedules and online data collection were controlled by a computer and an interface (MedPC, Med Associates Inc.) located outside the animal rooms.

During the entire operant conditioning phase, animals were housed in the operant conditioning cages. Operant conditioning training was conducted daily during the first hour of the dark phase. Food was removed and the bodyweight of animals was gradually decreased to 90% of their free feeding bodyweight. Approximately 3 h after operant conditioning sessions additional chow was given.

At the start of each session, the retractable lever was extended into the chamber and delivered pellets under a FR-1. After three sessions on a FR-1 schedule, animals received reinforcement sessions according to a VI-15 schedule until stable performance for at least three days (7 sessions). The level of stable performance was corrected for spontaneous initial lever press activity.

2.6. Statistical analysis

Group data of the offensive aggression test and operant conditioning data are expressed as averages with standard error of the mean and analyzed using a two-tailed student *t*-test. To analyze correlations between various behaviors (i.e., operant behavior, offensive aggression and attack latency time) two-tailed Pearson correlation coefficients were used. Comparing correlations was done by converting the correlation coefficient to a *z*-score and