Physiology & Behavior 144 (2015) 116–123

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

The influence of social environment in early life on the behavior, stress response, and reproductive system of adult male Norway rats selected for different attitudes to humans

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HIGHLIGHTS

- The influence of social disturbance in early life was studied in adult grey rats.
- Social disturbance reduces aggressive behavior in unselected males.
- Social disturbance decreases stress reaction in aggressive males.
- Social disturbance decreases basal corticosterone level in tame males.
- Social disturbance decreases testis weights in unselected and aggressive males.

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 12 March 2015
Accepted 13 March 2015
Available online 14 March 2015

Keywords:
Rats
Behavior
Tame
Aggressive
Early-life environment

ABSTRACT

The influence of social disturbance in early life on behavior, response of blood corticosterone level to restraint stress, and endocrine and morphometric indices of the testes was studied in 2-month Norway rat males from three populations: not selected for behavior (unselected), selected for against aggression to humans (tame), and selected for increased aggression to humans (aggressive). The experimental social disturbance included early weaning, daily replacement of cagemates from days 19 to 25, and subsequent housing in twos till the age of 2 months.

The social disturbance increased the latent period of aggressive behavior in the social interaction test in unselected males and reduced relative testis weights in comparison to the corresponding control groups. In addition, experimental unselected rats had smaller diameters of seminiferous tubules and lower blood testosterone levels. In the experimental group, tame rats had lower basal corticosterone levels, and aggressive animals had lower hormone levels after restraint stress in comparison to the control. The results suggest that the selection in two directions for attitude to humans modifies the response of male rats to social disturbance in early life. In this regard, the selected rat populations may be viewed as a model for investigation of (1) neuroendocrinal mechanisms responsible for the manifestation of aggression and (2) interaction of the hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal systems in stress.

1. Introduction

Social environment is significant for the formation of adequate behavior and stress response not only in neonatal development but also in adolescence [1–3]. The term *adolescence* means the transition from childhood to maturity, when sexual maturation is accompanied by rearrangement of the hypothalamus–pituitary–adrenal (HPA) axis; further development of the limbic system and brain cortex; and gradual cultivation of social, cognitive, and exploratory behavior [4–7]. In this period, an individual is highly responsive to various stressors, in particular, to changes in social environment, which involve primarily the interaction between the mother and its descendants and later, among the youth [2, 8]. Disturbance of such interactions in adolescence can cause stress [9]. Its consequences in adulthood are anxiety [10–12], unmotivated aggression, and changes in HPA axis functions in various directions [1]. However, social disturbance exerting mild or relatively short-term action in adolescence can lead to habituation and adaptation without

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http://dx.doi.org/10.1016/j.physbeh.2015.03.018
0031-9384/

inducing any deviations in adults. In addition, changes may be missed in case of repeated disturbance in adolescence and adulthood [1].

Recent studies indicate that social environment in adolescence affects the reproductive function of mature animals [3,8]. Male rats exposed to 1-h isolation followed by cagemate replacement at ages within 30–45 days have lower blood testosterone levels and less pronounced reproductive behavior [8]. Guinea pig males kept in pairs with females also show reduced testosterone production and altered behavior in comparison to animals grown in the litter [3]. It is reasonable to suggest that the effects of social disturbances on the reproductive function are mediated by kisspeptin (Kiss1), the hormone involved in the feedback between sex steroids and secretion of gonadotropin-releasing hormone in the hypothalamus, is essential for the regulation of the hypothalamic–pituitary–gonadal axis and sexual maturation [13–15]. It has been shown that most Kiss1 mRNA-positive cells in the periventricular nuclei of mouse males express mRNAs for steroid estradiol and androgen receptors [16].

Experimental data demonstrated the dependence of the effect of social disturbances in early life on the behavioral phenotype of the animals. In particular, adult male Wistar rats bidirectionally bred for high (HAB) or low (LAB) anxiety-related behavior on the elevated plus-maze differed in the consequences of periodic maternal deprivation. HAB rats demonstrated reduced anxiety-related behavior, but the levels of anxiety in LAB rats were enhanced in comparison to their corresponding unstressed controls. The corticotrophin (ACTH) and corticosterone hyper-responses seen in control rats of the HAB line compared with those of the LAB line became attenuated in HAB rats after maternal deprivation, whereas maternal deprivation did not significantly alter neuroendocrine responses in LAB rats [17]. Investigation of the behavior of adult Wistar rats and wild-type Groningen ones (*Rattus norvegicus*; originally wild trapped animals and bred under laboratory conditions for over 45 generations) also showed that adolescent Wistar males are more vulnerable to being defeated by a mature male than wild-type Groningen ones [18].

Earlier studies conducted at our laboratory with Norway rat populations selected for many generations for the reduction and enhancement of the aggressive–fearful response to humans (tame and aggressive, respectively) demonstrated that the selection was accompanied by shifts in a broad range of physiological and behavioral indices [19–21]. For example, tame animals demonstrated lower activity of the HPA axis and mitigation of intraspecies aggression in the resident–intruder test in comparison to aggressive and unselected animals [22,23]. It was reasonable to suggest that the effects of social disturbance in early life on behavior, stress response, and gonadal function would be different in unselected rats and those bidirectionally bred for behavior to humans.

The goal of our work was to study the influence of social disturbance in early life on anxious and aggressive behavior, stress response, and the reproductive system in tame, aggressive, and unselected rats.

2. Materials and methods

2.1. Animals

Experiments were performed with males of two unique gray rat (*R. norvegicus*) populations, which had been selected for either elevated aggressiveness (aggressive) or the absence of the aggressive–fearful response to humans (tame) for 78 generations. Wild-type rats of the seventh or eighth generations kept in the vivarium of the Institute of Cytology and Genetics were taken as an unselected control sample. Animals were kept in standard cages (50 × 40 × 30 cm) under natural photoperiod and given food and water ad libitum. Blood sampling, the restraint stress test, and replacement of cagemates were performed from 10:00 to 14:00.

2.2. Experimental social environment in early life

The experimental social environment included disturbances formerly described in the literature with some modifications: early weaning, replacement of cagemates, and keeping in twos [3,8,24]. In the group exposed to social disturbances (hereafter termed experimental) males of the same behavioral phenotype but different litters were placed in twos on day 19 of life, or day 1 of cagemate replacement. Six cages of twos for unselected, aggressive, and tame rats were generated. Personal identification marks were pricked in ears of all pups. On day 2 of replacement, one male from each cage was transferred to a neighboring cage; thus, the original cagemates were replaced. On day 3, the original pairs were restored by adding the second mate of the original pair to that transferred before. This cagemate exchange within a population was done daily for six days. Then the males were kept in twos until the age of 2 months.

Experiments were done with 24 males of each behavioral phenotype. Of each group, 12 males at the age of 2 months were subject to behavior tests: the open-field test and, on the next day, the social interaction test. The other 12 males were subject to the restraint test. In the control groups, kept in the standard social environment, 12 males of each population were weaned at the age of 30 days and kept in fours or fives with no less than two of one litter until the age of 2 months.

2.3. Behavioral tests

The behavior test was done as males reached the 2-month age. Only rats whose birthdays differed by no more than 1 or 2 days were tested in one day. All behavioral tests were conducted from 14:00 to 18:00. Each group included 10 to 12 males. The behavior was videotaped and analyzed using a computer program, developed at the IC&G [25] which allows for the assessment of the latency, the number and the total duration of behavioral patterns.

2.4. Open-field test

The open field was a white circle 100 cm in diameter surrounded by 40-cm high transparent walls. The arena was uniformly illuminated. An animal was set in the field center in a transporting cage, the floor of the cage was pulled out, and the cage was immediately removed. The test lasted for 5 min. The estimated parameters included the time spent in the center, the duration of horizontal and vertical locomotion, and duration of self-grooming.

2.5. Social interaction test

Two rats of the same behavioral phenotype which were not familiar with each other, were placed to the arena of open field. The males in a pair had approximately equal body weights. The interaction test lasted for 5 min. The duration of aggressive behavior (attacks, chasing, and vertical postures), social interaction (approach, sniffing, grooming a partner, following a partner), and their latent periods were characterized for each pair.

2.6. Restraint stress and blood sampling

Male rats were placed to individual cages two days prior the stress experiment. Restraint of movement was used as the stressor. It was done by placing an animal to a tight plastic case for 30 min. Blood was sampled from the tail vein into a test tube with EDTA immediately after the stress. Two or three days later, the animal was euthanized by decapitation, and a blood sample was taken to determine the basal levels of corticosterone and testosterone. Blood was centrifuged, and the plasma was stored at −20 °C until analysis.