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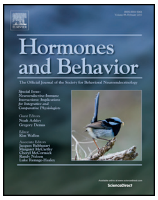

Stress responsiveness and anxiety-like behavior: The early social environment differentially shapes stability over time in a small rodent

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ABSTRACT

The early social environment can profoundly affect behavioral and physiological phenotypes. We investigated how male wild cavy offspring, whose mothers had either lived in a stable (SE) or an unstable social environment (UE) during pregnancy and lactation, differed in their anxiety-like behavior and stress responsiveness. At two different time points in life, we tested the offspring's anxiety-like behavior in a dark–light test and their endocrine reaction to challenge in a cortisol reactivity test. Furthermore, we analyzed whether individual traits remained stable over time. There was no effect of the early social environment on anxiety-like behavior and stress responsiveness. However, at an individual level, anxiety-like behavior was stable over time in UE- but not in SE-sons. Stress responsiveness, in turn, was rather inconsistent in UE-sons and temporally stable in SE-sons. Conclusively, we showed for the first time that the early social environment differentially shapes the stability of behavioral and endocrine traits. At first glance, these results may be surprising, but they can be explained by the different functions anxiety-like behavior and stress responsiveness have.

1. Introduction

Members of the same species represent a major part of the social environment and are known to crucially influence animals throughout life (Sachser et al., 2013, 2011). This social environment can for example support welfare and health (Hennessy et al., 2009; Sachser et al., 1998) but can also result in severe stress, eventually leading to disease and even death (von Holst, 1998). Interestingly, the social environment affects not only adult individuals but also offspring during the prenatal and early postnatal phases (Guibert et al., 2013; Kaiser and Sachser, 2005; Mommer and Bell, 2013). Behavior, body constitution, and endocrine reactions to stressful situations can be altered by parental effects to prepare offspring to fit future challenges (Mousseau and Fox, 1998).

In wild cavies (*Cavia aperea*), sons of mothers living in an environment with frequent exchange of social interaction partners display an infantilized behavioral profile. That is, they more frequently show behavioral patterns that are usually displayed by juveniles (e.g., *playing*). In contrast, sons of mothers living in an environment with a low degree of social interactions show higher levels of aggressive, attentive and social orientation behavior (Siegeler et al., 2011). Concerning hormonal development, early social instability was shown to cause a delayed increase in testosterone (T) concentrations shortly before sexual maturity while it did not affect baseline cortisol (C) values (Siegeler et al., 2013, 2011). Both behavioral and endocrinological findings were interpreted as adjustment to the prevailing environment: a repeated exchange of social partners resembles high population densities with a high degree of competition and consequently high levels of aggression. Infantilized sons appear to fare better under such conditions by displaying a delayed development and avoiding agonistic encounters with others. In contrast, an environment with a low degree of social interactions mimics a low population density with few social interactions. Sons born in this situation have no benefit in behavioral infantilization but rather invest energy into traits that facilitate early reproduction (Siegeler et al., 2011). However, these differences may not be the only adaptations to the current environment. There are other important traits that could be adaptively shaped, for example, anxiety-like behavior or stress responsiveness, which have been found to be highly sensitive to early social experiences in other rodent species (Champagne and Curley, 2005; Curley et al., 2011; Patin et al., 2005; Vallée et al., 1997).

In the last decades, a broad range of studies described the temporal stability of traits in several different taxa while in search of “animal personalities,” “temperaments,” or “behavioral syndromes” (Caspi et al.,

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2005; Cockrem, 2013; Réale et al., 2007; Zipser et al., 2013). Trait stability appears contradictory to the obvious benefits of phenotypic flexibility. Animals face different challenges throughout their life, and environments are likely to change over time. Therefore, keeping traits flexible allows individuals to spontaneously adapt to heterogeneous conditions, which should bring a major fitness advantage (Wolf et al., 2007). However, phenotypic flexibility comes at a cost as it requires high amounts of energy and is, against expectations, quite costly in terms of fitness (Stamps and Groothuis, 2010). Thus, the trade-off between costs and benefits determines whether or not individuals form stable traits (Wolf et al., 2007). Interestingly, only a few studies in this field addressed the issue of a possible influence of early experiences on trait stability in later life (Del Giudice, 2015; Günther et al., 2014a).

In the present study, we tested male wild cavies born in an unstable (UE) or in a stable social environment (SE) to detect the effects of early experiences on anxiety-like behavior, stress responsiveness, and the stability of these traits over time. For this purpose, we exposed each subject to two tests that characterize anxiety-like behavior and stress responsiveness at two different time points in life (Zipser et al., 2014, 2013). Individual stress responsiveness was estimated by analyzing the total and maximum C reactivity in a stressful situation. In addition, plasma T values were concurrently measured with stress responsiveness assessment. We hypothesized that UE- and SE-sons differ in their anxiety-like behavior and stress responsiveness in early and later life. Moreover, we expected that individuals show differences in the temporal stability of both of these traits as a result of their early social experiences.

2. Material and methods

2.1. Animals

The experiments were conducted with wild cavies belonging to the species *C. aperea* ERXLEBEN, 1777, derived from a breeding stock established at the Department of Behavioral Biology, University of Münster. The animals were descendants from feral cavies trapped in the province of Buenos Aires, Argentina, in 1995 and from lineages belonging to the Universities of Bayreuth and Bielefeld, Germany. Because cavies have a uniform brown pelage that does not allow for individual differentiation, they were marked individually by bleaching the fur with 32% hydrogen peroxide.

All experiments were conducted in accordance with the institution's animal care and use guidelines and approved by the national and local authorities (LANUV-NRW; reference number: 84-02.05.20.12.211). All procedures complied with the regulations covering animal experimentation within the EU (European Communities Council DIRECTIVE 2010/63/EU).

2.2. Housing conditions

All animals were kept under the following standardized conditions: temperature 22 ± 2 °C, relative humidity approximately 50 ± 10%, and light/dark cycle 12:12 h, with the light phase starting at 07:00 am. Commercial guinea pig diet (Höveler Meerschweinchenfutter 10700, Höveler Spezialfutterwerke GmbH & Co. KG, Dormagen, Germany, and Altromin 3023, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany), hay, and water were available ad libitum. This diet was supplemented weekly with oat flakes (Fortin Mühlenwerke GmbH & Co. KG, Düsseldorf, Germany). Vitamin C was added to the water twice per week. All animals were housed in wooden enclosures (height of the walls = 80 cm). The floors were covered with wood shavings for bedding (Allspan Olympia-Einstreu, Allspan GmbH, Karlsruhe, Germany) and cleaned every 4 weeks. Further, the animals were provided with a carton box as shelter.

2.3. Housing conditions of pregnant and lactating females

Sixteen groups were formed, consisting of one male and two female wild cavies along with their preweaning offspring. Within these groups, eight unstable and eight stable social environments were assigned (for more details see *2.4 Establishment of unstable and stable social environments*). Each group lived in a 1.5 m^2 enclosure. Pups were removed from their mothers after weaning (age 20 ± 1 days).

2.4. Establishment of unstable and stable social environments

2.4.1. Unstable social environment (UE)

In the eight groups, one of the two females was transferred to the clockwise neighboring enclosure every second week. With a 1 week shift, the remaining female was rotated counter-clockwise in the same manner. Males remained in their enclosures. This regular exchange of females between different groups led to a change in group compositions once per week. Preweaning offspring was transferred together with lactating females.

2.4.2. Stable social environment (SE)

In contrast to the unstable groups, the group composition of stable groups remained constant throughout the study. To prevent handling bias, all females and their preweaning offspring were handled in the same manner as the unstable females at corresponding times.

2.5. Housing conditions of sons

The experiment was conducted with 28 sons originating from females that gave birth to at least one litter beforehand in the according social environment (UE-sons: 14; SE-sons: 14). In the group of UE-sons, two animals shared the same mother (UE-mothers: 13), whereas in the group of SE-sons, three animals shared the same mother (SE-mothers: 12). None of the subjects were from the same litter. Sons from the same social environment were kept from the age of 20 ± 1 days onward in groups of two in 0.5 m^2 wooden enclosures. Group mates originated from different natal groups and were unfamiliar with each other. When partner animals differed in age by >3 days, the older animal of a pair stayed alone in the enclosure till its partner was weaned (maximum age difference: 11 days). It was taken care that the time of single housing was kept to a minimum (mean days in single housing ± SD: UE-sons 1.50 ± 1.52 days; SE-sons 0.79 ± 0.63 days).

2.6. Experimental design

Two different tests were conducted, always in the same order for each subject, at two different time points in life. The first testing block started when the animals had reached an age of 52 (±1) days and lasted up to an age of 54 (±1) days. This testing phase was termed "early life" as it precedes sexual maturation, which occurs around 80 days of age (Trillmich et al., 2006). The second testing block started when the individuals reached day 122 (±1) of age and lasted up to day 124 (±1) of age. This testing phase was named "later life" as it is set considerably after sexual maturation and at the beginning of adulthood (Günther et al., 2014b; Trillmich et al., 2006). Each testing block started with a dark–light test, for assessing anxiety-like behavior, followed by a cortisol reactivity test two days later. Only one of the two housing partners was tested on the same day to exclude disruptive effects on the remaining individual. Both tests were conducted in a wooden enclosure (100 × 100 × 80 cm). The housing room of the experimental animals was not entered 2 h prior to testing. Further, testing was conducted in a different room than the animals' housing room in which unfamiliar cavies were housed. The lightning intensity in all the tests was 290 ± 10 lx.