

Original Article

Long-term consequences of early development on personality traits: a study in European rabbits

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Early-life parameters such as litter size and growth are frequently associated with an animal's behavioral performance or motor skills as well as with its stress responsiveness. All these traits can be involved in the ontogeny of behavioral phenotypes, and therefore, we wanted to know whether features such as early growth also show long-term correlations with the animals' behavioral responses to challenges around maturity. We collected data on the early postnatal development of individually marked European rabbits living in a field enclosure and conducted 2 standardized behavioral tests shortly before the animals matured. In small enclosures, we experimentally tested their behavioral responses 1) in this novel environment and 2) to the confrontation with predator odor. Animals, which were more exploratory during the novel environment test, showed lower behavioral signs of anxiety during the predator test. Both responses were correlated with individual pup body mass, with subjects with higher body mass being more exploratory in the first test and showing lower levels of anxiety in the second. The animals' current body mass or age when being tested were not correlated with any of their responses. First, the correlated responses of the animals during the different contexts of the applied tests strongly suggest the existence of behavioral phenotypes in European rabbits. Second, and most importantly, our study provides evidence that an animal's early development can exert long-term effects on its personality type, although it is not clear whether body mass per se or some correlated physiological features drive the observed relationships. *Key words:* animal personality, behavioral phenotype, early growth, novel environment, *Oryctolagus cuniculus*, predator odor. [*Behav Ecol* 22:1123–1130 (2011)]

INTRODUCTION

In recent years, a growing number of studies have found that distinct behavioral phenotypes exist within many animal species by demonstrating individual differences that occur consistently across time and/or across contexts (Gosling 2001; Sih et al. 2004; Bergmüller 2010). This phenomenon, which is commonly referred to as animal personality, temperament, or coping style, has been frequently reported to occur not only in birds and mammals but also in other vertebrate taxonomic classes, such as in fish, and even in invertebrates (Gosling and John 1999; Stamps and Groothuis 2010). The existence of stable individual differences gives rise to various questions on the cause, function, and adaptive value of animal personality. However, an important first step for understanding the ultimate function of the existence of this phenomenon is to increase our knowledge on its ontogeny (Stamps and Groothuis 2010; Trillmich and Groothuis 2011).

As yet, few studies have focused on the determinants and underlying mechanisms involved in shaping the development of different personality or behavioral phenotypes. Although many studies clearly show that behavioral phenotypes can have a genetic basis (Sluyter et al. 1996; Sokolowski 2001; Dingemans et al. 2002; Van Oers et al. 2004), it becomes increasingly clear that nongenetic effects are also involved in the emergence of individual differences in personality

types. Maternal effects have the potential to influence behavioral traits in offspring. For example, higher concentrations of yolk androgens stimulate territorial behavior in black-headed gull (*Chroicocephalus ridibundus*) chicks (Müller et al. 2009), and maternal stress during pregnancy in mammalian species can exert long-term consequences on personality traits or behavioral profiles, such as on anxiety levels in novel situations (Weinstock 2001; Kaiser and Sachser 2005, 2009; Götz and Stefanski 2007).

In addition to maternal effects, individual features of the offspring can be expected to contribute to behavioral development (Hudson, Bautista, et al. 2011). For example, many studies have shown the importance of sibling interactions for the development of behavioral and social skills (Bekoff 1977; Nunes, Muecke, Lancaster, et al. 2004; Nunes, Muecke, Sanchez, et al. 2004; Nicolás et al. 2011). In addition, the behavioral development of an animal can be directly influenced by features of its early physical and physiological development (Dimitantos et al. 2007; Rödel and von Holst 2009; Rödel and Meyer 2011). In mammalian species giving birth to litters of variable sizes, offspring born in larger litters usually have slower postnatal growth and physiological development, mainly due to the lower share of parental or maternal resources (Mendl 1988; Hudson and Trillmich 2008; Rödel et al. 2008; Prager et al. 2010). Such a slower development might potentially constrain the individual's behavioral responses in challenging situations. For example, studies in different mammalian species show that young with low body mass often have reduced motor skills and behavioral performance (Salas 1972; Gramsbergen and Westerga 1992; Hindell et al. 1999; Muciño et al. 2009). This interplay between an

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animal's physical state and its behavior during the early development could have long-lasting effects in priming an animal's behavioral phenotype (McElreath et al. 2007). For example, the results of a recent study in laboratory rats (*Rattus norvegicus*) indicate that heavier young, which manage to successfully master challenging situations, adopt a more offensive and bolder behavioral style, whereas lighter individuals are less offensive (Rödel and Meyer 2011).

Given the scarcity of studies dealing with the impact of early environmental conditions on animal personality, there is a need for studies under field or seminatural conditions that describe the development of personality traits (Stamps and Groothuis 2010; Hudson, Bautista, et al. 2011). We conducted a long-term study in European rabbits (*Oryctolagus cuniculus*) growing up in a large field enclosure where we related features of early individual development during the nest period with the animals' behavioral responses in standardized tests conducted around maturity. We measured the animals' behavioral responses in a novel environment test and a predator test where subjects were confronted with predator odor (fox feces). These procedures have been previously and successfully used in a study on European rabbits (Rödel et al. 2006), and it has been shown that animals of this species respond behaviorally and physiologically to the odor of a predator (Monclús et al. 2005; Monclús, Rödel, Palme, et al. 2006; Monclús, Rödel, and von Holst 2006). We tested 1) whether individual variation in an animals' early development (measured as body mass measures before weaning) correlated with boldness or exploration behavior in the novel environment test and signs of anxiety in the predator test conducted around maturity and 2) whether behavior in the novel environment test correlated with that of the predator test, which would be consistent with the existence of personality types (Sih et al. 2004).

MATERIALS AND METHODS

Study animals

The study was conducted on European rabbits, which were descendants of wild animals caught in South Germany in 1984. All animals used in our experiments were born and grew up in the seminatural environment of a field enclosure of 2.0 ha in size, situated next to the campus of the University of Bayreuth (Franconia, Germany). The population in the field enclosure was established in 1986 (von Holst et al. 2002). Vegetation in the enclosure consisted of grassland interspersed with groups of trees and a pond in the middle. The number of animals at the onset of the breeding season usually ranged from 18 to 35 per hectare and was 26 during the year of study. According to field data, the density in our enclosure was high but within the range of wild rabbit population densities (Wallage Drees and Michielsen 1989; Palomares 2001). Like in most European rabbit populations in temperate zones, the animals of our study population did not actively take part in the reproductive process within their year of birth (Tablado et al. 2009), and thus, the animals used for the experiments in autumn/winter were hereafter referred to as "subadults."

In addition to the burrows and breeding stops dug by the rabbits (around 40–50), the area contained 16 artificial concrete warrens with interconnected chambers and removable tops. These were used by the rabbits as the main warrens of their group territories and also for breeding. The whole study site could be observed from 2 towers, and all animals could be identified by their individual ear tags. Before we started our study (conducted in 2004), we installed a double electric fence in order to protect the animals against the access of mammalian predators.

Data collection in the field enclosure

Every morning during the breeding season (starting in March), we checked for newborn litters and considered this postnatal day 1. To do this, we prepared all natural warrens and breeding stops dug by the animals with artificial vertical openings to the nest chambers, which we covered with concrete flagstones.

On postnatal day 12, around 1 week before the young leave the breeding burrow, the nestlings were sexed, weighed, and marked individually with colored plastic tags in both ears (Dalton Rototag: $20 \times 5 \times 1$ mm; Dalton Continental GmbH, Bocholt, Germany). As it has been previously shown for European rabbits (Rödel et al. 2008), the body mass on day 12 of the pups born during the breeding season of the year of study was negatively correlated with litter size ($r = -0.46$, $n = 225$, $P < 0.001$). When the animals reached an age of about 3–4 months, they were marked with larger plastic ear tags (Dalton Rototag: $35 \times 10 \times 2$ mm) and with a colored aluminum tag.

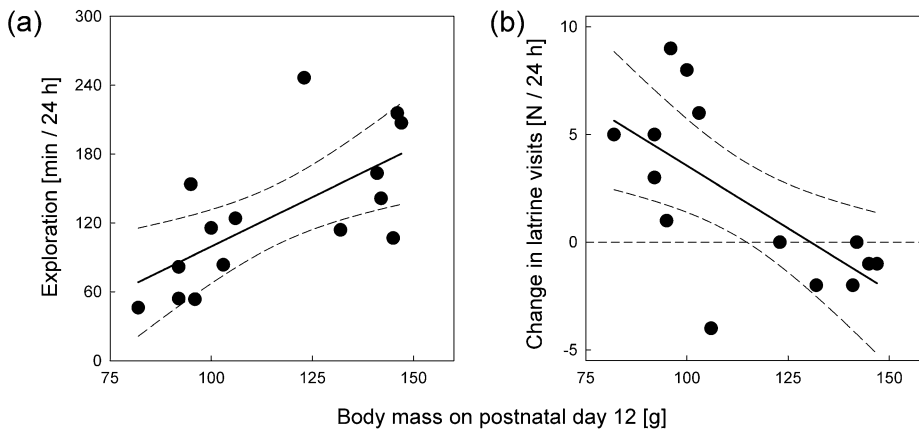
Experimental animals

All experiments were conducted within 1 year, that is, with animals born during the same breeding season. We live-trapped subadult animals for our experiments with wooden traps baited with salted peanuts set early in the morning. This was done during 3 different trapping sessions between mid-September and mid-November in 4-week intervals. We checked the traps from the distance every 30 min to ensure a very low retention time of the trapped animals. The animals were stored singly in gunny sacks in a silent and dark room until 6 of the focal animals were captured. The animals were then transported to the Department's animal facilities. In total, we trapped and translocated 15 animals (7 females and 8 males), which were between 130 and 241 days old when the experiments started (Figure 1). The animals stemmed from 10 different litters; thus, the data set included 2 pairs and 2 trios of litter siblings. Note that at this time (autumn), only a small number of the originally born animals were still alive (cf. Seltmann et al. 2009). Postweaning mortality was mainly due to the action of diseases and the strong predation pressure by birds of prey. During the experiment, one animal died during the second week of the habituation period, most probably due to rabbit hemorrhagic disease. Thus, we only had a sample size of $n = 14$ for the predator test (Figure 1).

Experimental housing conditions

For the experiments, we placed the animals singly in small outdoor wire mesh enclosures (360×460 cm) with sandy soil. Digging was prevented by a wire mesh layer underneath the sand, and a wire mesh on the top was used to exclude raptors. Each of these enclosures contained an artificial concrete burrow consisting of a tube (length: 150 cm and diameter: 20 cm) and a chamber with a removable top (diameter: 60 cm). In total, 6 of these enclosures were available for the experiments. We covered the wire mesh between the different enclosures with wooden blinds in order to prevent social interactions among the animals. To avoid contact with predators, the whole area was additionally surrounded by a 4-m high wall.

Water was provided ad libitum in the center of each enclosure. We placed 2 wooden boxes ($30 \times 30 \times 30$ cm) in each enclosure as feeding sites for the rabbits and ensured that they were at the same distance from the entrance of the burrow. One side of the box was left open. In each box, we placed 2 concrete feeding bowls. The outer bowl contained food pellets (Solikanin Plus, Muskator-Werke GmbH, Düsseldorf, Germany), and rabbits could access the food without entering the box. During

**Figure 1**

Pup body mass and adult behavior. Correlation between the body mass of European rabbits measured on postnatal day 12 and their behavioral responses (a: exploration) in a novel environment test and (b: changes in defecation rate) during a predator test conducted when the animals had reached an age of 4–7 months. Regression lines with 95% confidence intervals are given; see Table 1a,b and text for statistics.

the predator test (see below), we placed fox feces in the inner bowl of one of the boxes, whereas the inner bowl of the other box was left empty. Fox feces were collected from captive animals of the Zoological Garden in Hof/Saale (Franconia, Germany). See Rödel et al. (2006) for more details on this experimental design.

On the top of each of the enclosures, we installed a video camera allowing us to observe the whole area. We ensured that the location of the camera allowed us to register the behavior of the rabbits when feeding at the boxes. In addition, we fixed a red light bulb (40 W) in each enclosure. Thus, we were able to continuously videotape the animals' behavior all-day round during each 24-h period, using time-lapse recorders. All recordings were started at around noon.

Experimental procedure

Novel environment test

About 30 min after being caught in the field enclosure, the animals were entered singly into the concrete warrens of the experimental enclosures. We first blocked the entrance of the warrens with gunny sacks and waited for another 30 min in order to provide the animals enough time to recover from the transport to this novel environment. Then, we simultaneously opened the entrances of the burrows of all 6 enclosures and left the animals undisturbed by any human presence. Video recordings were started immediately, and we videotaped the animals for the first 24 h after entering them into the experimental enclosures (Figure 1). We focused on 2 variables in order to describe their behavioral response to this novel environment test.

Start of activity. The lapse of time between the opening of the burrow of the experimental enclosure and the moment when the animal left the burrow for the first time.

Exploration. The time outside the burrow that the animal spent moving or stopping while sniffing and looking around.

Predator test

After the animals were habituated to the enclosures for 14 days, we again videorecorded them for 24 h in order to get basal values of their individual behavioral patterns. Two days later, in the morning of the 16th day of the experiment, we then confronted the animals with the odor of a potential predator by presenting fox feces in the empty bowl next to the feeding bowl of the box where the animals preferably fed during the previous week. Video recordings (for 24 h) were immediately started again after the presentation of the fox feces. For analysis, we then calculated the absolute changes between the 2 successive video recordings with respect to the following 3 variables:

Change in time spent outside the burrow. The change in the time that the animals spent outside the burrow, regardless of their current activity.

Change in latrine visits. The change in the number of times the animal visited the latrine and obviously defecated. Note that 1 or 2 latrines could be found in all the enclosures when the control experiment started, that is, all the animals chose a conspicuous place where they predominantly defecated.

Change in scanning while feeding. The change in the number of times the animals showed any signs of alertness while feeding. We recorded an animal's behavior as a scanning event when it stopped feeding and raised the head, turned around, looked at both sides, looked back, or was standing at the hind legs. We calculated the absolute change in the number of scans per hour feeding time during both periods. Details are given in (Monclús et al. 2005).

Ethical note

Almost all the animals gained weight during the experiment. The average starting mass of the animals when being entered into the small enclosures was 1164 g (± 132 standard deviation [SD]), and the final mass after the experiments was 1244 g (± 164 SD). Thus, the average weight gain was 7.7% (minimum: -1.9%, maximum: 23.7%) within 16 days, which is within the normal range for European rabbits of this age class (Monclús and Rödel 2009). After the experiments, the animals were retransferred to outdoor enclosures. Permissions for population biology studies on European rabbits and for animal experiments were provided by the Government of Middle Franconia, Germany (211-3894a; 621-2531.32-5/05).

Data analysis

The videos were blindly analyzed by 2 persons naïve to the experimental design. Both persons analyzed recordings of the different experimental periods in a random order, and we checked for observer effects by including observer identity in the multivariate models (see below) as a fixed factor. However, there were no significant effects, and thus, this factor was excluded before calculating the final models. All statistical analyses were done using the software R version 2.11.1 (R Development Core Team 2010).

First, we tested for changes in the different behavioral responses before and after confrontation with fox odor by Wilcoxon tests. We used nonparametric statistics here because some variables deviated from a normal distribution (checked by Shapiro–Wilk test).

Second, we used multivariate mixed-effects models for testing the effects of 5 different predictor variables: litter size (covariate), sex (factor with 2 levels), nestling body mass (covariate; measured on postnatal day 12), and age and body mass measured at the onset of the experiments (covariates) on the animals' behavioral responses in the different test situations (see above for the calculation of these response variables). The effects of age and body mass measured at the onset of the experiments were tested separately in order to avoid including these collinear predictors within the same statistical models (see separate statistics on these effects in the text). In addition, we included litter identity as a random factor because some of the focal animals stemmed from the same litters (see above) and thus shared the same conditions during their early development. As a consequence, we were running different models during the 2 tests, dependent on the number of response variables (as given in Table 1a,b). Analyses were done by multivariate linear mixed-effects models (LMM) using the R package lme4 (Bates 2005). The program R does not directly provide *P* values for LMM calculated with this package. Thus, we extracted the *P* values and also the parameter estimates by Markov chain Monte Carlo sampling based on 10 000 simulation runs (Baayen et al. 2008) using restricted maximum likelihoods.

It has been frequently emphasized that family-wise error adjustments are required when using multiple testings (here: multiple linear mixed-effects models) if one final conclusion or decision is drawn (Bender and Lange 2001), although the use of excessive alpha-level corrections has been frequently criticized (Moran 2003; Nakagawa 2004). In order to control for such a potential inflation of type-I error within our 2 experiments (novel environment test and predator test; see Table 1a,b), we calculated multivariate analyses of covariance (MANCOVAs) (with *P* values based on Pillai's trace) including the different response variables in one model (Overall and Klett 1972). Unfortunately, MANCOVA calculations including random factors are not (yet) available in R, and thus, we omitted the random factor (litter identity) from these calculations. In both cases, the predictor variable, which was found to be significant for the linear mixed-effects model,

was also significant in the MANCOVA model, indicating no notable problems with type-I error inflation with respect to the results on the 2 different experiments (see Table 1a,b). We also checked for multicollinearities among all predictor variables by calculating variance inflation factors (VIF) for each model. All VIF values were lower than 2 indicating no problems with multicollinearities (Fox and Monette 1992).

Third, we tested for consistencies across time and contexts by mixed-effects models, again including litter identity as a random factor (see description above). In particular, we aimed to focus on consistencies between the behavioral variables measured during the 2 test situations, which were found to be related to features of the animals' early development.

Normality of the residuals of all parametric models was checked visually by normal probability plots and with the Shapiro-Wilk test, and we assured the homogeneity of variances and goodness of fit by plotting residuals versus fitted values (Faraway 2006). For significant mixed-effects models, we calculated Nagelkerke's Pseudo- R^2 (based on maximum likelihoods), which can be used as a measure of explained variance for this kind of analysis (Nagelkerke 1991).

RESULTS

Behavioral responses

Novel environment test

Latency times until the animals left the artificial burrow system for the first time after being entered into the small enclosures varied between 3.7 and 256.6 min, and the average was 71.0 min (± 16.8 standard error [SE]). Within the first 24 h after being entered, the animals showed exploration behavior (moving around while sniffing) for on average 126.4 min (± 15.8 SE), varying between 46.2 and 246.5 min, and adding up to on average 8.7% of the total observation time.

Predator test

A comparison between the animals' behavior during the 24-h recordings before and after confrontation with fox feces did not reveal a statistical differences in the time spent outside the

Table 1

Multivariate linear mixed models on the effects of different predictor variables on the behavioral responses measured during the (a) novel environment test and (b) predator test

	Response variable	Predictor variable	β_{MCMC}	95% _{lower}	95% _{upper}	P_{MCMC}
(a) Novel environment test ($n = 15$)	Start of activity	Sex (male)	29.66	-112.94	48.80	0.43
		Body mass day 12	0.53	-1.31	2.27	0.53
		Age	0.39	-0.80	1.76	0.51
	Exploration	Sex (male)	40.03	-14.50	89.61	0.13
		Body mass day 12	1.75	0.65	2.92	0.004
		Age	-0.38	-1.21	0.39	0.31
(b) Predator test ($n = 14$)	Change in time spent outside the burrow	Sex (male)	3.99	-13.24	20.06	0.62
		Body mass day 12	-0.17	-0.58	0.23	0.38
		Age	0.08	-0.23	0.36	0.53
	Change in latrine visits	Sex (male)	1.48	-2.71	5.77	0.45
		Body mass day 12	-0.11	-0.20	-0.02	0.027
		Age	-0.03	-0.10	0.04	0.29
	Change in scanning while feeding	Sex (male)	3.86	-3.68	12.00	0.30
		Body mass day 12	-0.11	0.08	0.31	0.26
		Age	0.01	-0.14	0.14	0.97

P values and parameter estimates (including 95% confidence intervals) were calculated by 10 000 Markov chain Monte Carlo simulation runs. A backward elimination of nonsignificant predictors (not shown) did not lead to different results than obtained by calculating *P* values for the full model including all main effects, although *P* values of the 2 significant effects decreased. In order to consider potential the family-wise error inflations of multiple models within the 2 tests, we calculated 2 MANOVAs including all respective response variables. Results revealed significant effects for the predictor "body mass day 12" in both cases (a: $F_{2,12} = 5.11$, $P = 0.025$; b: $F_{3,10} = 4.86$, $P = 0.024$), indicating no notable problems with error inflation. Significant effects are reported in bold.

burrow (Wilcoxon test: $Z = 1.73$, $n = 14$, $P = 0.084$; before presentation, mean = $50.23\% \pm 5.51$ SE; after presentation, mean = $43.7\% \pm 5.98$ SE) or in the frequency of latrine use ($Z = 1.79$, $n = 14$, $P = 0.074$; before presentation, mean = 7.1 ± 1.4 SE; after presentation, mean = 9.0 ± 1.3 SE). There was also no significant increase in the frequency of scanning after the confrontation with fox odor ($Z = -1.49$, $n = 14$, $P = 0.14$) with average scanning rates per hour of $3.98 (\pm 0.64$ SE) before and $4.49 (\pm 1.78$ SE) after the fox odor confrontation. These results were surprising because significant effects have been found in previous studies (Monclús et al. 2005).

Effects of individual characteristics

Novel environment test

The latency to the start of activity was not correlated with pup body mass, sex, or with their age when tested (Table 1a). In addition, there were no significant effects of litter size or of the body mass of the animals measured at the onset of the experiment ($P_{\text{MCMC}} > 0.10$). The latter predictor variables were always tested in separate models because of the strong biological relationship between litter size and pup body mass (cf. Rödel et al. 2008) and of age and body mass when tested (cf. Rödel, Bora, Kaetzke, et al. 2004).

However, there was a significant correlation between pup body mass measured on postnatal day 12 and the time that the animals spent exploring their novel environment ($R^2_{\text{Nagelkerke}} = 0.330$), that is, animals with a higher pup body mass explored longer than lighter individuals (Figure 1a).

Predator test

Also here, we did not find correlations with the tested predictor variables (see Table 1b); including litter size and body mass when tested ($P_{\text{MCMC}} > 0.10$), except for the significant effect of pup body mass on the changes in the frequency of latrine visits ($R^2_{\text{Nagelkerke}} = 0.402$; statistics in Table 1b). Animals with a higher body mass on postnatal day 12 did not change or even decreased their frequency of latrine visits, whereas animals with a lower pup body mass increased the frequency of visits in the course of this experiment (Figure 1b).

We also checked if the decrease in latrine use and thus of the animals' purported defecation rates was just due to changes in feeding behavior. However, the data did not support this alternative hypothesis: There was neither a significant change in feeding time before and after the confrontation with fox feces (Wilcoxon test: $Z = -0.094$, $n = 14$, $P = 0.93$) nor a significant correlation between the individual changes in feeding time and the changes in the frequency of latrine visits (LMM: $R^2_{\text{Nagelkerke}} = 0.092$, $\beta_{\text{MCMC}} = 0.28$, $n = 14$, $P_{\text{MCMC}} = 0.27$).

Correlations across contexts

There was a significant negative correlation between the 2 behavioral responses, which were found to be related to the animals' pup body mass. Those animals that spent more time exploring after entering the novel environment showed a lower increase in the frequency of latrine visits 2 weeks later when confronted with the odor of fox feces (LMM: $R^2_{\text{Nagelkerke}} = 0.306$, $\beta_{\text{MCMC}} = 0.04$, $n = 14$, $P_{\text{MCMC}} = 0.024$; Figure 2). All other correlations between the parameters measured during the 2 tests were not significant ($P_{\text{MCMC}} > 0.10$).

DISCUSSION

Conditions experienced early in life can have profound effects during later life (Lindström 1999; Lummaa and Clutton-Brock

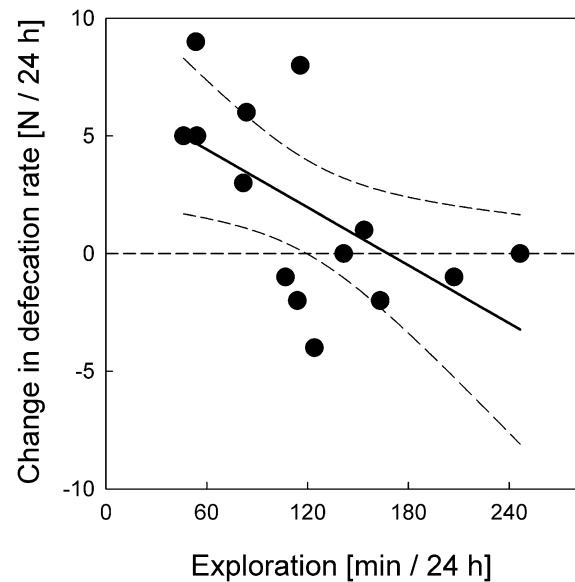


Figure 2

Consistency between contexts. Correlation between the time that subadult European rabbits spent exploring during the novel environment test and the change in defecation rate during the predator test conducted 2 weeks later. Regression line with 95% confidence intervals is given; see text for statistics.

2002), and such long-term fitness consequences have also been shown in European rabbits (Rödel et al. 2009). One of the drivers of such long-lasting effects might be the impact of early-life parameters on an individual's behavioral phenotype (Stamps and Groothuis 2010). With our study, we provide evidence for such purported long-term effects and show how these behavioral traits were correlated across contexts: Animals with higher body mass during preweaning life were more exploratory and less anxious around reaching maturity as quantified by their responses in standardized behavioral tests. Other factors such as sex, litter size, and the body mass of the animals when being tested did not notably influence their behavioral responses.

When confronted with a simulated predator presence, European rabbits (Monclús et al. 2005; Monclús, Rödel, and von Holst 2006) and also many other vertebrate species typically tend to decrease activity and increase vigilance or scanning in order to reduce the probability of direct predator encounters (Endler 1991; Kats and Dill 1998). In our study, we did not find statistical evidence for such respective changes, which might be partly accounted to our rather moderate sample size. In addition, there was no significant decrease in the change in latrine visits, which is frequently considered as an indicator of anxiety (Hall 1934; Archer 1973). However, the direction of changes in this emotionality response was related to early-life characteristics of the animals: Animals with a lower preweaning body mass showed higher signs of anxiety, whereas individuals with higher preweaning mass tended to be less anxious when confronted to simulated predator presence.

We propose that early-life differences in behavior or experience between heavier and lighter individuals might be an important mechanism underlying the observed long-term correlations. Heavier juveniles are usually more successful or dominant in agonistic encounters or while play fighting with same-aged conspecifics (Rödel and von Holst 2009; Eccard and Rödel 2011). In contrast, lighter animals might be predisposed from early stages to have higher levels of anxiety as

they might be more prone to negative interactions with conspecifics or predators (Waite 1987). This might result in positive feedback loops leading to the adoption and manifestation of distinct personality types early in life (Sih et al. 2004; McElreath et al. 2007). Such effects of early experience on the development of personality traits have been, for example, observed in rainbow trout (*Onchorhynchus mykiss*) (Frost et al. 2007), and a study in juvenile laboratory rats showed that the juvenile body mass was positively related to an increase in exploratory behavior between successive open field tests conducted during different age classes (Rödel and Meyer 2011). Interestingly, and in accordance with our hypothesis, the subadult body mass of the rabbits in our study was not related to any of their behavioral responses.

It has been suggested that hormones might be important mediators of the animals' behavioral phenotypes (Koolhaas et al. 1999, 2010; Sih et al. 2004). As it has been shown in domestic rabbits and laboratory rats, the body mass or growth rate around weaning, which we use here as a proxy for the animal's early development, is also related to their hormonal development (Rödel et al. 2010; Hudson, Maqueda, et al. 2011). Thus, it might be feasible that differences in hormonal profiles and not (only) the body mass or size itself were the drivers of the observed long-term effects.

Although we cannot completely rule out that the effects described here are genetically driven, we think that such effects were of rather minor importance. In animals with variable litter sizes, such as rabbits, variation in weaning body mass is mainly attributed to the number of offspring in the litter (Rödel et al. 2008), with individuals born in larger litters being typically smaller due to the lower share of milk or, more generally, maternal resources obtained (Mendl 1988; Hudson and Trillmich 2008), and such a correlation was also apparent in this study (see "MATERIALS AND METHODS" section). At least in European rabbits, such litter effects are unlikely to have a strong genetic basis because litter size of individual females fluctuates strongly within the breeding season (Rödel, Bora, Kaiser, et al. 2004; Eccard and Rödel 2011).

We found a link between the individual responses of the animals measured in 2 successive tests related to different functional contexts: Animals, which were more exploratory and thus bolder in the novel environment test, were also less anxious when confronted with predator odor. Such a correlation between the responses in 2 different contexts can be interpreted as an indication for the existence of personality types (Sih et al. 2004; Bergmüller 2010). A possible explanation might be that animals that explore more might reduce their anxiety as the control of the situation is one of the main factors that have been shown to buffer the stress response (Sapolsky 1992; Boissy 1995). However, given the correlational nature of the result, it remains open whether there was indeed a causal relationship (coupling) between exploration behavior and anxiety or if the ontogeny of both personality traits is just driven by the same feature of the animals' early ontogeny, namely their pup body mass or some correlated physiological (hormonal) traits that we did not measure here.

CONCLUSIONS AND IMPLICATIONS

Our study highlights the importance of features of an animal's early development in priming its behavioral phenotype during later life. Further tests using cross-fostered individuals with experimentally manipulated postnatal growth will help to rule out or to assess the impact of maternal and prenatal effects on the relationship described here, and measuring hormonal profiles might help to better understand the underlying mechanisms.

Our results not only have implications for our understanding of the ontogeny of personality types in mammals but may also be relevant for the design and interpretation of biomedical studies with laboratory mammals. Using subjects without knowing their early development is a common practice; however, the assumption that standardized breeding conditions produce standardized individuals is unlikely to be true (Hudson, Bautista, et al. 2011). As we can show here, natural variation in weaning mass or related traits of the early development have the potential to influence the behavioral responses of European rabbits in standardized tests and possibly also in other small mammals commonly used in the laboratory. Such individual differences might have the potential to affect the outcome of psychobiological tests conducted with laboratory mammals.

In addition, the long-term effects described in this study might constitute an important mechanism of how features of the early development can impact on an individual's later life. It is well known that a lower growth of an animal before or around weaning can have persistent effects on its physical and/or physiological development with potential consequences for health, fecundity, and survival (Sedinger et al. 1995; Lindström 1999; Lummaa and Clutton-Brock 2002). Our study suggests that there is also a link between early growth and the ontogeny of the individual behavioral phenotype (cf. Rödel and Meyer 2011), which in turn has the potential to affect the fitness of an animal (reviewed in Smith and Blumstein 2008).

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