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Cortisol during adolescence organises personality traits and behavioural syndromes

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ABSTRACT

Despite the growing evidence for the importance of developmental experiences shaping consistent individual differences in behaviour and physiology, the role of endocrine factors underlying the development and maintenance of such differences across multiple traits, remains poorly understood. Here, we investigated how an experimental manipulation of circulating glucocorticoids during early adolescence affects behavioural and physiological variation and covariation later in life in the precocial cavy (*Cavia aperea*). Plasma cortisol concentrations were experimentally elevated by administering cortisol via food for 3 weeks. Struggle docility, escape latency, boldness, exploration and social behaviour were then tested three times after individuals attained sexual maturity. In addition, blood samples were taken repeatedly to monitor circulating cortisol concentrations. Exogenous cortisol affected mean trait expression of plasma cortisol levels, struggle docility and escape latency. Repeatability of cortisol and escape latency was increased and repeatability of struggle docility tended to be higher (approaching significance) in treated individuals. Increased repeatability was mainly caused by an increase of among-individual variance. Correlations among docility, escape latency and cortisol were stronger in treated animals compared to control animals. These results suggest that exposure to elevated levels of cortisol during adolescence can alter animal personality traits as well as behavioural syndromes. Social and risk-taking traits showed no correlation with cortisol levels and were unaffected by the experimental manipulation, indicating behavioural modularity. Taken together, our data highlight that cortisol can have organising effects during adolescence on the development of personality traits and behavioural syndromes, adding to the increasing evidence that not only early life but also adolescence is an important sensitive period for behavioural development.

1. Introduction

While consistent among-individual differences in behaviour, i.e., animal personality (Dingemanse and Wolf, 2010; Wolf and Weissing, 2010), are common across taxa, our knowledge about the mechanisms driving and maintaining these differences is still rather limited (Dingemanse and Wolf, 2010; Groothuis and Trillmich, 2011; Koolhaas et al., 2010; Stamps and Groothuis, 2010a, b). Variation in personality arises from the combined influence of genetic and environmental factors (Dingemanse and Dochtermann, 2014; Dobzhansky et al., 1977; Han and Dingemanse, 2015). Hence, understanding the contribution of developmental experiences in shaping adult personality traits is fundamental to a comprehensive understanding of the generation of consistent individual differences (Groothuis and Trillmich, 2011; Stamps and Groothuis, 2010a, 2010b).

Because phenotypic plasticity may involve costs (Snell-Rood, 2013), the adjustment of personality traits may potentially be limited to sensitive windows during development (Bateson, 1979). Sensitive windows are defined as periods or developmental stages in which experiences shape phenotypic characteristics to a larger extent than in other periods or stages (Fawcett and Frankenhuis, 2015). The canalisation of behaviour within sensitive windows may represent an evolutionary adaptation when the costs and benefits of sampling and reacting to information vary between life stages (Panchanathan and Frankenhuis, 2016). So far, most attention has been given to early life periods, usually the pre-natal and early post-natal life (Bateson, 1979; Curley and Branchi, 2010; Fawcett and Frankenhuis, 2015) while recently, also the early adolescent period has been identified as one such sensitive window in which many species adjust to their environment (Blakemore and Mills, 2014; Ellis et al., 2012; Sachser et al., 2013; Sebastian et al., 2010).

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Across taxa, there is strong evidence that glucocorticoids (GCs) mediate environmental challenges including stressors through pleiotropic effects on multiple neurological, physiological and behavioural traits (Hau et al., 2016). Brain circuits involving the prefrontal cortex and parts of the limbic system, such as the hippocampus and amygdala, play a central role in stress reactivity as they contain high densities of gluco- and mineralocorticoid receptors, which bind adrenal steroids that are regulated by the hypothalamic–pituitary–adrenal (HPA) axis (Herman et al., 1997). During adolescence, all these brain structures undergo structural and functional maturation (Brydges, 2016; Uematsu et al., 2012), which is likely to be affected by variation in the main glucocorticoids, cortisol and corticosterone (Lupien et al., 2009; McEwen, 2007).

Environmental effects such as predation pressure, population density, food availability or environmental predictability that are experienced throughout development, can have canalising effects on the hormonal phenotype that last into adulthood, thereby altering behaviour and fitness across the life span (Brown and Spencer, 2013; Kapoor et al., 2006; Love et al., 2005; Monaghan and Haussmann, 2015). Hence, glucocorticoids are prime candidates by which environmental conditions experienced during early adolescence create differences in adult personality traits among individuals.

Most studies investigating effects of GCs on personality traits to date focus on the comparison of the mean phenotypic response of a trait in control versus experimentally stressed animals. Exposure to corticosterone during development, for example, leads to increased activity and novel environment exploration in adult Japanese quail (*Coturnix coturnix japonica*) (Zimmer et al., 2013). In small rodents, stress or direct exposure to stress hormones during adolescence generally leads to more anxious adults (for review see McCormick and Green, 2013).

Notably, already two decades ago, the importance of variation around the mean has been pointed out, as this variation provides the basis on which selection can operate (Boake, 1989; Hayes and Jenkins, 1997). Although this notion was for a long time somewhat neglected, researchers have now started to quantify the contribution of within-versus among individual variation in shaping behavioural variation (Dingemanse, Dochtermann, 2013; Westneat et al., 2015). Within-individual variation indicates flexibility and measurement error (Dingemanse, Dochtermann, 2013; Jenkins, 2011). Among-individual variation, on the other hand, indicates the extent to which individuals differ in their mean trait expression. Nutritional stress during the nestling stage of zebra finches (*Taeniopygia guttata*), for example, increases differences in exploration and activity between individuals (i.e. increasing among-individual variance) (Careau et al., 2014a). The proportion of among-individual variance divided by the total phenotypic variance is known as trait repeatability (Falconer and Mackay, 1996). Its quantification is one of the cornerstones of animal personality research as repeatability can set the upper boundary for heritability and thus indicates the evolutionary significance of a trait (Falconer and Mackay, 1996). Recent research indicates that the relative contributions of these two variance components and hence repeatability can show sex-specific and experience-specific variation within species (Briffa, 2013; Careau et al., 2014a; Han and Dingemanse, 2017; Jenkins, 2011; Royauté and Dochtermann, 2017). For example, hermit crabs (*Pagurus bernhardus*) exposed to a perceived predation threat show increased repeatability by decreasing within-individual variation (Briffa, 2013). Similarly, dietary stress increases repeatability in anti-predator behaviour of house crickets (*Acheta domestica*) by decreasing within-individual variation (Royauté and Dochtermann, 2017).

In addition to effects on single personality traits, exposure to stressful environments during development has the potential to alter the covariance between traits and hence affect behavioural syndrome structure (Killen et al., 2013). Pesticide exposure has been shown to weaken correlations between personality traits in jumping spiders (*Eris militaris*) (Royauté et al., 2015). Similarly, dietary stress during development uncoupled correlations between different personality traits in

great tits (*Parus major*) (Carere et al., 2005) and between activity, metabolic rate and stress-induced corticosterone in zebra finches (Careau et al., 2014b). On the other hand, food deprivation of European bass (*Dicentrarchus labrax*) led to a stronger correlation between risk-taking and metabolic rate (Burton et al., 2011) and unpredictable food conditions revealed an association between metabolic rate and territory acquisition in Atlantic salmon not being present in controls (*Salmo salar*) (Reid et al., 2011). Despite the existing evidence that environmental characteristics can influence the covariance structure, the developmental mechanisms that shape the emergence or break up the existence of behavioural syndromes are poorly understood so far (Groothuis and Trillmich, 2011; Han and Dingemanse, 2015).

Here we propose that circulating glucocorticoids during early adolescence (also called pre-puberty) represent a key mechanism by which stressful experiences shape personality traits, levels of among- and within individual variation as well as among-individual covariation between personality traits (i.e. behavioural syndromes, structural consistency). To test this, we manipulated levels of circulating cortisol (CORT) for a period of 3 weeks during early adolescence to simulate chronic stress in wild cavies (*Cavia aperea*). After the end of the treatment, behaviours indicating stress-coping, risk-taking and social tendencies as well as baseline cortisol levels were measured three times throughout life. We then compared mean trait expression, trait variances and correlations among traits between treated animals and control animals.

We hypothesise based on the literature (1) that a three-week CORT elevation modifies the developmental trajectory resulting in more anxious, less risk-taking and potentially less social individuals. We further hypothesise (2) that the CORT manipulation leads to an increased repeatability compared to untreated animals. We hence investigate if changes in repeatability estimates arise due to effects on among- or within-individual variance components. In addition to changes in among-individual variation, elevated stress-hormone levels may affect within-individual variation, i.e. individual flexibility (Briffa, 2013; Careau et al., 2014a; Royauté and Dochtermann, 2017). Finally, (3) we test if the manipulation of CORT during development has the potential to alter behavioural syndromes up into adulthood (Careau et al., 2014b).

2. Material and methods

2.1. Animals and maintenance

Experimental animals were bred in outdoor enclosures during spring by adding one male to two females each. After weaning, juvenile cavies ($n_{\text{mothers}} = 24$; $n_{\text{juveniles}} = 60$; ♀26, ♂33) were individually marked with a pit tag (ID-100, TROVAN, passive transponder system; Euro ID, Weilerswist, Germany; dimensions: diameter: 2.12 mm; length 11.5 mm) inserted subcutaneously into the interscapular region and kept in 24 unrelated same sex groups throughout the experiment. Siblings were divided such that half of them were later assigned to the control group and the other half to the treatment group.

Group enclosures were located in two rooms directly adjacent to each other. Enclosures could be divided into multiple compartments by trap doors allowing a separation of individuals for behavioural testing. Each compartment measured 0.8 m² and contained a brick stone, a water bottle, a feeder, hay and an opaque shelter. Wood chips were used for bedding. Except for behavioural testing, the trap doors stayed open, allowing animals to move freely within the enclosure and interact with each other. Four males became aggressive when approaching adulthood and were thus separated prior to the last test phase. They had still visual, olfactory and auditory contact to their former group mates.

Animals were kept under natural light conditions with additional artificial light from 6 am to 7 pm, at constant temperatures of $20 \pm 2^\circ\text{C}$. Hay, guinea pig pellets (Höveler, Germany) and water were available ad libitum and supplemented with fresh greens (apples,

carrots, bell-pepper, cucumber, grass and branches) three times a week.

2.2. Cortisol manipulation

In cavies, weaning occurs at around 21 days of age and animals reach sexual maturity around 45–60 days of age. The experimental CORT manipulation started at an age of 33 ± 2 days, thus during early adolescence, when animals had an average body mass of 310 ± 8 g. All animals sharing an enclosure were either assigned to the treatment or the control group.

Three milligram cortisol powder (Ref. Nr. 352450050; Arcos Organics) were applied to a slice of cucumber for the treatment group ($n = 30$; ♀13, ♂17) while control animals received a same sized slice of cucumber without cortisol. The CORT powder quickly infiltrated the watery cucumber and evoked no aversion in the animals. The amount of cortisol administered was calculated based on results from pilot studies testing dosages of 5–12 mg cortisol on slightly older animals (see [Appendices A, B](#)). The 5 mg dosage induced CORT levels comparable to an acute stressor ([Guenther and Trillmich, 2013](#)). In cavies basal cortisol levels decrease with age (unpublished data), to receive a similar peak concentration in the younger experimental animals, we chose to use 3 mg. The animals received either the control or the treatment for two out of 3 days for a total of 21 days. This procedure has been shown to result in a robust increase of baseline cortisol levels while avoiding supra-physiological elevation (see [Appendix B](#)). On experimental days, individuals were separated by closing the trap doors of the enclosures in their home cage at 9 am. Subsequently, a slice of cucumber (with or without cortisol) was presented on the brick stone of the enclosure. Every 15 min thereafter, the experimenter checked if the cucumber was consumed by the animal. Trap doors were opened and animals could again move freely and interact with each other when cucumber slices were consumed. Usually, the cucumber was consumed quickly within 34 ± 12 min. On six occasions (4 treatment, 2 control), individuals did not readily consume the cucumber. In those cases, the animals remained separated during the night while maintaining visual, olfactory and auditory contact to their group mates. The next morning, the cucumber was consumed in all cases.

2.3. Personality assays

Behavioural testing was conducted twice during adolescence and once more when the animals were fully adult. The first round started 1 week after the end of the CORT treatment (early adolescence; age: 61 ± 3 days). Every animal was subjected to a novel object test, a free exploration test, a struggle docility test, a hand-escape test, and a social interaction test in a balanced design, resulting in 4 sequences in which each test is followed and preceded by each possible other test ([Díaz-Uriarte, 2002](#)). The test sequence was the same in all three test rounds. Animals completed all behavioural tests of a test round within 2 weeks. To minimise potential carry-over effects between tests, animals were given at least 1 day rest after each test. The second test round was conducted 1 month later (late adolescence; age: 92 ± 3 days) and the final test round was again conducted 1 month later when the animals were fully adult (age: 123 ± 3 days). Behavioural tests were conducted from 9 to 12 am and 2–5 pm. Previous studies confirmed no time-of-day effect ([Guenther et al., 2014b](#); [Guenther and Trillmich, 2013](#)). Temporal and cross-context consistency of all behavioural tests has been validated in earlier studies ([Guenther et al., 2014a,b](#); [Guenther and Trillmich, 2015](#)). All behavioural tests were conducted by two well-trained observers blind to the treatment.

2.3.1. Novel object test

To test for boldness, an individual was separated from its group by closing a trap door in its home enclosure. A novel object was then introduced about 30 cm in front of the shelter under which individuals usually hide from the experimenter. A 4 cm green eggcup was used as

novel object during the first test round, a 10 cm yellow plastic duck was used for the second test round and a 10 cm grey plastic cylinder was used during the third test round. The animal's interactions with the object were video-recorded for 1 h. As a measure of boldness, we used the number of touches within 15 min after the object was first touched by the individual.

2.3.2. Free exploration

Exploration was tested by giving the animal a free choice to explore an unknown area. The individual was introduced into an enclosure (0.8×0.8 m) equipped with a feeder, a shelter and a water bottle at 9 am and left alone for 5 h to habituate to this new enclosure. At 2 pm, a trapdoor connecting the enclosure with an unknown area (0.8×3.0 m) was opened and the animal was given 3 h to explore this new area. The number of trips into this area was recorded by a movement detector located behind the trap door.

2.3.3. Struggle docility

The individual was removed from the enclosure and the time it spent struggling when held on its back in the hand of an experimenter was measured for 30 s before the animal was placed back in the home enclosure. Individuals that struggled for a long time, actively cope with this stressful situation, hence, high values are interpreted as low docility.

2.3.4. Hand-escape latency

The individual was captured from its enclosure and weighed. Thereafter, the animal was placed on the open hand of the experimenter to measure how the animals react to this mildly stressful situation. The hand was held approximately 5 cm above ground in the enclosure of the animal. The latency to leave that hand was measured for 60 s. If the animal had not left the hand voluntarily within this time, the animal was placed under a shelter. Remaining on the hand of the observer ("freezing-behaviour") was interpreted as passive stress-coping.

2.3.5. Sociopositive and aggressive behaviour

For the social encounter test, the subject was separated by closing a trap door in its enclosure. A second shelter was introduced into this compartment to allow both the focal and the stimulus animal to hide if they lacked motivation to interact with each other. After the focal animal was gently chased under one shelter, a same-sex stimulus animal was introduced beneath the second shelter. Stimulus animals were unfamiliar to the focal animals, but of the same age and size (four males and four females) and did not participate in other experiments. Upon introduction of the stimulus animal, the number of aggressive (fixation, demonstrating a curved body posture, chasing, teeth chattering, attacking and biting), sociopositive (nudging, naso-nasal and naso-anal sniffing) and social avoidance (evade) interactions initiated by the focal animal were scored for 15 min. Behaviour was recorded live by the experimenter through continuous sampling, using ethograms based on [Rood \(1972\)](#). In case severe fighting occurred, the test was interrupted and the focal animal scored the maximum amount of aggressive interactions observed during all tests. This happened once in the second test round and once in the third round.

2.4. Basal cortisol level

One week after each round of behavioural testing, a blood sample was collected from one animal per enclosure per day. Blood samples were taken at $12:00 \pm 30$ min within 3 min after catching an animal to avoid an increase of cortisol due to disturbance. To collect the sample, the animal's marginal ear vein was punctured and about 70 µl blood was collected into heparinized capillaries. Plasma was separated after the blood was centrifuged for 10 min at 8000 rpm and then stored at -20°C until further analysis.

Analysis of cortisol was done using a competitive enzyme

immunoassay (RE52061 IBL, IBL International GmbH, Hamburg, Germany) using specific antibodies against cortisol (for details of the procedure see: Kaiser et al., 2003; Künzl et al., 2003). The antibody used cross-reacted with relevant steroids as follows: prednisolone 29.8%, 11-desoxycortisol 8.48%, cortisone 4.49%, prednisone 2.12%, corticosterone 1.99%, 6b-hydroxycortisol 1.03%. Samples were evenly distributed across seven assays. The intra-assay % CV was 3.8%, the inter-assay % CV was 6.9%.

2.5. Ethical note

All experimental procedures were in accordance with German animal protection laws. Facilities were approved (2014) by the local government authority responsible for health, veterinary and food monitoring (Gesundheits-, Veterinär- und Lebensmittelüberwachungsamt Bielefeld). The experiments were performed under licence 84-02.04.202016.A071 LANUV, NRW, Germany. After the study, all animals remained at the institute under stock conditions.

2.6. Statistical analyses

All analyses were performed using R version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>) with the package “MCMCglmm” for Bayesian mixed models (Hadfield, 2010) and the package “gap” to control for false discovery rates.

2.6.1. Effect of cortisol treatment on average trait expression and trait repeatability

To calculate the effect of the cortisol treatment on the development of an individual's mean phenotype (assessed traits: body mass, exploration, boldness, docility, hand-escape latency, sociopositive behaviour, aggressive behaviour and baseline cortisol), we used univariate Bayesian mixed models (Hadfield, 2010). Treatment, sex, test round (1–3) and the two-way interactions between treatment and sex and treatment and test round were fitted as fixed effects. In addition, individual ID nested in mother ID were fitted as random effects. Stimulus ID was fitted as additional random effect in the models for sociopositive behaviour and aggressiveness. Body mass, hand-escape latency, struggle docility and baseline cortisol levels were fitted assuming a Gaussian distribution. Models for boldness, exploration and sociopositive behaviour were fitted with a Poisson distribution and the model for aggressiveness had to be fitted as binary model because aggressive interactions did only occur in < 50% of all social confrontation assays. For Gaussian and Poisson models, we used slightly informative inverse-Wishart priors, assuming the residual variance to be 10^3 . For the binary model, the variance was assumed to be 1. All models were run with 525,000 iterations, a burn-in of 25,000, and a thinning interval of 500. This yielded Monte Carlo Markov chains with a sample size of 1000 and low autocorrelation. Our hypothesis was that the cortisol treatment would influence the developmental trajectory of personality traits which would be detected through a significant treatment $\times$ test round interaction. To assess significance of fixed-effects, we used the MCMC-derived estimate of the p values. We report the posterior mode for each fixed effect estimates along with their 95% credible intervals (CI). To control for multiple testing (eight models in total), we applied a Bayesian version of the false discovery rate (Wakefield, 2007).

To estimate treatment dependent repeatability, we calculate the posterior mode of $R = V(\text{ID})/V(\text{ID}) + V(\text{units})$, with $V(\text{ID})$ being the among-individual variance and $V(\text{units})$ being the within-individual (or residual) variance. Variance components were estimated separately by treatment (Nakagawa and Schielzeth, 2010). A trait was considered repeatable if the CI clearly deviated from zero. To compare repeatabilities of treated animals with control animals, we also calculated the MCMC posterior distributions of the differences in repeatability

estimates between treatments ($\Delta R = R(\text{control}) - R(\text{cort})$) following (Montiglio and Royauté, 2014; Royauté et al., 2015). Positive ΔR indicates higher repeatability in the control animals. This difference was considered “significant” if the posterior mode did not include zero.

To determine whether treatment differences in repeatability were caused by differences in among- or within-individual ($V(\text{ID})$ or $V(\text{units})$) variance components, we repeated these calculations for each variance component separately.

2.6.2. Effect of cortisol treatment on trait correlations

To test if the correlations between traits differed by treatment, we fitted Bayesian multi-response mixed models on each treatment group separately. This procedure allowed us to compare among- and within individual correlations between treatments, following (Dingemanse and Dochtermann, 2013). All six behavioural traits and baseline cortisol were fitted as response variables within one model. The model conditions as well as fixed and random effects were as described above. We assessed the magnitude of among- and within-individual correlation ($r(\text{ai})$ and $r(\text{wi})$) based on the posterior mode of their estimates and used the 95% credible intervals as an indication of the precision of the estimates. As above, we compared the effect size of the difference in posterior correlation estimates between treatments ($\Delta r = r(\text{control}) - r(\text{cort})$).

We tested the convergence of univariate and multivariate models by applying Gelman and Rubin's (1992) test using two separate chains with overdispersed starting values.

3. Results

3.1. Effect of cortisol treatment on average trait expression and trait repeatability

The CORT treatment significantly affected the development of baseline CORT levels (interaction treatment $\times$ test round, estimate $\pm$ CI: $\beta = -221.81 [-329.91; -80.21]$, $p\text{MCMC} = 0.028$), struggle docility ($\beta = -6.7 [1.6; 9.81]$, $p\text{MCMC} = 0.03$) and hand-escape latency ($\beta = 5.59 [0.15; 10.64]$, $p\text{MCMC} = 0.04$) (Fig. 1). It had no effect on body mass development ($\beta = 7.36 [-4.76; 19.27]$, $p\text{MCMC} = 0.21$), boldness ($\beta = 0.08 [-0.32; 0.52]$, $p\text{MCMC} = 0.71$), exploration ($\beta = -0.43 [-1.15; 0.31]$, $p\text{MCMC} = 0.24$), sociopositive behaviour ($\beta = -0.08 [-0.86; 0.66]$, $p\text{MCMC} = 0.84$) or aggressiveness ($\beta = -32.72 [0. -98.14; 36.33]$, $p\text{MCMC} = 0.70$).

Sex-specific effects of the CORT treatment (interaction treatment $\times$ sex) were never detected, but sexes differed in mean trait expression for several traits. Males were heavier than females ($\beta = 44.26 [7.13; 89.68]$, $p\text{MCMC} = 0.03$), had lower CORT levels ($\beta = -361.69 [-617.60; -161.19]$, $p\text{MCMC} < 0.001$) and were more explorative than females ($\beta = 0.94 [0.17; 1.84]$, $p\text{MCMC} = 0.03$).

Repeatability was higher in treated animals compared to control animals for baseline CORT levels, hand-escape latency and tended to be higher (p -values of 0.08 and 0.05) for struggle docility (Table 1). Repeatability of other behaviours did not differ between control and treated animals.

The observed differences in repeatability were caused by an increase in among-individual variance in all three variables while the within-individual variance components did not differ between treatments (see Table 1). This indicates that the differences between individuals were larger in CORT treated animals compared to control animals, while there was no difference in their flexibility.

3.2. Effect of cortisol treatment on trait correlations

Under control conditions across all ages, we found two behavioural syndromes, one including struggle docility, hand-escape latency and baseline CORT level (Fig. 2a) and a second syndrome including the two risk-taking traits (boldness, exploration) and the two social traits. The

Fig. 1. Effect of CORT treatment on development of baseline CORT levels, hand escape latency and struggle docility. Values are presented as raw data, lines represent regression curves. Black dots and lines correspond to cortisol treated animals, grey dots and lines correspond to control animals.

experimental CORT manipulation resulted in a strengthening of the correlations between cortisol and stress-coping traits (Fig. 2b). The strength of correlations between risk-taking and social traits was not affected by the treatment. However, in CORT-treated animals, we detected a significant correlation between struggle-docility and boldness

($r_{\text{treat}} = 0.38 [0.12:0.53]$; $r_{\text{control}} = -0.03 [-0.33:0.28]$) and a significant correlation between struggle-docility and exploration ($r_{\text{treat}} = 0.33 [0.05:0.61]$; $r_{\text{control}} = -0.05 [-0.42:0.50]$) that were not present in the control. This resulted in a significant difference of correlation estimates between control and treatment (Fig. 2b).

Table 1

Repeatability (R), among-individual variance (V_{among}) and within-individual variance (V_{within}) estimated for cavies treated with exogenous cortisol during early adolescence (T) and control animals (C). Given are the point estimates and their accompanying credible intervals (CI) separately for the control and treatment groups. The p -values ($P_{(\Delta R)^*}$) indicate if estimates for the treatment and control group differ from each other.

Trait	Group	Repeatability			Among-individual variance			Within-individual variance		
		R	CI	$P_{(\Delta R)^*}$	V_{among}	CI	$P_{(\Delta R)^*}$	V_{within}	CI	$P_{(\Delta R)^*}$
Baseline cortisol level	C	0.07	0–0.14		124.0	73.8–379.1		1604	1262–2430	
	T	0.36	0.08–0.47	0.03	893.6	578.1–2053.4	0.04	2313	1792–3450	0.92
Hand-escape latency	C	0.02	0–0.16		2.33	0.5–30.0		218.5	165.5–300.6	
	T	0.29	0.18–0.51	0.04	132.5	11.0–312.6	0.03	220.4	160.8–428.8	0.74
Struggle docility	C	0.18	0.05–0.37		3.15	0.71–12.4		23.6	16.9–33.4	
	T	0.35	0.13–0.58	<i>0.06</i>	10.3	3.3–22.8	<i>0.08</i>	17.7	13.5–28.3	0.19
Boldness	C	0.19	0.14–0.37		16.1	1.7–52.2		97.3	70.3–128.8	
	T	0.12	0.08–0.25	0.27	15.0	3.2–65.5	0.46	153.7	113.6–228.2	0.99
Exploration	C	0.28	0.18–0.52		12.7	1.0–170.7		2037.6	1252.1–3085.7	
	T	0.20	0.16–0.44	0.86	14.1	7.1–216.1	0.30	2851.1	2015–3712.6	0.84
Sociopositive behav.	C	0.25	0.11–0.41		3.7	1.3–8.5		13.1	8.7–16.7	
	T	0.22	0.10–0.43	0.32	2.3	0.7–7.4	0.31	12.7	9.0–17.7	0.52
Aggressiveness	C	0.25	0.10–0.40		2.4	0.8–4.6		6.9	5.2–10.2	
	T	0.19	0.09–0.36	0.29	1.8	0.6–4.8	0.48	9.5	7.4–14.3	0.94

Bold estimates indicate significant differences between treatment and control, estimates in italic indicate trends ($0.09 < p > 0.05$).

Fig. 2. A – Behavioural syndrome structure of control animals. Arrows indicate significant correlations. Numbers next to arrows indicate the posterior mode of correlation coefficients (CI). B – We calculated if the correlation estimates differ between control and treated animals by calculating the difference between control and treatment: $\Delta r = r(\text{control}) - r(\text{cort})$. Values left from zero indicate a stronger correlation in treated animals compared to control animals. P* gives the percentage of estimates excluding zero thus providing a measurement of significance.

4. Discussion

The aim of this study was to test whether a prolonged but temporary experimental CORT elevation during early adolescence affects the development of personality traits and behavioural syndrome structure into adulthood. We found that the CORT manipulation affected trait means, trait variances, and trait correlations in a domain-specific pattern.

4.1. Personality type and cortisol concentrations are affected by early cortisol manipulation

Substantial evidence has been gathered across taxa showing that exposure to GCs during early development affects a multitude of physiological and behavioural parameters later in life (mammals: (Stefanski et al., 2005; Ward, 1972; Weinstock et al., 1998), birds: (Groothuis et al., 2005; Henriksen et al., 2011; Schoech et al., 2012), fish: (Shama and Wegner, 2014)). In line with our predictions, we found that

individuals experiencing elevated circulating cortisol levels during early adolescence developed a more passive stress-coping type, indicated by long freezing behaviour on the hand of an observer (Hand-escape test) and by less active escape attempts when held by an observer (Struggle-test).

The CORT treatment also led to a decrease in baseline circulating cortisol levels, with the effect persisting into adulthood. The dampening effect of chronic stress on baseline GCs is known in the literature and has been reported from various species (Cyr and Romero, 2007). Similarly, exogenous manipulation of CORT often results in supra-physiological peaks (e.g. Bonier et al., 2007). By providing CORT in pulsatile manner, plasma levels remained elevated over the three-week period within the natural range of the species, avoiding a reduction due to a triggering feedback mechanism of the HPA axis (see Fig. A3).

In cavies and their domesticated form, guinea pigs (*Cavia aperea f. porcellus*), low baseline CORT values indicate higher cortisol responsiveness to acute stress (Guenther and Trillmich, 2013). Cavies born into autumn-like environmental conditions, indicating unfavourable food and weather conditions, regularly develop lower baseline CORT concentrations, heightened cortisol responsiveness under stressful situations and a more fearful, less risk-taking personality compared to animals born into spring (Guenther et al., 2014b; Guenther and Trillmich, 2013), suggesting that these developmental trajectories might be adaptive adjustments to the environment individuals face. Our results indicate that differences in baseline cortisol levels during adolescence may bring about these consistent and potentially adaptive adjustments.

The treatment affected the baseline CORT level and associated behavioural stress-coping traits, but had no effects on other personality traits such as risk-taking and social behaviours. From the classical coping-style literature it has become evident that HPA-axis related traits reflect coping with potentially threatening or stressful, often uncontrollable, situations (Coppens et al., 2010; Koolhaas et al., 1999). The “fear-reaction” in such situations is predominantly elicited by the “forced” and often uncontrollable component of situations in which individuals are forced to react to a stressor rather than by the novelty of the situation (Lister, 1990). Anxiolytic drugs that bind to serotonin receptors (5-hydroxytryptamine; 5-HT receptors) or modulate these receptors have been shown to affect “fear-reactions” in forced or uncontrollable situations while behaviours related to encounters of novel situations (e.g. free exploration, boldness) appear insensitive to such drugs (Griebel et al., 1993). These findings indicate a difference in the neurobiological pathway by which these behaviours are controlled and hence a potential difference in their functional domains. While both stress-coping situations (escape latency, struggle-dolity) in this study measure a forced reaction, neither of the two risk-taking situations (exploration, boldness), nor the social encounter (aggressiveness, sociopositive behaviour) contained a forced component.

4.2. Cortisol influences individual differentiation and repeatability

In accordance with earlier studies in cavies, we found all measured traits to be repeatable, usually in both treatments with the exception of two traits that were only repeatable in stressed animals. The magnitude of reported estimates are in line with earlier findings for most of the measured traits (Guenther et al. 2014a,b; Guenther & Trillmich 2015).

We found an increased repeatability in cortisol-treated animals for baseline CORT concentrations, hand escape latency and a non-significant tendency also for struggle docility while the repeatability of other traits was not affected. An increased repeatability of behavioural and physiological traits in response to a stressful environment during development was reported in several other studies across different taxa (Careau et al., 2014a; DiRienzo and Montiglio, 2016; Lichtenstein et al., 2016; Royauté and Dochtermann, 2017 but see Han and Dingemanse, 2017; Lichtenstein et al., 2016). This suggests that developmental stress programs not only average trait expression but also trait variation

throughout life and that both effects are mediated by GCs. Surprisingly few studies have investigated the causes and consequences of variation in repeatability despite the abundance of research addressing the ecological and evolutionary significance of animal personality (Réale et al., 2010a; Wolf and Weissing, 2012). Higher trait repeatability might indicate the potential for faster evolutionary change as repeatability usually sets an upper limit for heritability (Falconer and Mackay, 1996). Increased repeatability as a consequence of developmental stress is in line with quantitative genetic studies showing that heritability is environment dependent (Wood and Brodie, 2015) and commonly higher under stressful or unfavourable environments (Charmantier and Garant, 2005; Hoffmann and Merilä, 1999).

Developmental effects on trait repeatability provide interesting insights for ecological and evolutionary dynamics; however, since R is a ratio, the precise biological implications depend on which levels of variation are affected. The among-individual variance represents those aspects of the phenotype that are stable over the measurement period, arising from genetic and non-genetic factors such as maternal or permanent environment effects. Increased among-individual variance, such as found here for cortisol level and stress-coping behaviours, indicates that individuals differ more from each other. This finding supports our initial hypothesis that GCs would increase among-individual variance and is in line with other studies reporting increased among-individual variance in personality traits due to developmental stress (Careau et al., 2014a; Royauté and Dochtermann, 2017). Several mechanisms could lead to an increase in among-individual variation. Individuals might for example differ in the absorption rate of the administered cortisol which would increase the variability of effects of cortisol. An increase in among-individual variance could also indicate the release of cryptic genetic variation, variation that was already present but is not expressed under benign environmental conditions (Flatt, 2005). Such changes can be restricted to the lifetime of individuals in which case they would increase repeatability but not heritability or they can be transmitted to the next generation, hence influencing heritability and evolutionary trajectories (Bonduriansky and Day, 2009; Day and Bonduriansky, 2011).

Several other studies investigating the effects of early environmental conditions on development of personality traits reported a change of within-individual variance, i.e., in the level of individual stability. Han & Dingemanse (2017) and Royauté and Dochtermann (2017) report a decrease in within-individual variance following a diet restriction during early development while the perceived level of predation reduced stability in hermit crabs (Briffa, 2013). We did not find an effect of CORT elevation on behavioural or physiological stability. This discrepancy between our results and results reported by others suggest that environmental stress acting via GCs directly affects the amount of individual differentiation and stability, but that such effects may be function- or species-specific or depend on the stressor itself or the severity of the stressor. While moderate stress often increases trait variance, a severe stressor can have such a restrictive effect on behaviour or physiology that it greatly reduces variation (Killen et al., 2013). Future studies are clearly required to assess the generality of developmental stress effects on trait variances and to what extent this is mediated by variation of GCs.

4.3. Cortisol influences trait correlations

We found stress-coping behavioural traits to correlate with each other and with CORT in control animals. Likewise, risk-taking behaviours (exploration and boldness) were correlated with each other but were independent of CORT and stress-coping traits under control conditions. These distinctive behavioural correlations have been shown in wild cavies before under varying social and environmental conditions (Guenther et al., 2014b; Guenther & Trillmich, 2015; Guenther, 2018). In the current study, a quite high proportion of animals in a given age class did not express behaviours like struggle docility or aggressiveness.

Nevertheless, we found the same trait-correlations across all age classes that were reported in previous studies. Hence, these trait correlations appear to be a general phenomenon in this species and to be present across a wide range of ecological and social conditions. We could also show that aggressiveness and sociopositive behaviour correlate with risk-taking traits. In general, trait correlations among behaviours related to risk-taking and social traits were stronger under control conditions (indicated by a shift to the right in Fig. 2b) while trait correlations involving stress-coping traits and cortisol were usually stronger in treated animals (indicated by a left-shift in Fig. 2b). Exposure to cortisol during early adolescence significantly strengthened trait correlations among stress-coping traits and cortisol and revealed correlations between struggle docility and both risk-taking traits, effectively merging the two behavioural syndromes to one syndrome under perceived stress.

From an ultimate perspective, the coupling of stress-coping traits and risk-taking in an environment that is perceived as stressful might be advantageous for survival. Exploration and boldness have been shown to be good indicators of dispersal in other studies (Cote et al., 2010; Dingemanse et al., 2003). Hence, more explorative/bold individuals may be more likely to disperse and are also more likely to cope with a stressful situation like a predator more actively when they experienced a stressful environment during early adolescence. This is, however, speculative as no data on dispersal and/or personality related survival are available for this species.

Studies investigating the effects of developmental stress on among-individual correlations so far report contrasting results. While food restriction during development uncoupled correlations between behaviour and physiology in zebra finches (Careau et al. 2014b), and between aggression and exploration in great tits (Carere et al. 2005) it had no effect on trait correlations in spiders (Lichtenstein et al. 2016) or crickets (Royauté and Dochtermann, 2017). In accordance with developmental effects on variances of single traits, effects on trait correlations may depend on the investigated traits, the species, the applied stressor or the severity of the stressor. Moderate stressors have been found to amplify or reveal correlations between physiology and behaviour while severe stressors often break or attenuate correlations (for

review see Killen et al. 2013). In the present study, CORT levels were elevated to acute stress levels for a period of 3 weeks which did not result in changes of growth trajectories that are often observed under severe chronic stress. In addition, the strengthening of correlations fit patterns often observed under moderate stress, hence, we conclude that our treatment was a moderate stressor that nevertheless exerted long-lasting changes in personality traits and behavioural syndromes.

Finding an influence of GCs only on particular personality traits and correlations among them, adds to the growing evidence that behavioural syndromes and potentially syndromes including other functional traits such as pace-of-life syndromes are usually population and/or environment dependent (Bell, 2005; Montiglio and Royauté, 2014; Sweeney et al., 2013). The behavioural syndrome concept and closely related concepts such as the pace-of-life syndrome concept hypothesise that traits (behavioural, physiological and life history traits) form one suite of correlated (i.e., integrated) traits (Réale et al., 2010b; Sih et al., 2004). Although trait correlations in general and behavioural syndromes in particular have been shown to influence evolutionary trajectories (Dochtermann and Dingemanse, 2013; Lande and Arnold, 1983; Walsh and Blows, 2009), our data suggest that evolutionary consequences might only affect specific functional modules. Personality traits may be integrated into discrete functional modules, in which traits are correlated because they share developmental or causal pathways (Réale et al., 2007; West-Eberhard, 2003) or have been jointly shaped by selection for a particular function (Araya-Ajoy and Dingemanse, 2014) but are independent of other traits. The existence and potential ecological and evolutionary importance of such behavioural modules have thus far not received attention in personality research although modularity has been recognised as a key driver for evolvability, allowing populations to rapidly adapt to environmental change (Clune et al., 2012).

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Appendix A. Cortisol elevation in response to dose and body mass

To determine the acute response to an experimental cortisol elevation and the appropriate dose for the experimental treatment, we conducted three pilot studies. First, animals received a single dose of CORT by the cucumber method (0, 5, 8, 10 and 12 mg) at 9:00 am ($n = 5$ per group). A blood sample was taken 3 h later as described in the Methods section. We found a linear increase of the cortisol concentration depending on the dose received (Fig. A1a).

In a second pilot study, we tested if the cortisol concentration 3 h after receiving a dose of cortisol depended on the body mass of the individuals. Adolescent males of the same age (92 ± 5 d) received a single dose of 5 mg CORT at 9:00 am and a blood sample was taken 3 h later. One day later, the animals were weighed. There was no association (determined by linear regression) between body mass and cortisol concentration following an experimental CORT manipulation (Fig. A1b).

Fig. A1. Baseline cortisol level 3 h after animals received a dose of cortisol. A - The higher the dose of cortisol, the higher the cortisol level 3 h later ($df = 4,24$; $p < 0.001$). B - There was no association between body mass of same aged males and their response to a cortisol elevation of 5 mg ($df = 1,16$; $p = 0.58$).

In a third pilot study we investigated the response to an experimental CORT manipulation within 24 h (Fig. A2). Animals of the treatment group received a 5 mg dose of cortisol with a piece of cucumber at 9:00 am while the control group received a piece of cucumber without CORT. Blood samples were taken either 1 h after the feeding ($n = 5$ per group), 3 h after feeding ($n = 5$ per group), 5 h later ($n = 5$ per group), 8 h later ($n_{\text{control}} = 4$; $n_{\text{treat}} = 5$) and 24 h later ($n_{\text{control}} = 5$; $n_{\text{treat}} = 4$).

The feeding of CORT results in an acute increase of cortisol concentration within an hour. This increase is followed by a steep decrease which results in baseline levels after 24 h.

Fig. A2. Cortisol concentration in animals receiving a single dose of either control or 5 mg CORT during 24 h. $N = 5$ animals per time-point.

Appendix B. Long-term response to cortisol elevation

To investigate if the experimental CORT manipulation would result in a robust cortisol increase for 3 weeks, we conducted another pilot study. Animals ($n = 5$ per group) received either a control, 5 mg or 10 mg CORT two out of 3 days for a total of 21 days. Blood samples were taken on the first day of the treatment and every 3 days thereafter (Fig. A3, measurements 1–8). A last blood sample (measurement 9) was taken 1 week after the end of the treatment.

Fig. A3 shows an elevated cortisol concentration for animals receiving 5 or 10 mg CORT compared to control animals. One week after the end of the CORT treatment, animals of all groups did not differ statistically, indicating that cortisol levels of CORT treated animals decreased within a week after the last CORT feeding.

Fig. A3. Cortisol concentration of animals receiving either a control, 5 mg CORT or 10 mg CORT for two out of 3 days for 21 days in total. Measurements 1–8 were taken during the CORT treatment while measurement 9 was taken 1 week after the end of the treatment. Arrows indicate the start and the end of the treatment.

Appendix C. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yhbeh.2018.06.010>.

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