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„Effects of age and social confrontations on cortisol concentrations and metabolic rate in male guinea pigs“

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Abstract

Social confrontations of unfamiliar conspecifics are a well-established method for the study of social stress and have been proven to elicit endocrine responses like increase in cortisol concentration. The energy requirements to deal with such agonistic encounters, however, have not been studied so far. The current study investigated changes in metabolic rate (MR) measured in oxygen consumption in response to social confrontations of unfamiliar male guinea pigs, its effect on cortisol concentrations, and its relations to behaviour. As behavioural strategies of rank acquisition in male guinea pigs have been shown to change with increasing age and experience, social confrontations were conducted during adolescence and in full adulthood. Twelve group-housed focal males of the same age were singly confronted with an unfamiliar older male guinea pig. MR and cortisol concentrations were measured before and after the social confrontation, social interactions were recorded during group housing and during social confrontations. Despite the high social interaction rates, MR declined from pre- to post confrontation measurements and were unaffected by cortisol levels. At the age of 180 days, thus in full adulthood, the strongest cortisol increase in response to the social confrontation was measured, while at the same time MR significantly decreased. A possible explanation of this finding is an energetical trade-off caused by the limitation of food resources. As agonistic encounters are energetically costly the energy left for MR may be restricted, which been suggested in a previous study showing similar relationships in wild cavies. Underlying physiological mechanisms, however, are poorly understood and require further investigations.

Keywords: guinea pigs, metabolic rate, cortisol, adolescence, social behaviour, social rank

1 Introduction

1.1 Energy generation and use in animals

For a proper functioning of an organism energy is required continuously. Therefore, polymers and monomers are broken down in the cells of the animal organism (catabolism). Parts of the broken molecules are used to generate/ regenerate new cells (anabolism), the rest is used to generate energy in the form of adenosine triphosphate (ATP) and waste products like CO_2, H_2O and NH_3, which are deposed by the body. A central molecule in the energy production of animal organisms is oxygen, which is utilized to break down the essential energy source glucose. With increasing energy requirement more glucose has to be split, and consequently more oxygen is required. Therefore, oxygen consumption is a reliable indicator for the MR of an animal (DeLany, 2017; Speakman, 2013).

1.2 Factors influencing energy expenditure of an animal

The energy required by an animal varies and is determined by a number of factors. First, since each cell of the body requires energy for its metabolism, the absolute energy expenditure positively correlates with the absolute body mass. However, when corrected for the body mass (oxygen consumption (VO_2) per kg of body mass), the relative energy requirement decreases with increasing body mass, due to changes in body composition. This also accounts for developing animals where the relative energy expenditure decreases with progressing age (Holliday et al., 1967; Poczopko, 1979). One explanation is that cells of different organs differ in their metabolic activities. For example, while fat cells are known for a low MR, hepatocytes, neurons, and heart cells have a particularly high metabolism. Holliday et al. (1967) found out that liver, heart, brain, lungs, and kidney, which only account for 7% of mass per body mass in adult humans and 15% in infants, are responsible for ca. 79% (or 70-80%) of the basal metabolic rate (BMR). Based on such findings it can be assumed that the relatively bigger sizes of these organs in infants are a major factor for their relatively higher BMR. Even the fact that adults have relatively more of the metabolically active muscle tissue cannot compensate this, since only 17,4% of the BMR can be derived from it (Aschoff, 1963), which is a lot less that the

metabolic activity of the 5 mentioned organs. Similar relations of organ sizes and BMR have been found in laboratory mouse strains where 52% of between-strain and within-strain variation of BMR could be explained by variations of the masses of heart, kidney, liver, and small intestines (Konarzewski and Diamond, 1995). Since small animals in comparison to bigger animals, both intra- and interspecifically, have bigger sizes of such metabolically hyperactive organs in relation to their total body mass, this could at least partly explain the allometric scaling.

Another reason could be the fact that small animals have a bigger surface area to volume. Consequently, their relative heat loss is higher than the heat loss of bigger animals, for which they have to compensate by increased heat production, thus by a higher metabolic activity. This effect has been subject of studies for a long time, where it had been discussed that heat loss and heat production should scale with about 2/3 power in mammals (Calder, 1984; Clarke et al., 2010; Glazier, 2010, 2014; White and Seymour, 2004).

1.2.1 Developmental factors influencing the MR

Crucial determinants of the BMR of an adult animal are intrinsic and extrinsic factors affecting the early developmental conditions of the organism, ranging from genotype, maternal effects, hormonal influences, disease, parasite load, food quality and quantity, and environmental conditions. Basically, any kind of stressor a developing animal is exposed to has the potential to alter the BMR expressed in adulthood (reviewed by Burton et al., 2011).

1.2.2 Effects of physical activity on MR

Muscle work strongly increases the MR, but also after a phase of enhanced physical activity the MR remains elevated for some time (EPOC = excess post exercise oxygen consumption). The strength of this effect depends on the intensity and duration of previous muscle work (Børsheim and Bahr, 2003) and the duration of elevation of MR can range from a few minutes up to 48 hours. The EPOC phase consists of a first phase of rapid decline of MR right after the physical activity followed by a phase of slow decline back to RMR. As reviewed by Børsheim

and Bahr (2003) different studies investigating this effect report highly variable results, indicating both, a high interindividual variability as well as strong dependence of study design (e.g. exercise mode, physical fitness of participants).

1.2.3 Effects of hormones on MR

Various hormones, like growth hormone or insulin have also been found to influence MR (e.g. Ikeda et al., 2011; Menéndez and Atrens, 1989). In endocrine disorders affecting the thyroid hormones a strong alteration of the MR has been demonstrated (Teixeira et al., 2020). The effect of glucocorticoids on MR has been subject of various studies which were able to demonstrate that glucocorticoid levels increase when higher energy expenditure is demanded. Brillon et al. (1995) showed that the infusion of cortisol overnight in humans increased the plasma glucose level and the resting energy expenditure. Haase et al. (2016) conducted a meta-analysis on cortisol-dominant mammals and found a proportional correlation of baseline cortisol concentrations with mass-specific MR. Body mass significantly correlated with baseline and elevated cortisol levels. In contrast to this meta-analysis, Francis et al. (2018) did not find strong evidence that glucocorticoids are linked to the mass-specific MR in vertebrates. However, they found the same correlation as Haase et al. (2016) for baseline glucocorticoids and body mass. Cortisol levels also change in a circadian pattern with the peak in the morning and a decrease over night night in many mammals, including humans and the cortisol-dominant guinea pigs (Garris, 1979). In the corticosterone-dominant rats this pattern was not found (Critchlow, 1963). In wild guinea pigs a negative correlation between cortisol level and BMR has been found (Guenther et al., 2014). A possible reason for this is that there are energetic trade-offs, thus due to an upper limit in daily energy expenditure those individuals, which spend more time with energetically demanding activities, compensate with a low BMR.

1.3 The management of stress in social behaviour

In socially living animal species physiological stress responses to social stressors are omnipresent. Very often stress responses are elicited when animals compete for limited

resources like food or mating partners (Leary and Crocker-Buta, 2018; Reeder and Kramer, 2005). To avoid energetically costly fights, socially living animals establish social hierarchies where the access to limited resources is facilitated for dominant individuals. These dominance relationships are beneficial for the entire group, as with the decrease in agonistic encounters not only the likelihood of injuries is strongly reduced, but also significant amounts of energy can be saved (Border et al., 2021; Greenwood et al., 2014; Kondo and Hurnik, 1990; Sapolsky, 2005). In many socially living animal species the stratification into different hierarchical ranks starts with the onset of sexual maturity (Mutwill et al., 2021), but it takes until the animals reach full adulthood until their social ranks remain stable (Sachser, 1986; Zipser et al., 2013). During the adolescent life-phase animals seem to frequently change their tactics, which can be an explanation for the high fluctuation in social positions (Mutwill et al., 2019). This indicates that strategies to deal with agonistic interactions to get the best possible outcome are a result of learning and experience. This assumption is supported by the behaviour of older, more experienced males which restrained the access of younger males to mating partners (Bradley et al., 2005; East and Hofer, 2001; Spong et al., 2008; Wimmer and Kappeler, 2002). Thus, the developmental phase from puberty until the onset of adulthood can be considered a crucial phase for rank acquisition and consequently for the access to limited resources. This assumption is also supported by experiments on social animal species reared in isolation. Such individuals lack crucial social skills later in life when confronted with a conspecific (e.g. Harlow and Harlow, 1962; Immelmann et al., 1982). In general, especially social bonds with the mother and/ or siblings during infancy are considered as important for developing social skills (Immelmann et al., 1982). However, studies conducted on guinea pigs found the juvenile life stage to be essential for developing the ability to establish social hierarchies with unfamiliar conspecifics in a non-violent manner (Sachser, 1993; Sachser and Lick, 1991; Sachser et al., 1994). Sachser et al. (1998) showed that male guinea pigs, which were reared in isolation, were more aggressive in social confrontations with unfamiliar male conspecifics and got injured more frequently than males which were reared in social settings. In addition, even after a few days with another male guinea pig they did not establish a hierarchy. This shows that without the social skill to establish and respect a hierarchy an animal would suffer severe damages to its health, both, by constantly risking injuries as well as by the physical burden of long-time stress.

Studies have shown that whether an individual has a high or low social rank within its social group can manifest in its endocrine status (Gesquiere et al., 2011; Henry, 1982; Henry and Stephens, 1977; Sachser 1994a, b; Sachser et al., 1994; von Holst, 1998), due to different behavioural strategies. Dominants, who tend to act proactively by attacking, exhibit stronger increases, but return to a low baseline cortisol level faster, in contrast to subordinate individuals, who, behave in a more passive, conflict avoidant manner (reactive). This endocrine difference between males of different social ranks has also been found in baboons during times of stable hierarchical stratifications (Gesquiere et al., 2011; Sapolsky, 1982). Here as well, baseline cortisol concentrations were lower in dominant males, while their cortisol levels increased significantly stronger in response to a stressor. Similarly, experimental studies applying social confrontations showed that glucocorticoid levels are more strongly elevated in losers than in winners, while in winners it decreases back to a baseline level more rapidly (Sachser et al., 1994; von Holst, 1998).

However, studies that investigated the link between glucocorticoid secretion and dominance often had inconsistent results, which indicates that multiple variables affect baseline cortisol levels and cortisol responsiveness. In general, it seems that it is predominantly the energy requirement for acquiring and maintaining a social rank that determines the baseline glucocorticoid levels in dominants and subordinates (Gesquiere et al., 2011; Goymann and Wingfield, 2004).

Despite contradictive findings regarding the hormonal profiles of dominants and subordinates, the interplay between stressful social behaviour and endocrinology is well understood. However, only few studies addressed how the endocrine stress response to agonistic encounters alters with increasing age. Mutwill et al. (2021) investigated 3 endocrine parameters (testosterone, cortisol, and cortisol responsiveness) in intact male guinea pigs at the age from 120-240 days. They found no link between baseline cortisol levels and dominance, but their data indicate that cortisol responsiveness might be linked to dominance at a young age, while this link reverses in adulthood.

However, the actual energy demand, which is most accurately measured by respirometry, has been widely neglected so far. In particular, the alterations with age are barely understood.

1.4 The guinea pig as a model organism for the study of social stress

Guinea pigs are a well-suited model organism for the study of physiological stress responses and social stress. Measuring salivary cortisol levels has been demonstrated to be an advantageous method to assess stress responses in guinea pigs. It has been shown to strongly correlate with plasma cortisol levels (Nemeth et al., 2016b) and to reflect the biologically active form of cortisol, as it is unbound (Fenske, 1997). In addition, it is a non-invasive method that causes no handling- or stress-induced changes of the adrenocortical activity and due to its simple applicability salivary cortisol can be measured multiple times on the same individual (Fenske, 1997). Stressors like social confrontations with unfamiliar adult male lead to a significant increase in salivary cortisol level (Nemeth et al., 2016a, b).

Determining winners and losers of social confrontations of unfamiliar males is facilitated by the high interaction rates. Individuals stratify into dominants and subordinates, which is characterized by differences in expressed behaviours. Dominant males display more aggression than subordinates, e.g. they bite more often and are less often bitten (Sachser, 1990). Subdominants express more defensive and submissive behaviour (Sachser et al., 1998). Male guinea pigs living in colonies express rather stable hierarchies, where subordinates retreat when a dominant male approaches (Sachser et al., 1998). When two socially experienced males are confronted with each other, they establish a hierarchy quickly without displaying exceeding aggression. However, confrontations of unexperienced adult males have been shown to be characterized by high levels of aggression (Sachser and Lick, 1991; Sachser et al., 1998).

Despite the fact that multiple studies found that social confrontations lead to an increase in cortisol concentrations (e.g. Sachser and Lick, 1989; Nemeth et al., 2016a, b), the exact relationship between the degree of cortisol increase in response to social confrontations and success in the social confrontation, i.e. winning or losing it, remains unclear. Some studies found that glucocorticoid levels were higher in losers than in winners (e.g. Sachser et al., 1994), other studies, however, found no consistent differences in cortisol levels between dominant and subordinate guinea pig males (e.g. Sachser et al., 1998). It has been suggested that it is not an individual's success which determines the increase in cortisol concentration, but the amount of energy invested (Gesquiere et al., 2011; Goymann and Wingfield, 2004).

The effect of social confrontations on MR in guinea pigs, however, has not been focus of any studies yet. Generally, little literature on energy expenditure in guinea pigs exist. One study of wild cavies found a negative correlation between baseline cortisol level and RMR (Guenther et al., 2014), which may be explained by limited food resources causing trade-offs between energy required for demanding activities and energy available for maintenance of body functions (i.e. RMR, MR). Künkele and Trillmich (1997) investigated the effect lactation has on energy expenditure in the precocial guinea pig in comparison to altricial rodent species. In contrast to altricial species, in female guinea pigs it is mainly the first half of lactation which is characterized be high energy requirement.

1.5 Current study

So far, little is known about energy expenditure during stressful social encounters, to which extent it is affected by glucocorticoids, and how it changes from the onset of sexual maturity, until full adulthood, when the animals have acquired behavioural strategies to deal with agonistic encounters. The current study therefore aimed to investigate how an animal's MR is affected by changing cortisol concentrations caused by social interactions within its own social group as compared to an unfamiliar individual in a novel environment and how the interrelationships of physiological and behavioural parameters alter from an adolescent life stage to full adulthood. Therefore, the social interactions of male guinea pigs were observed in their own social male group and during social confrontations with unfamiliar adult male conspecifics. Energy expenditure was measured after taking them out of their social group and after the social confrontations, by indirect respirometry measuring their oxygen consumption in a flow-through respiratory chamber. To assess the strength of arousal of the animals, cortisol concentrations were measured before and after each respiratory measurement. At each experimental day the animals were weighted to control for the effect of body mass on the MR and the cortisol secretion. Social ranks have been shown to be linked with different behavioural phenotypes (proactive vs reactive) and physiological stress responses (Sachser, 1990; Sachser, 1994a, b; Sachser et al., 1994; Sachser et al., 1998), for which a hierarchy index was calculated for each individual male during both social settings.

As individuals vary strongly in their behavioural and physiological stress response as well as in baseline energy expenditure, interindividual differences were considered in the analyses.

Due to well-known developmental changes of baseline cortisol levels (i.e. increase from the juvenile life-stage to adulthood in male guinea pigs; Nemeth et al., 2018) as well as the increasing social experience over time in socially reared animals, age effects, like more distinctive behavioural and physiological differences between winners and losers of social confrontations with increasing age, were expected.

2 Material and methods

2.1 Ethical statements

Experiments were approved and permitted by the institutional board on animal ethics and experimentation (Faculty of Life Sciences, University of Vienna; no. 2018-026) and the Austrian Federal Ministry of Science and Research (BMBWF-66.006/0010-V/3b/2019).

2.2 Guinea pigs

For the experiments, sexually intact, male domestic guinea pigs (*Cavia aperea f. porcellus*) of two socially kept male groups (focal group: n=12; opponent group: n=7) have been used. While focal males were bred at the Department of Behavioural Biology, University of Vienna, and were descendants of nine females, opponent males were bought from different vendors. However, opponent males have been acquired three months prior to the start of the experiments, thus they were accustomed to their new environment and had enough time to form hierarchical structures with their group members. At the beginning of the study the focal animals were adolescent (age 3 months). The opponent males were already adult at the beginning of the study (age 6 months). The individuals were identified by their natural fur colourings. The enclosures of both groups were 2.4 m × 2 m and littered with woodchip bedding material. As hiding-facilities each enclosure was provided with two shelters. Ambient conditions were artificially controlled with the temperature being maintained at 22 ± 2°C, the humidity at 50 ± 5%, and the light conditions at a light-dark cycle of 12/12 h with an onset at

07:00 a.m. The animals were daily fed with 30g of guinea pig pellets (ssniff V2233, ssniffSpezialdiät GmbH, Soest, Germany) per individual. The group-specific amounts of food were provided in three feeding-pots. In addition, they were provided with hay once a day to promote abrasion of the teeth and intestinal activity. Vegetables such as cucumbers and carrots were provided Friday evening after the end of the last experiment of the week. Water was provided *ad libitum*.

2.3 Experimental procedures

2.3.1 Life-stages of the guinea pigs

The experiments were conducted in two age-blocks: in the first block the focal animals were about 90 days old, i.e., they were in their adolescent life-stage. The second experimental block was conducted when the focal animals were approximately 180 days old and adult. The males of the opponent group were already adult at the beginning of the first age block. During each age-block, oxygen consumption as an indicator of MR was measured twice in focal males: (1) after basal group housing condition and (2) following the social confrontation with an unfamiliar male of the opponent group. In addition, social interactions during group housing and social confrontations were analyzed in both male groups in order to assess the animal's social rank, amount and quality of initiated and received behaviours. Salivary cortisol concentrations were analyzed for both male groups as a control for physiological stress response (see figure 1 for timeline of the experimental procedure). As the opponent group consisted of fewer individuals than the focal group, some opponent males had to be used for social confrontations more than once per age-block. To ensure that these multiple assignments had no impact on the outcomes of the confrontations (i.e. exhaustion), there were at least some days in between the confrontations, during which the opponents could recover.

2.3.2 Experimental procedure

In a first step, starting at 9 a.m., the focal animal and the social opponent were video recorded in their regular social group for one hour in order to evaluate their status and behaviour in their social group. Beforehand, all houses and feeding pots were removed in order to ensure visibility of each individual all the time and to avoid food-related behavioral bias. Afterwards, body mass of the two individuals was measured and first saliva samples were taken for later analyses of salivary cortisol concentrations. After this group housing phase, oxygen consumption of the focal animal was measured for one hour (see “2.4.3. OxBox" for more details). To ensure equal conditions for the opponent male, it was placed in another transparent box of the same size and shape for the same time. Afterwards, the second saliva samples were taken. Both animals were then confronted with each other for 30 minutes and filmed in order to analyze behavioural responses to this setup (i.e. initiated and received aggressive, prosocial and dominance related behavior (hierarchical status defined by the Coulon Index: see “2.4.4. behavioral analyses”)). The social confrontations were conducted in a squared arena (1 m^2) built of laminated fiberboards with no shelter. After each social confrontation the litter (woodchip bedding material) was removed, and the arena was disinfected in order to get rid of odors which potentially could have elicited a behavioral and/or physiological response of the subsequent males. Afterwards, the third saliva samples were taken to evaluate the cortisol response to social confrontations. Previous studies already showed that in guinea pigs encounters with unfamiliar males elicit an HPA-axis response due to competition for the dominance rank (Nemeth et al., 2016a, b; Wallner and Dittami, 2003). After the social confrontation, the one-hour OxBox measurement was repeated for the focal animal and the opponent was again transferred into the plastic box for the same time. After this, the fourth and last saliva samples were taken. After these last measurements, all animals were returned to their social groups.

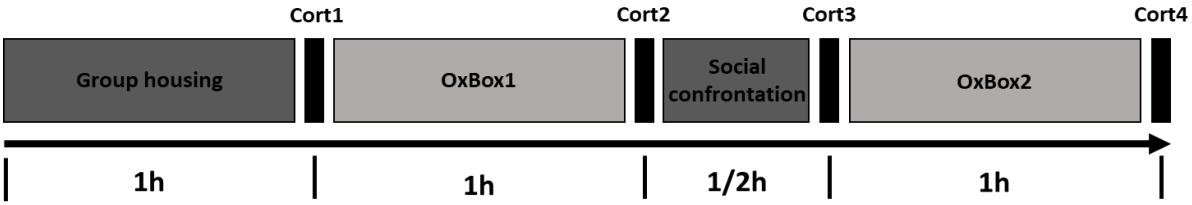

Figure 1 timeline of the experimental procedure: order and duration of experimental steps and saliva sampling (Cort 1-4).

2.4 Sample collection and analyses

2.4.1 Saliva sample collection

Saliva samples were collected by placing a cotton bud into the mouth of the guinea pig and gently spinning it for about 30 seconds until it was soaked with saliva. Then the soaked part of the cotton bud was cut off and put into a manipulated pipette tip, which was used as a holder (procedure: cut pipette tip in three equally big parts. Only middle part and narrow part next to tip were used. Tip-part was put into middle part. With the tip-part pointing downwards this construct was placed into a 1.5 ml Eppendorfer tube. Soaked cotton bud was put into this construct). The Eppendorfer tube then was centrifuged (10.000 rpm, 5 minutes) to extract saliva from cotton buds into the Eppendorfer tube. Pure saliva was frozen at -20 °C until hormone analyses.

2.4.2 Hormone analyses

Salivary cortisol concentrations were measured with biotin-strepdavidin enzyme-linked immunoassays (EIA) (Palme and Möstl, 1997). The cortisol-specific antibodies and enzymes were prepared at the University of Veterinary Medicine, Vienna, Austria and previously determined to be biologically valid in guinea pigs (Nemeth et al., 2016b). After thawing, the saliva samples were diluted with an assay buffer by the factor 1:50 and cortisol concentrations measured in 10 µl inputs. All samples were analyzed twice, in order to control for errors during processing. Intra- and interassay coefficient of variance were 21.93% and 19.54%, respectively.

2.4.3 OxBox

The oxygen consumption was measured by indirect calorimetry (oxygen consumption in ml per hour) in a flow-through system (OxBox 4.1 constructed at the Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria). The transparent box, in which the animals were placed for measurements, had a volume of 8 litres and ambient air was

constantly pulled through with a rate of 80 litres per hour, thus the air in the box was renewed ten times per hour. The oxygen consumption of the animal was measured by comparison of the oxygen concentration in the air leaving the box to the oxygen concentration of the ambient air. Reference measurements of ambient air for 120 seconds were conducted every 30 minutes. The actual oxygen consumption ranged between 0.5-1%. Prior to use, the OxBox was calibrated to ensure that all measures were corrected for temperature, humidity and pressure. Oxygen consumption was calculated as the means of 30 second intervals. For the analyses of MR, the first 10 minutes of oxygen consumption were excluded, since during this time the air inside the box is not yet adjusted to the oxygen consumption by the animal (the milieu of the ambient oxygen concentration is still present). However, the first 10 minutes were used for one analysis which focused the animal personality. This was based on Careau et al. (2008) where it was described that the first 10 minutes of MR measurements can indicate the stress responsiveness of individuals, as they have to adapt to the new environment and some animals respond exploratively, while others freeze in a stress response. The MR was then calculated as the mean oxygen consumption (ml/h) of the whole measurement period.

2.4.4 Behavioural analyses

Behaviour was quantified using Solomon Coder (version beta 17.03.22). Each video-analysis had a focal individual of which the interactions with conspecifics was recorded. The different behaviours were assigned to one of the following categories: (1) locomotion: walking or running. (2) prosocial behaviour: side by side, social grooming, nose nose, nose anal. (3) agonistic behaviour: head thrust, kick back, stand threat, teeth chatter, fight, chase, harassment. (4) dominant behaviour: displacement, ride, rumba rumble (ethogram based on Rood, 1972). Aggressive, prosocial, and dominance behaviours received by the focal animal were recorded as well. Locomotion and side by side were recorded in duration, all other behaviours as events. In order to evaluate the hierarchical status of the individuals within their social groups and during social confrontations, their Coulon Index (CI), which is defined as displacement active / (displacement active + displacement passive), was calculated. The hereby received values lie within an interval of 0-1, whereby a lower value indicates a low and a high value a high social status (Coulon, 1975; Zipser et al., 2013).

2.4.5 Statistical analyses

Statistical analyses were performed with R version 3.6.1 (R Development Core Team, 2018), using linear mixed effect models (package nlme). The parameters which were subject to the analyses were body mass, basal cortisol level (i.e. after group housing), post-social cortisol level (i.e. after social confrontation) (four cortisol measurements per animal per experiment ("measurement" 1-4): (1) pre OxBox1, (2) post OxBox1, (3) pre OxBox2, (4) post OxBox2), behaviour during group housing, behaviour during social confrontation and MR. MR measurements, which were only conducted in the focal animals, were calculated using three different calculation-methods ((1) ml/h, (2) ml/h/kg, (3) ml/h statistically adjusted for body mass as covariate). Group, age and pre- or post-social measurements were included as predictors, individual ID and mother ID were incorporated as random effects to correct for repeated measurements and kinship. Relations between parameters were considered by including covariates (e.g. cortisol, CoulonIndex, etc.; see result section). Model-fitting was conducted based on the Akaike Information Criterion (AIC). Post-hoc analyses were performed with Bonferroni corrections (package phia). In order to control for normal distribution and variance homogeneity Shapiro–Wilk normality tests and Levene tests for homogeneity of variance were performed and residuals were plotted for visual inspection. If necessary, data were transformed using the natural logarithm and models were fitted to heteroscedasticity of model residuals. Results were considered as significant if $p \leq 0.05$.

In order to control for the influence of circadian rhythmicity on the MR changes from pre- to post social confrontation measurements at the age of 180 days have been compared with data from the master's thesis of Fritscher (2021). This study was conducted on the same individuals at the same age and at the same time of the day, but without the stressor of the social confrontation in between.

3 Results

3.1 Physiological and behavioural comparisons of focal and opponent males

3.1.1 Body mass

Body mass was significantly affected by a group:age interaction (group: $F_{1,26} = 19.572$, $p < 0.001$; age: $F_{1,26} = 0.911$; $p = 0.347$; group:age: $F_{1,26} = 66.578$, $p < 0.001$). Focal group males had a lower body mass compared to opponent group males at an age of 90 days ($\chi^2 = 21.351$, $p < 0.001$), while no difference was detected at an age of 180 days ($\chi^2 = 0.120$, $p = 1$). Within the groups, only the mass of the focal animals significantly increased between an age of 90 and 180 days ($\chi^2 = 115.203$, $p < 0.001$), while it remained constant in the opponents ($\chi^2 = 0.994$, $p = 0.638$) (figure 2).

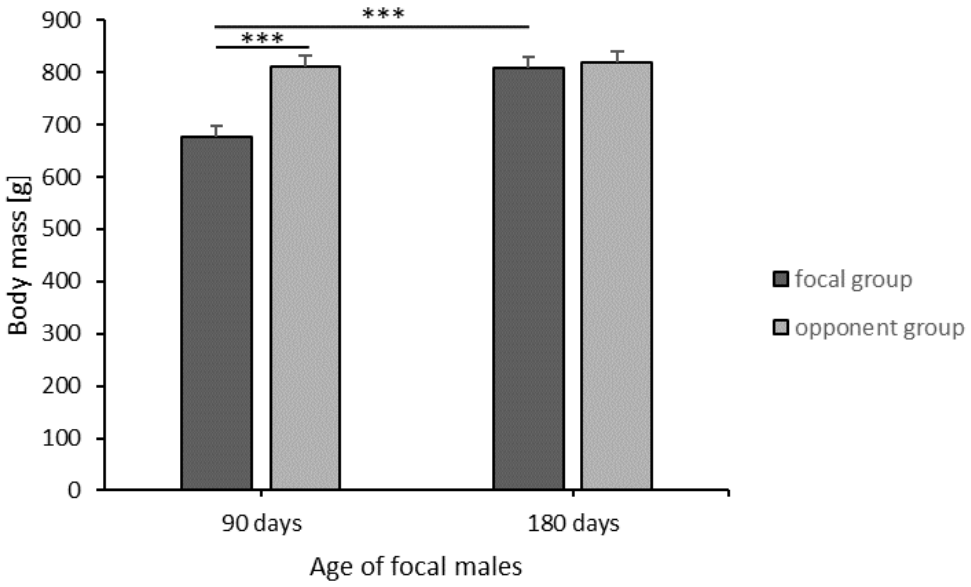

Figure 2 Mean body mass (± s.e.m.) of focal males during their adolescent and adult life-stage (age 90 and 180 days) and of the opponent males (adult) at the same time of the experiment. ***p ≤ 0.001.

3.1.2 Basal cortisol

Basal cortisol concentrations (i.e. first measurement prior to the social confrontation) significantly increased with age ($F_{1,26}$ = 9.767, p = 0.004) (figure 3) and did not differ between groups. Group and all other predictors were removed based on the AIC. However, on closer inspection, the increase with age was only significant in the opponent group (preAIC: opponent group: χ^2 = 10.404, p = 0.003; focal group: χ^2 = 2.244, p = 0.268).

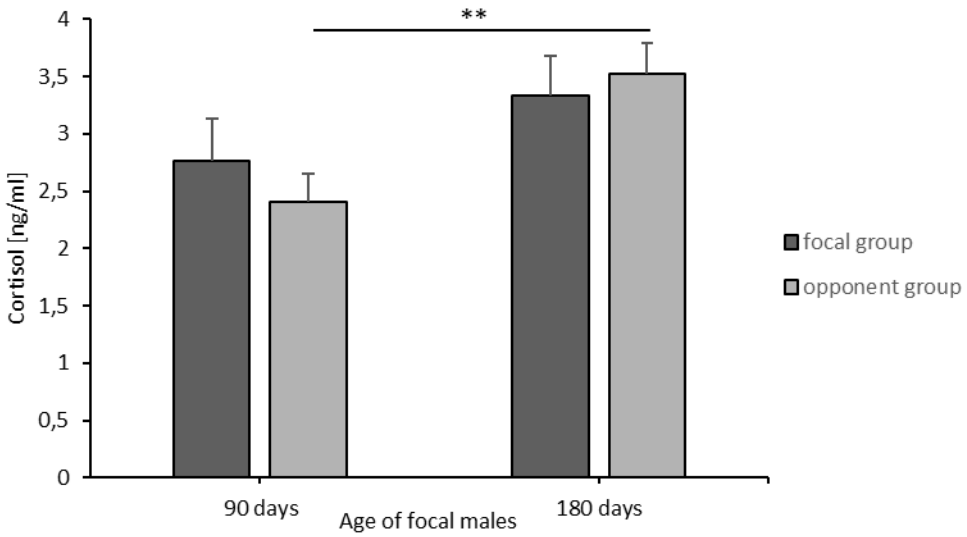

Figure 3 Mean basal cortisol level (± s.e.m.) of focal males during their adolescent and adult life-stage (age 90 and 180 days) and of the opponent males (adult) at the same time of the experiment. **p ≤ 0.01. (data log-transformed).

3.1.3 Changes in cortisol concentrations during the experiment

The analysis of cortisol concentrations over the 4 different measurements (preOxBox1, postOxBox1, preOxBox2, postOxBox2) revealed an age-effect and an interaction of group and measurement (age: $F_{1,164}$ = 31.411, p = < 0.001; group: $F_{1,164}$ = 0.069, p = 0.797; measurement: $F_{1,164}$ = 1.173, p = 0.322; group:measurement: $F_{3,164}$ = 5.007, p = 0.002).

The change of salivary cortisol concentrations over the measurements was only significant in the focal group (opponent group: χ^2 = 3.692, p = 0.593; focal group: χ^2 = 40.411, p < 0.001). However, this effect in the focal males was solely caused by changing cortisol concentrations

at an age of 180 days ($\chi^2 = 35.900$, $p < 0.001$). All other post-hoc comparisons were insignificant ($p > 0.640$). The pairwise analyses revealed a significant increase of salivary cortisol during social confrontation in the focal group ($\chi^2 = 25.075$, $p < 0.001$). This resulted in higher cortisol concentrations after social confrontations compared to the opponent group ($\chi^2 = 13.620$, $p = 0.001$; all other post-hoc comparisons $p > 0.062$; figure 4).

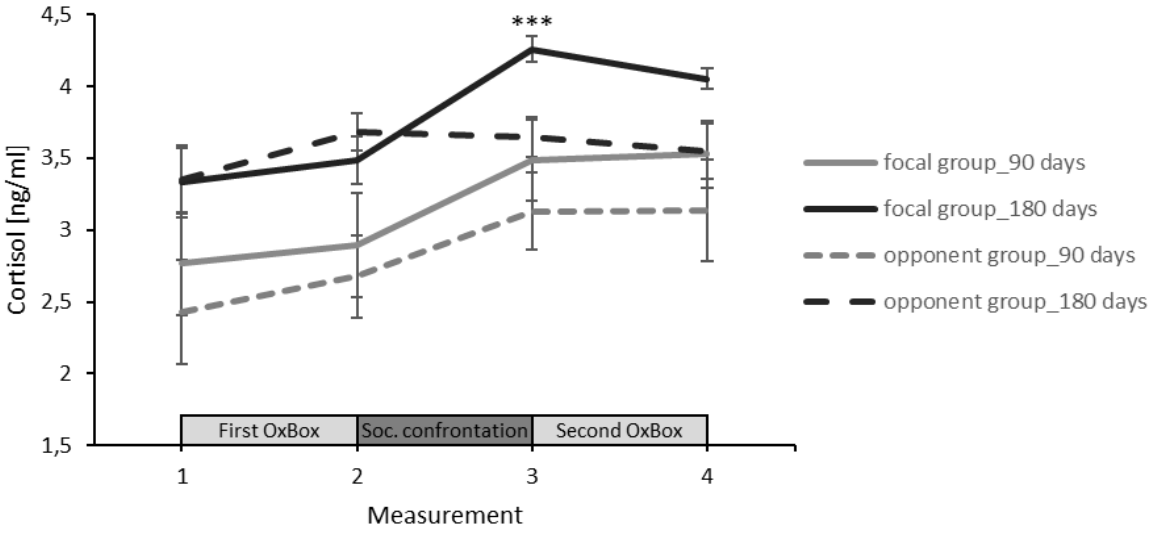

Figure 4 Mean cortisol concentrations (± s.e.m.) at the 4 sampling points (1 = GH/preOxBox1, 2 = postOxBox1/preSOC, 3 = postSOC/preOxBox2, 4 = postOxBox2) of focal males during their adolescent and adult life-stage (age 90 and 180 days) and of the opponent males (adult) at the same time of the experiment. ***p ≤ 0.001. (data log-transformed).

3.1.4 Behaviour

During group housing, the focal animals displayed significantly more sociopositive behaviour than the opponent animals ($F_{1,13} = 16.062$, $p = 0.002$) regardless of the age, which was eliminated due to model fitting (figure 5a).

During social confrontations, no significant effects were detected on sociopositive behaviour, but the group:age interaction missed the criterion of significance marginally (age: $F_{1,26} = 0.580$,

p = 0.453; group: $F_{1,13}$ = 0.219, p = 0.648; age:group: $F_{1,26}$ = 3.316, p = 0.080). This was caused by slightly increased sociopositive behaviour in the focal group at an age of 180 days (figure 5b).

With regard to aggressive behaviour, no significant effects were detected during group housing (all predictors removed due to model fitting based on the AIC) (figure 5c).

During social confrontations on the other hand, the opponents displayed significantly more agonistic behaviour than the focal animals (postAIC: $F_{1,13}$ = 6.733, p = 0.022). The predictors age and interaction between age and group were eliminated beforehand by model fitting (figure 5d).

In their dominance related behaviour no effects were found during group housing, i.e. all predictors were removed due to model fitting based on AIC (figure 5e).

No significant effects were detected during social confrontations either, but a tendency for decreasing frequencies with age was found (F = 3.122, p = 0.089) (figure 5f).

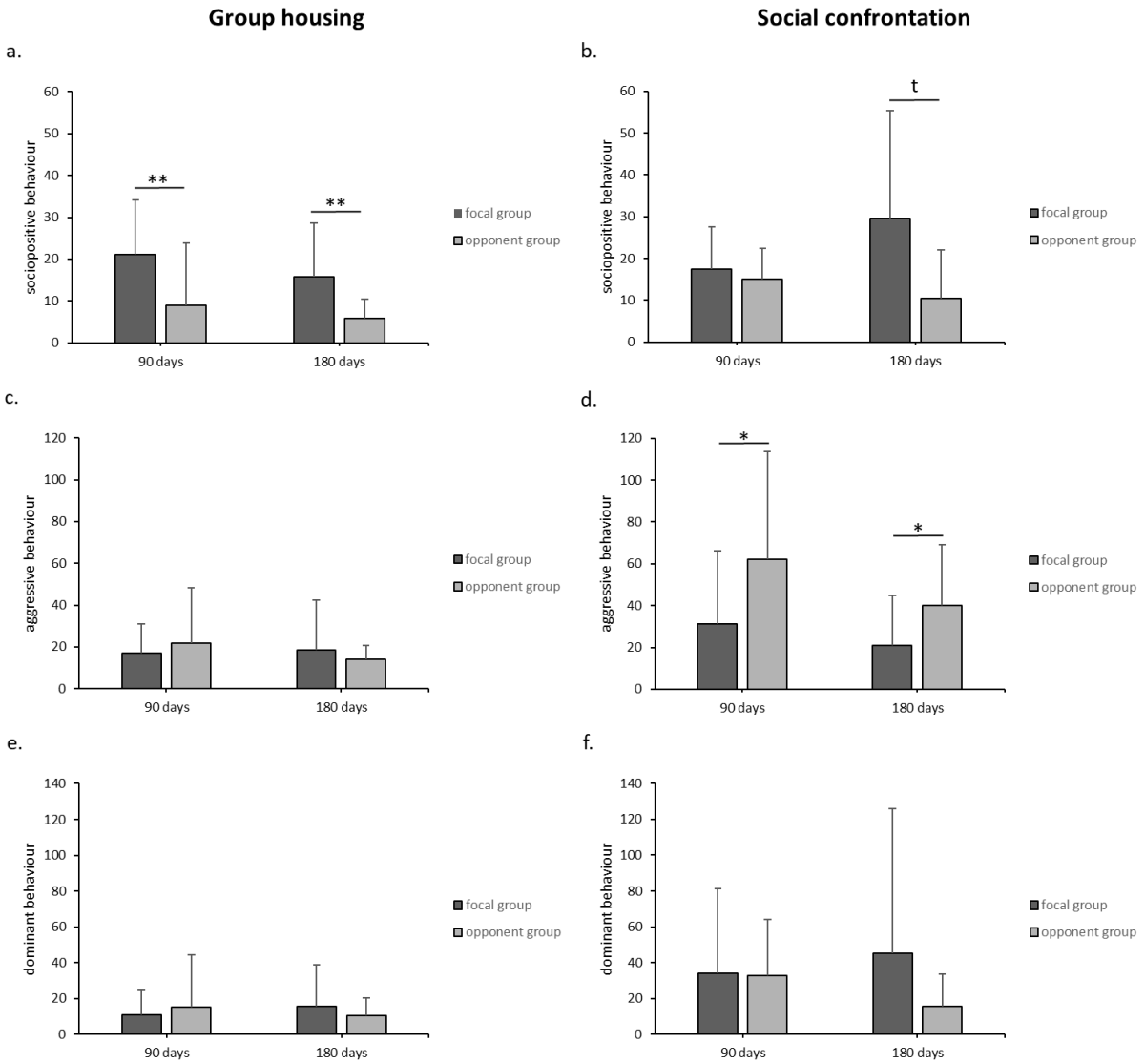

Figure 5 Mean frequencies of behaviours (± s.e.m.) within the categories 'sociopositive' (a and b), 'aggressive' (c and d) and 'dominance' (e and f) recorded during 1 hour of group housing (left graphs) and ½ hour of social confrontation (right graphs) for focal males at both age-blocks (90 days and 180 days) and opponent males. *p ≤ 0.0, *p ≤ 0.05, **p ≤ 0.01, t p ≤ 0.1.

3.1.4.1 Coulon-Index (CI) during social confrontations

The CI during social confrontations showed no differences between groups or ages (age: $F_{1,26}$= 0.032, p = 0.859; group: $F_{1,13}$ = 0.140, p = 0.714; age:group: $F_{1,26}$ = 0.068, p = 0.796) (figure 6).

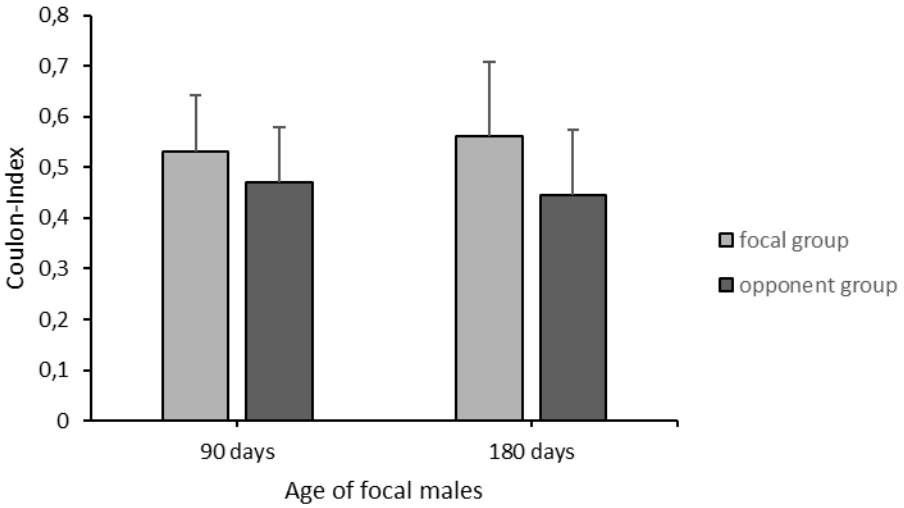

Figure 6 Mean Coulon Index (± s.e.m.) acquired during the social confrontation of focal males at the age of 90 and 180 days and opponents (adult) at the same time of the experiment.

3.2 MR in focal males

3.2.1 Comparison of 3 calculation methods for the MR

The analysis of absolute oxygen consumption (ml/h) of the focal group revealed an effect of age and measurement, while the interaction missed the criterion of significance marginally (table 1). MR significantly decreased from pre- to post-social confrontation at both ages (table 2, figure 7a). Moreover, MR prior to social confrontations significantly increased with age, while it did not differ in the post-social confrontation measurements (table 2, figure 7a).

When the body mass of the individuals was considered in the analysis by dividing the absolute oxygen consumption by their body mass (ml/h/kg), a significant change of the MR with age and measurement was found, as well as an interaction between those two predictors (table 1). The post-hoc Bonferroni test revealed a significant decrease in the MR from pre- to post-social confrontation in both, the adolescent, and the adult measurements, but it decreased stronger during adulthood (table 2, figure 7b). A decrease in the MR with age was significant in pre- and post-social confrontation measurements, but stronger pronounced in the latter one (table 2, figure 7b).

Contrary to the two other calculation methods, the correction for the body mass by inclusion in the model calculation did not reveal an age effect (table 1). Measurement as well as the interaction between age and measurement, however, were significant as well (table 1). The additional parameter body mass in this calculation method positively affected the MR (table 1). The post-hoc Bonferroni analysis revealed a significant decrease in the MR from pre- to post-social confrontation in the 90 days as well as in the 180 days measurements with a stronger effect at an age of 180 days (table 2, figure 7c). The MR significantly decreased with age in the post-social condition, while prior to the social confrontation age showed no influence (table 2, figure 7c).

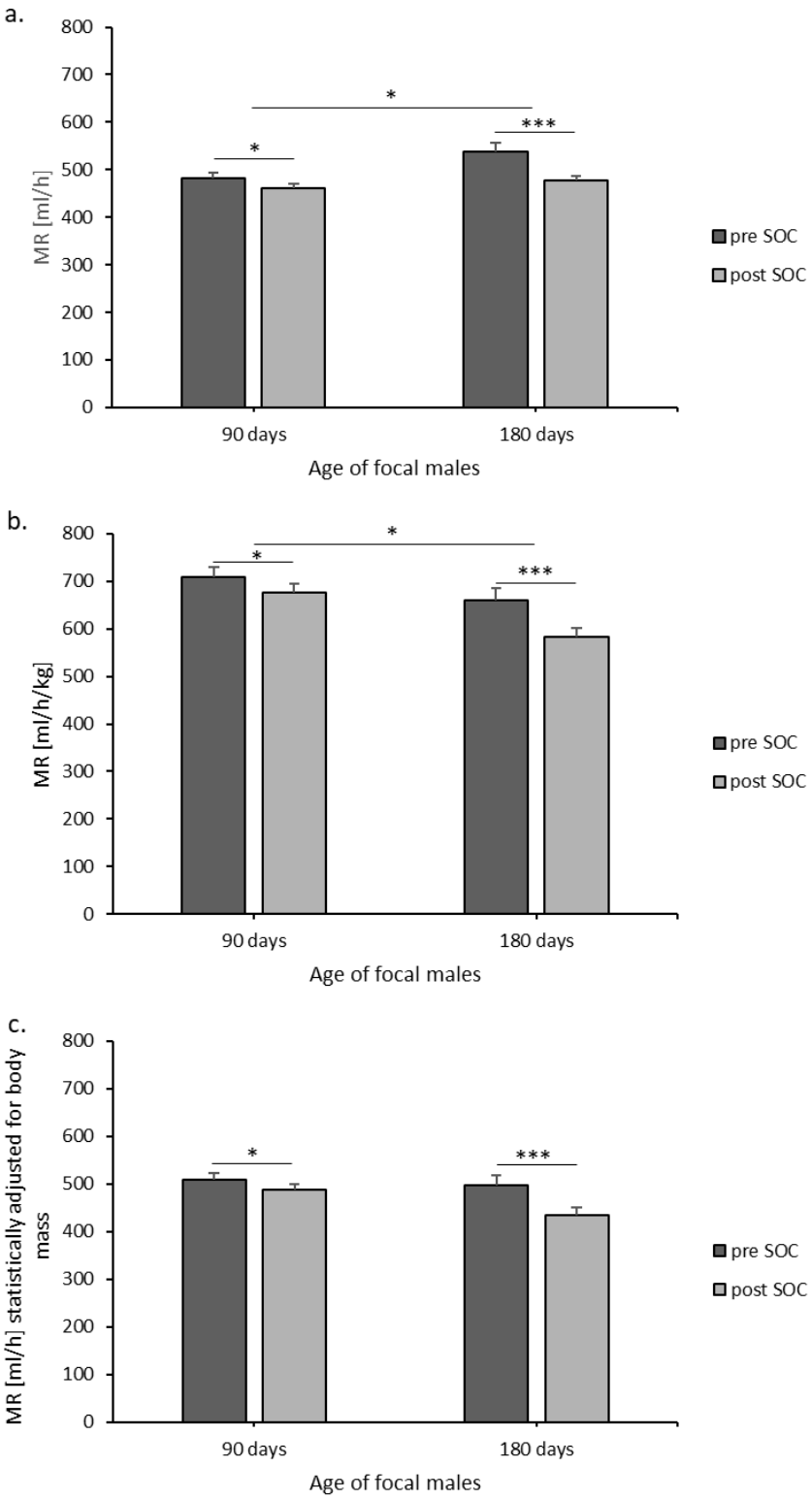

Figure 7 Comparison of metabolic rate (MR) measurements (means ± s.e.m.) prior to (pre) and after (post) the social confrontation (SOC) at the ages 90 and 180 days depending on the calculation model (a) ml/h, (b) ml/h/kg, and (c) ml/h statistically adjusted for body mass. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

Table 1 Statistics (ANOVA tables) for analysis of MR using the three different calculation methods (ml/h, ml/h/kg, and ml/h statistically adjusted for body mass (BM)).

	MR_ml/h		MR_ml/h/kg		MR_ml/h ~ BM	
	F-value	p-value	F-value	p-value	F-value	p-value
Age	7.159	0.012	5.568	0.025	0.213	0.648
Measurement	5.008	0.032	6.889	0.013	6.811	0.014
Age:Measurement	3.196	0.083	4.211	0.048	4.691	0.038
Body mass					21.742	<0.001

Table 2 Post-hoc comparisons of MRs (with Bonferroni corrections) using the three different calculation methods (ml/h, ml/h/kg and ml/h statistically adjusted for body mass (BM)).

Post-hoc analysis		MR_ml/h		MR_ml/h/kg		MR_ml/h ~ BM	
		χ^2	p	χ^2	p	χ^2	p
Pre-social vs. post-social	90 days	5.474	0.039	7.529	0.012	7.622	0.012
	180 days	10.260	0.003	17.380	<0.001	14.629	0.000
90 days vs. 180 days	Pre-social	7.825	0.010	6.086	0.027	0.238	1.000
	Post-social	3.548	0.119	71.873	< 0.001	10.187	0.003

3.2.2 First 10 minutes in the OxBox

When only the first 10 minutes in the OxBox were analyzed, the effects were still similar to the analyses of the entire hour. For the first 10 minutes highly significant effects of age were found in both, calculations of absolute oxygen consumption (ml/h) and in oxygen consumption in relation to body mass (ml/h/kg): Oxygen consumption in ml/h increased with age (postAIC: F = 10.942, p = 0.002) and oxygen consumption in ml/h/kg decreased with age (postAIC: F = 44.460, p = < 0.001). In both calculation models a highly significant decrease of MR after the social confrontation was found (post AIC: ml/h: F = 37.816, p =< 0.001; ml/h/kg: F = 50.559, p = < 0.001).

3.2.3 Effects of cortisol concentrations on MR

Considering cortisol concentrations in MR analyses revealed absolutely no effects (Table 3). Model fitting resulted in the same models as outlined above (Table 1).

Table 3 Effects of cortisol concentrations on MR: full model statistics (ANOVA tables) for the three different calculation methods (ml/h, ml/h/kg, ml/h statistically adjusted for body mass (BM)).

	ml/h		ml/h/kg		ml/h ~ BM	
	F-value	p-value	F-value	p-value	F-value	p-value
Age	0.450	0.508	2.540	0.122	1.087	0.306
Measurement	2.150	0.154	2.190	0.150	3.063	0.092
LOGCortisol	2.410	0.132	0.064	0.802	0.268	0.609
Age:Measurement	0.144	0.708	0.044	0.836	0.161	0.691
Age:LOGCortisol	2.087	0.160	1.048	0.315	0.966	0.334
Measurement:LOGCortisol	1.201	0.283	0.416	0.524	0.934	0.342
Age:Measurement:LOGCortisol	0.842	0.367	0.370	0.548	0.647	0.428
Body mass	-	-	-	-	17.418	0.000

3.3 Physiological and behavioural correlates (with MR) in focal males

To further investigate the increase in cortisol concentrations and decrease in MR during social confrontations, behavioural and physiological relationships were determined in focal males.

3.3.1 Effects of behaviour on the CI in focal males

Various behaviours of the focal males correlated with their CI. Their CI increased from group housing to the social confrontation ($F_{1,28}$ = 17.288, p < 0.001). Individuals with a high CI received significantly more prosocial behaviour from other males ($F_{1,28}$ = 4.507, p = 0.043), while they were less likely to receive agonistic ($F_{1,28}$ = 19.314, p < 0.001) or dominant ($F_{1,28}$ = 5.318, p = 0.029) behaviours. Additionally, they displayed more agonistic behaviours towards other males ($F_{1,28}$ = 33.871, p < 0.001). Age, active prosocial behaviour and active dominant behaviour did not influence the CI (age: $F_{1,28}$ = 0.895, p = 0.352; sociopositive active: $F_{1,28}$ = 0.016, p = 0.901; dominance active: $F_{1,28}$ = 0.366, p = 0.550).

3.3.2 Effects of the CI on cortisol concentrations

For the focal group no influence of the CI on salivary cortisol concentrations was detected; all effects in relation to age or the social condition (social confrontation or group housing) remained insignificant (full model: age: $F_{1,29}$ = 1.386, p = 0.249; measurement: $F_{1,29}$ = 0.304, p = 0.586; CoulonIndex: $F_{1,29}$ = 0.125, p = 0.726; age:measurement: $F_{1,29}$ = 0.060, p = 0.808; age:CoulonIndex: $F_{1,29}$ = 0.286, p = 0.597; measurement:CoulonIndex: $F_{1,29}$ = 0.042, p = 0.839; age:measurement:CoulonIndex: $F_{1,29}$ = 0.006, p = 0.940).

3.3.3 Correlations between CI, cortisol response, and MR response during social confrontations

Further analyses regarding relationships between the CI, cortisol responses, and MR responses during social confrontations revealed no significant effects in the focal group. None of the performed models, including the respective predictors, differed from the null model (always p > 0.118).

3.4 Relationships between group housing and social confrontations

3.4.1 CI

The individual CI measured during group housing predicted the CI during social confrontations, although model fitting yielded no significant effects (postAIC: age: $F_{1,9}$ = 1.269, p = 0.289; CoulonIndex_GH: $F_{1,9}$ = 0.175, p = 0.689; age:CoulonIndex_GH: $F_{1,9}$ = 2.010, p = 0.190).

The performance of a post hoc analysis revealed that the CI measured for group housing positively affected the CI measured during social confrontations at an age of 180 days (χ^2 = 8.770, p = 0.003), while no such effect was found at an age of 90 days (χ^2 = 0.21, p = 0.647) (figure 8).

The CI of the opponent had no influence on the CI outcomes during social confrontation, thus this predictor was eliminated during model fitting based on AIC.

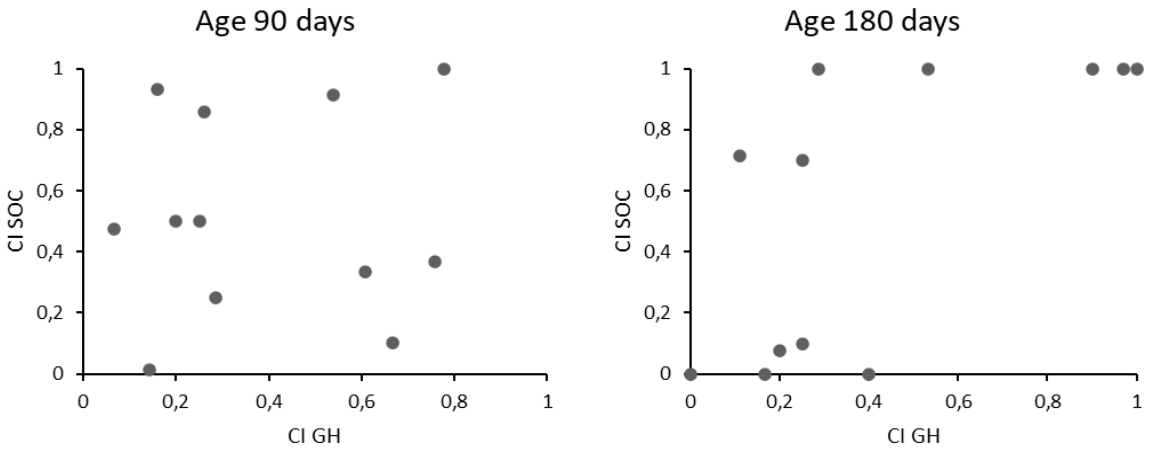

Figure 8 Relationships between Coulon Indices (CI) during group housing (GH) and social confrontations (SOC) in the focal males during adolescent (90 days) and adult (180 days) life-stages.

3.4.2 Cortisol concentrations

Salivary cortisol concentrations measured during group housing positively affected concentrations measured during social confrontations (age: $F_{1,10}$ = 5.839, p = 0.036; (LOG)cortisol GH: $F_{1,10}$ = 10.192, p = 0.010) (figure 9). However, individuals with high cortisol

concentrations during group housing showed a similar level during social confrontations, but individuals with a lower cortisol level showed an increase in response to social confrontation (figure 10).

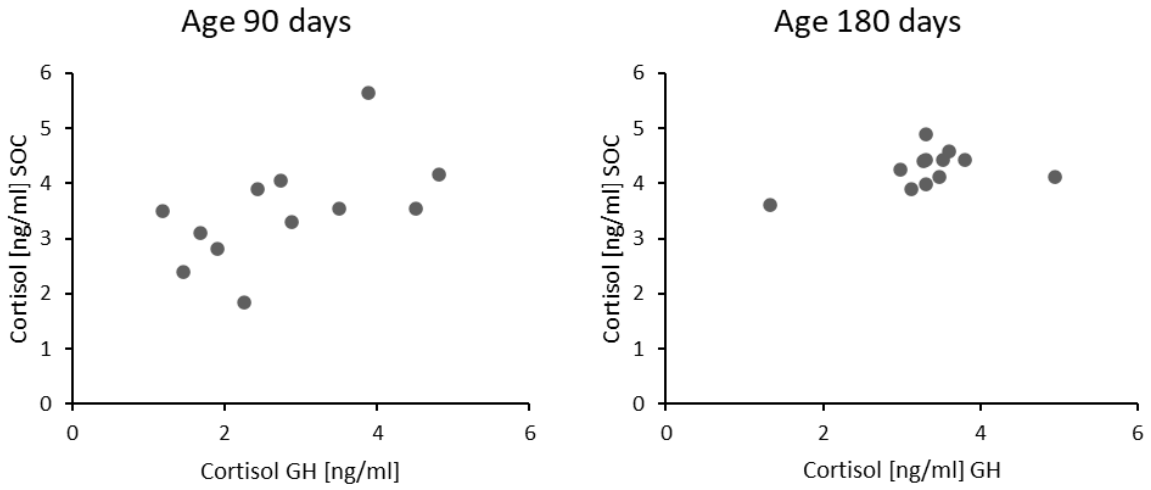

Figure 9 Relationships between salivary cortisol concentrations in response to group housing (GH) and social confrontations (SOC) in the focal males during adolescent (90 days) and adult (180 days) life-stages (data log-transformed).

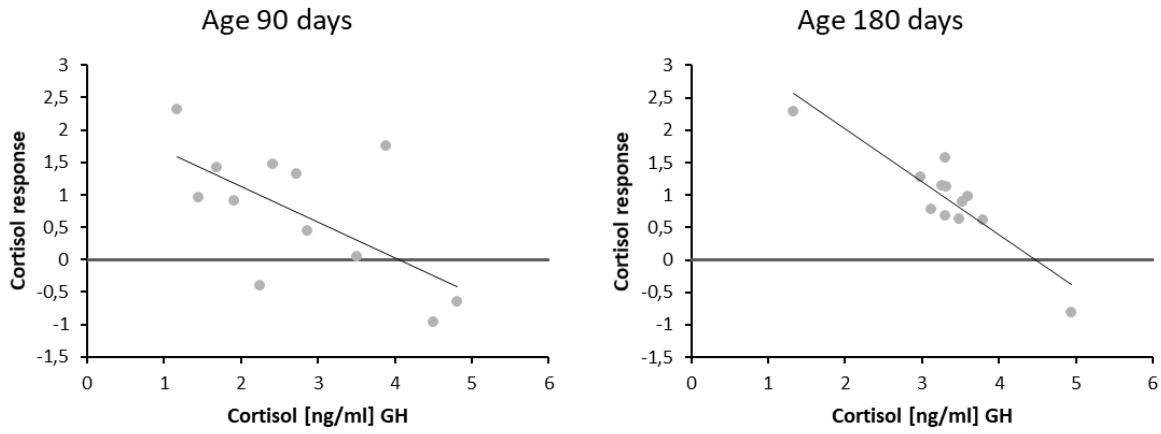

Figure 10 Effects of cortisol concentrations during group housing (GH) on the cortisol response (log of cortisol concentrations after social confrontations divided by

concentrations before social confrontations) in the focal males during adolescent (90 days) and adult (180 days) life-stages (data log-transformed).

3.4.3 MR

The MR after social confrontations was positively affected by the prior MR measured after group housing: individuals in general showed either high or low MR as compared to conspecifics irrespective of experimental conditions ($F_{1,10}$ = 21.005, p = 0.001) (figure 11). The absolute change in MR from pre- to post-social confrontation showed a strong tendency for a positive correlation with the change in MR of the 2.5h-measurements ($F_{1,9}$ = 4.953, p = 0.053) (figure 12).

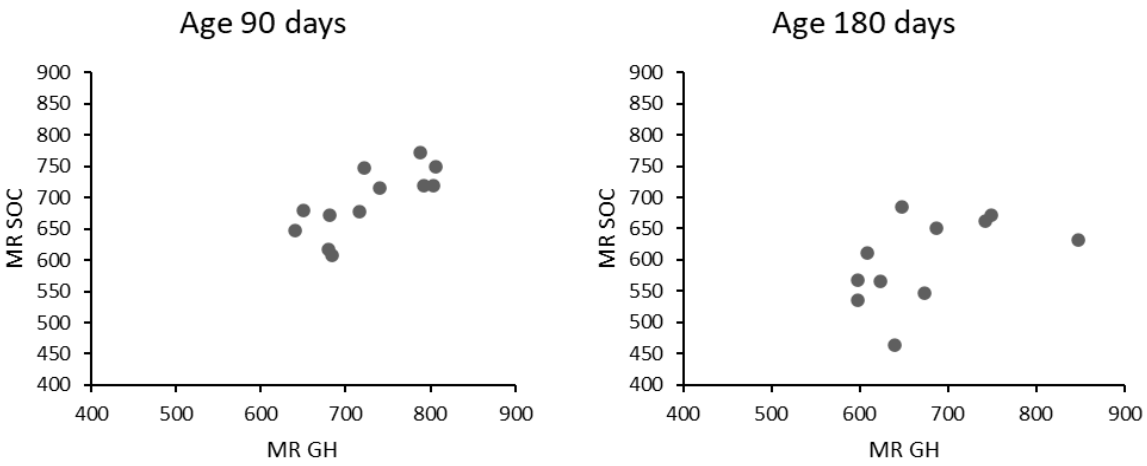

Figure 11 Relationships between metabolic rates (MR) after group housing (GH) and social confrontations (SOC) in the focal males during adolescent (90 days) and adult (180 days) life-stages.

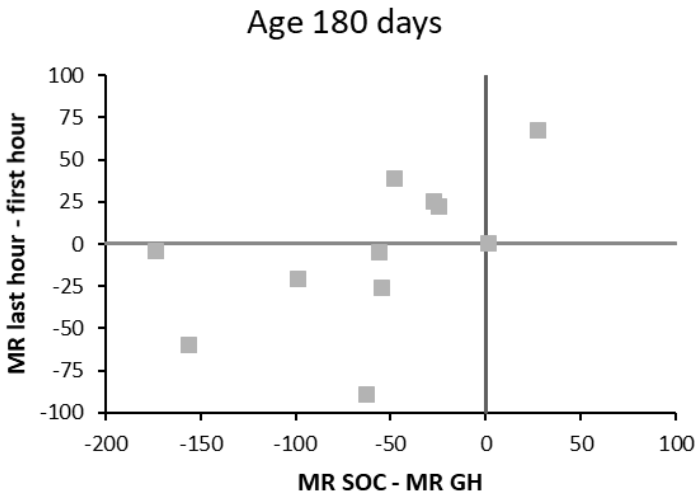

Figure 12 Correlation of the change in metabolic rate (MR) from group housing (GH) to social confrontation (SOC) detected in the current study (x-axis) and the change in MR from the first to the last hour of a 2.5 hour measurement of the same individuals at the same age and time of the day by Fritscher (2021) (y-axis).

4 Discussion

Social confrontations of unfamiliar conspecifics are a valuable method for the study of social stress, aggressive and dominance behaviour, preconditions for success in rank acquisition, as well as physiological mechanisms involved. Studies applying this approach were able to assess various factors which predict the likelihood of success or failure in rank acquisition as well as consequences for physiological profile and health in dominant and subordinate individuals (e.g. Henry and Stephens, 1977; Sachser and Lick, 1989; Stefanski and Hendrichs, 1996; von Holst, 1986). Apart from obviously beneficial parameters for acquiring high ranks like physical conditions (e.g. health and strength), it has been shown that behavioural strategies to succeed in agonistic encounters have to be learned, thus experience is essential (e.g. Harlow and Harlow, 1962; Immelmann et al., 1982). In male guinea pigs the juvenile life stage has been shown to be essential for developing skills necessary for rank acquisition in adulthood (Sachser, 1993; Sachser and Lick, 1991; Sachser et al., 1994).

Whether an animal was successful in agonistic encounters can manifest in physiological parameters. For instance, winners of agonistic encounters express higher levels of

testosterone (e.g. Beehner et al., 2006; Oyegbile and Marler, 2005; Sachser, 1987; Sapolsky, 1982) and in guinea pigs it has been shown that directly after an agonistic encounter between a resource holder and an intruder the intruder express higher levels of norepinephrine (Sachser, 1987). Studies that investigated the alteration of glucocorticoid concentrations in response to social confrontations found that the loser had higher levels afterwards than the winners and that in winners it decreased back to baseline more rapidly (Sachser et al., 1994; von Holst, 1998). However, results of further studies indicate that it is mainly the amount of energy required to deal with agonistic encounters which determines the level of increase in glucocorticoid concentration rather than success or failure (Gesquiere et al., 2011; Goymann and Wingfield, 2004). In the current study no significant correlations between cortisol levels and hierarchy were found for which none of the previous studies can be confirmed.

While the endocrine responses to social confrontations are well studied, the changes in MR in response to this type of stressor have not been focus of research so far. Based on the fact that positive correlations between cortisol levels and MR have been demonstrated before (Jimeno et al., 2018), the assumption was that social confrontations would lead to an increase in MR. Surprisingly the opposite was found to be true. It is possible that in the current study the social confrontation did not elicit as much of a stress response as expected, as the cortisol concentrations also did not always increase from measurements before the encounter to measurements afterwards. The only exception was at the age of 180 days, where the cortisol levels of the focal males increased in response to the confrontation.

At the first experimental block at the age of 90 days neither the focal males nor the opponents showed an increase in their cortisol concentrations during social confrontation. At the age of 180 days, the focal males expressed a significant stress response, while the opponents again did not differ from the salivary cortisol levels measured before. Simultaneously, the MR decrease was more strongly pronounced at the age of 180 days. Those findings contradict the expectation as previous studies indicated the confrontation with an opponent to cause a stress response (e.g. Nemeth et al., 2014; Nemeth et al., 2015) and that stressors of different nature (i.e. physiological and psychological stressors) which elicit a certain level of increase in glucocorticoids enhance the MR to the same extent (Jimeno et al., 2018). The results of the current study may be caused by asymmetries in age, strength, and experience between the two male groups. During the first experimental block at the age of 90 days the focal males

were still growing and of lower body mass than the opponents, thus may not have been perceived as a threat by the opponents. Moreover, at this age the focal males were in a life-stage where proper competition for rank does not yet occur. At the age of 180 days the focal males had reached the same body mass as the opponent males and could be considered fully adult. Previous investigations on guinea pig males, however, showed that they have to reach the age of seven months to acquire a high social status (Sachser, 1986). The focal males of the current study were a little younger at this experimental block (i.e. six months), thus they might still have been too young for rank acquisition. In addition, the opponent males have been used in confrontations for other studies before, which might have been an advantage in the encounters with the focal males. Despite the fact that the focal males have been reared in a male group they did not have the opportunity to gather proper experiences in agonistic encounters, as they have never been confronted with an older, more experienced male. Thus, they were only experienced in fights with similarly strong and equally experienced males. However, the outcomes of the CI indicate that the focal males got more experienced in agonistic encounters from the 90 days to the 180 days confrontations, as those focal males, which had a higher rank in their own social group were able to keep their rank in the confrontations. Additionally, the comparison of the CI outcomes from the social confrontations at the age of 180 days indicate that focal males did already reach an age where the stratification into dominant and submissive individuals did already happen. While during the group housing CI values of all ranges occurred, the social confrontations led to a bifurcation into either low or high CI values, thus in either low- or high-ranking focal males. 5 focal males reached a CI of 1, which is the highest CI possible, 3 focal males failed to express any dominance and reached a CI of 0. The remaining 4 focal males also had either a high or a low CI (ca. 0.7 and 0.1, respectively), thus not a single male was not assignable to be either a dominant or a submissive male. The CI values, however, neither correlated with cortisol levels, not with MR.

The cortisol increase due to the social confrontation at the age of 180 days may have been caused by the way the focal males behaved. The fact that focal males with a high social rank during group housing were able to maintain it indicates that they behaved proactively, for which more energy might have been required. This proactivity could have elicited the significantly higher aggression of the opponent males. The increased sociopositive behaviour

of the focal males may have served to appease the opponents. However, it is also possible that the aggression of the opponents was the cause of the cortisol increase in the focal males.

Surprisingly, the decrease in MR after the social confrontation was more strongly pronounced at the age of 180 days than at 90 days, despite the increase in cortisol concentrations. Since cortisol does not seem to have affected MR, there must have been other factors influencing the MR. It cannot be ruled out that the time delay between the social confrontations and the respiratory measurements played a role, despite the fact that the first 10 minutes of OxBox measurements did not differ from the full hour.

Generally, MR was rather constant on an individual level, thus individuals that had low or high MR after group housing had similarly low or high levels after the social confrontations. The interindividual variation on the other hand, was high.

Guenther (2014) found a negative association between cortisol and BMR in wild guinea pigs. In this paper it is discussed that animals exposed to a stressor require more energy to deal with this stressor and consequently, as resources are limited, the amount of energy that can be invested in BMR decreases. Since the focal males in the current study also were limited in their food, it is possible that physiological stress responses negatively affected the availability of energy for other physiological processes which led to a decrease in MR. It would have been interesting to see how the MR would have changed if unlimited food resources had been available. Another possible explanation is, that a circadian rhythm existed in the focal group, that had a stronger influence than the stress induced by an unfamiliar male. Despite the fact that most studies on the circadian rhythmicity of oxygen consumption in guinea pigs found no patterns of daily respiratory rate variability (e.g. Kramer et al., 1999), authors sometimes described that a few animals did express a rhythm (Mortola, 2004; Stupfel et al., 1981). The studies that found a circadian pattern, however, were conducted on very small sample sizes and should be interpreted cautiously (Mortola (unpublished observation): n = 2; Stupfel et al. (1981): n = 1). The existence of a circadian rhythm of MR in the individuals of this study is also supported by the strong tendency for a correlation ($p = 0.053$) with the first and last hour of MR measurement over 2.5 hours by Fritscher (2021) conducted on the same individuals at the same time of the day and the age of 180 days, but without the stressor of an unfamiliar conspecific. A decrease in MR during measurements lasting for multiple hours is also found in

other studies (Careau et al., 2008) where a decrease of arousal is assumed to be the cause. However, the correlation of MR measurements between the findings of Fritscher (2021) and the current study cannot be explained by a decrease of arousal, which makes a circadian effect a more likely cause.

For cortisol some studies did not find circadian rhythms in guinea pigs (e.g. Banjanin et al., 2004), but others did at least to some degree. Garris (1979) found lower cortisol levels late in the light phase and higher levels close to the onset of light, which confirms differences in measured cortisol levels in different times of the day by other studies (Gala and Westphal (1967): afternoon; Dalle and Delost (1974): morning). During the time period from 8 a.m. till 14 p.m., thus the time range where the experiments of the current study were conducted, cortisol levels declined in the measurements by Garris (1979). Consequently, it is possible that the social confrontations prevented a decrease in the cortisol concentrations.

The fact that stress responses were lower as expected might also be due to the housing condition of the male groups. In most other social confrontation studies the males have been housed either isolated from other males (either in single cages (e.g. Wallner and Dittami, 2003;) or with one or more females (e.g. Sachser, 1993; Sachser and Lick, 1989; Sachser and Lick, 1991; Stefanski and Hendrichs, 1996), or in social groups mimicking the natural social structures of guinea pigs, where multiple male and female guinea pigs of different ages were housed together (e.g. Sachser, 1987; Sachser, 1993; Sachser and Lick, 1991) before the social confrontation started. In the latter setting the males express well defined linear hierarchy structures (Sachser, 1987), for which the inter-male aggression is usually rather low (Davis, 1971; Kondo and Hurnik, 1990; Rood, 1972; Sachser, 1987), leading to low stress-levels (Sachser and Lick, 1991; Sachser et al., 1994; Sapolsky, 2005). In guinea pigs it has been demonstrated that the baseline cortisol levels of males raised in mixed-sex colonies or alone with one female is the same, but in social confrontations with unfamiliar males the cortisol levels of colony-raised males remain at baseline, regardless of whether they were successful. In unexperienced males raised with females the winners of such confrontations return to a baseline cortisol level within a few days, while the cortisol level remains elevated in the loses (Sachser et al., 1998).

In the current study, however, 12 males were housed together without any females. The high rate of interactions in the guinea pig males of the current study strongly differed from interaction rates of other studies conducted on guinea pigs. For example, Mutwill et al. (2021), who determined the dominance ranks of the males in a mixed sex group from retreats in agonistic encounters, describe in their methods that they used the video-analyses conducted over 2 hours in the afternoon to assess the hierarchies, because of a higher level of interactions as compared to the morning. They recorded the behaviour in the group housing during the morning (8-10 a.m.) merely as a backup, in case they did not reach an amount of 10 retreats in the afternoon. Comparing this with the retreat frequencies of the males in the currents study demonstrates strikingly how much the behaviours in the male groups differed, as here behaviour during group housing was measured in the morning for only one hour and yet the displacement rate exceeded the amount of 10 most of the time. Consequently, it is likely that the males of the current study already had elevated stress levels close to a plateau during group housing, for which a measurable increase in physiological stress responses due to the social confrontation was difficult to achieve. Our findings are supported by a study of Beer and Sachser (1993) who found an increase in aggression and stress level with increasing group sizes in male guinea pig groups.

As a decrease of arousal during the 1h OxBox measurement was considered as a possibility for having found no increase in MR after the social confrontation, the MR levels during first 10 minutes in the OxBox were analysed and compared with the entire hour. Previous studies (Careau et al., 2008) pointed at the first minutes of respiratory measurements to give valuable information about an animal's personality. However, in the current study the first 10 minutes did not differ from the whole hour measurement, which also indicates that the social confrontation did not cause further increases in the focal males' stress levels.

4.1 Limitations of the current study

For a possible detection of differences in physiological stress-levels between dominant and subordinate males a social confrontation lasting for multiple days would have been required, as the cortisol levels haven been shown to increase in all opponents in a first phase, regardless of success, while in winners it is lower than in losers after a few days (Sachser and Lick, 1989). It would have been interesting to know if MR differences appeared between winners and

losers of the confrontation once a hierarchy is established and hierarchy-borne differences in cortisol concentrations occur.

In addition, for the study of the development of physiological responses to social stress a different housing, either singly, singly with a female or in mixed-sex groups with different ages would have been beneficial, as it is difficult to detect any physiological changes caused by a stressor, when the stress level was already high beforehand. Due to the multi-male housing it is also possible that an hierarchy-based difference in stress-levels existed before the experiments started. Standardized conditions before the onset of the social confrontation would have been beneficial.

A sample size of more than 12 individuals might have allowed to find more effects, since the strong interindividual variations in cortisol concentrations and MR could mask effects elicited by the experiment.

As it is more accurate to consider cortisol as a hormone that provides energy for energy expenditure (either actual energy demands or anticipated energy demands) it may have been advantageous to not only record locomotion of an individual, but specify if it is walking or running, since the latter is more energetically demanding.

4.2 Conclusion

Stressful agonistic encounters and increases in cortisol concentrations are not necessarily attended by increases in MR. Similarly, to the BMR measurements of Guenther et al. (2014) we found that cortisol levels negatively affected MR. One factor that may have caused this effect is the restriction in food resources. Generally, however, differences in experimental setups and confounding factors that may cause contradictory findings regarding the relationships between cortisol levels and MR are poorly understood. More research in needed to investigate underlying physiological mechanisms.

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Zusammenfassung

Soziale Konfrontationen unbekannter Artgenossen sind eine gut erprobte Methode zur Untersuchung von sozialem Stress und konnten endokrine Reaktionen, wie Erhöhungen der Kortisol-Konzentration aufzeigen. Die energetischen Anforderungen, um mit derartigen agonistischen Auseinandersetzungen umzugehen, sind hingegen noch unerforscht. Die aktuelle Studie untersuchte daher die Änderungen im Sauerstoffverbrauch, als Maß für die Stoffwechselrate in Reaktion auf soziale Konfrontationen unbekannter Meerschweinchen Männchen, deren Zusammenhang mit Kortisol Konzentrationen, sowie den Bezug dieser Variablen zum Sozialverhalten. Da sich bei männlichen Meerschweinchen gezeigt hat, dass sich die Verhaltensstrategien der Rangakquirierung mit zunehmendem Alter und Erfahrung ändern, wurden die sozialen Konfrontationen während der Adoleszenz und im Erwachsenenalter durchgeführt. Zwölf gleichaltrige Männchen aus einer gemeinsamen Männchen-Gruppenhaltung wurden hierfür einzeln mit einem älteren, unbekannten männlichen Meerschweinchen konfrontiert. Stoffwechselrate und Kortisol Konzentrationen wurden vor und nach den sozialen Konfrontationen gemessen. Soziale Interaktionen wurden sowohl in der eigenen Männchen-Gruppe als auch während der sozialen Konfrontationen aufgezeichnet. Trotz der hohen Interaktionsrate sank die Stoffwechselrate von den Messungen vor der sozialen Konzentration zu Messungen danach und wurden von Kortisol-Anstiegen nicht beeinflusst. Im Alter von 180 Tagen, also im vollen Erwachsenenalter, wurde der stärkste Anstieg im Kortisol gemessen, während zeitgleich die Stoffwechselrate stark abnahm. Eine mögliche Erklärung hierfür könnte sein, dass die nicht unlimitierte Futterverfügbarkeit die Energiereserven begrenzt hat. Die hohen energetischen Kosten der agonistischen Auseinandersetzungen könnten die für die Stoffwechselrate verfügbare Energie reduziert haben, wie es von Autoren einer vorangegangenen Studie an wilden Meerschweinchen interpretiert wurde. Welche physiologischen Mechanismen einem solchen Zusammenhang zugrunde liegen könnten, ist aber unklar, weshalb weitere Untersuchungen erforderlich wären.

Schlagwörter: Meerschweinchen, Stoffwechselrate, Kortisol, Adoleszenz, Sozialverhalten, sozialer Rang