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„Effects of dietary fatty acids on social behavior in guinea pigs (*Cavia aperea f. porcellus*)"

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„The truths of organic nature are of a charming and awesome beauty,
the deeper you penetrate into their details and special features,
the more beautiful they become.”

Konrad Lorenz
Altenberg, summer 1949

„Denn die Wahrheiten der organischen Natur sind von liebenswürdiger und
ehrfurcht-gebietender Schönheit, und sie werden immer schöner,
je tiefer man in ihre Einzelheiten und Besonderheiten eindringt.“

Konrad Lorenz
Altenberg, im Sommer 1949

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Abstract

Fatty acids (FAs) influence the fluidity of neuronal membranes and therefore play an important role in the structure and functions of the brain. This suggests that FAs also have an impact on social behavior as well as the hypothalamic-pituitary-adrenocortical system (HPA-axis). In the present study we investigated the effects of unsaturated and saturated FAs on social behavior and the HPA-axis in guinea pigs (*Cavia aperea f. porcellus*). Sexually intact adult domestic guinea pigs (N=63), kept in isosexual groups, were used in this study. In addition to their standard food supply, the subjects received an orally administered diet rich in: 1) unsaturated (UFA) or 2) saturated FAs (SFA; per group: ♂ N=11, ♀ N=10) during a 60 day period. The control groups received the same amount of water. Behavioral parameters included agonistic and socio-positive behaviors as well as locomotion. The dominance hierarchy of the treatment groups was determined and changes of individual rank positions within each group were recorded. Furthermore, bodyweight and saliva cortisol concentrations were repeatedly measured and plasma fatty acid concentrations were analyzed. The findings of this study revealed that the intake of a diet high in SFAs resulted in a more pronounced decrease in both agonistic and socio-positive interactions during the course of the study compared to the control group. Similar patterns were found in individuals supplemented with UFAs, however less pronounced compared to the SFA group. Control animals, in particular males, showed relatively high rates of agonistic and socio-positive encounters not only in the beginning but also at the end of the study. High frequencies of agonistic behavior were associated with more socio-positive interactions, suggesting that socio-positive behavior might be used as a form of appeasement after agonistic encounters. High frequencies of agonistic and socio-positive interactions were paralleled by longer durations of locomotor activity. Furthermore, saliva cortisol concentrations decreased in the SFA group over the course of the study, whereas no clear effect of dietary manipulation was found in the UFA group. Interestingly, the control group showed rather stable and low cortisol concentrations, indicating that either frequent conflicts did not cause elevated stress levels or that stressful situations were counteracted by socio-positive behavior. The dietary manipulations

did not affect the stability of the dominance hierarchies in the three groups. Body mass increased throughout the study and did not differ between the groups, indicating that the dietary treatment did not cause detectable differences in the body condition of the animals. Moreover, plasma fatty acid concentrations showed an increase of total plasma UFAs or SFAs in line with the different treatments. The n-6:n-3 ratio was lowest in the UFA and clearly elevated in the SFA group. Considering reported beneficial effects of low n-6:n-3 ratios on brain functions and metabolism, long term positive effects would rather be expected in the UFA than in the SFA group. In general, our findings suggest that the guinea pigs, in particular males, receiving a diet rich in fatty acids were able to maintain stable dominance relationships with decreasing conflict rates over the course of the study. This could be energetically advantageous especially when food resources are limited. Surprisingly, the effects were even more pronounced in the SFA compared to the UFA group. Thus, the hypothesis that high SFA intake would lead to increased aggressive behavior could not be supported in this study.

Introduction

Fatty acids (FAs) are vital components in nutrition. They play an important role in metabolism as essential parts of membranes, in storage and transport of energy, and as gene regulators[1]. Thus, the FA composition of membrane lipids has the ability to change neuronal membrane fluidity and hence neuronal functions[2]. Because of these mechanisms, FAs have an important impact on the structure and functions of the central nervous system[3–5]. The term 'fatty acids' comprises saturated (SFA), mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids. The latter is divided into omega-3 (n-3) and omega-6 (n-6) FAs as well as omega-9 (n-9) FAs, which are counted also among MUFAs. While saturated fatty acids (SFAs) can be synthesized *de novo* within the body, most unsaturated fatty acids (UFAs) have to be acquired via the diet[4]. In particular, PUFAs, such as n-3 and n-6 FAs, are known as 'essential fatty acids'. These nutritional components are important to maintain normal physiological functions[6].

An increasing number of studies reveal an UFA-based influence on agonistic and exploratory behavior, locomotion, emotional status, and cognitive functions[7]. Cynomolgus monkeys, for example, displayed more agonistic behavior towards conspecifics when consuming a low fat, low cholesterol diet[8]. This was also the case in other studies, in which diets deprived of n-3 PUFAs resulted in an increase in the aggression level of male German Shepherds dogs and rats[7,9]. In contrast, another study revealed an increase in agonistic behavior in male mice and rats when treated with high quantities of n-6 PUFAs[10]. Stress-related behaviors, such as depressive-like behaviors, anxiety, and cognitive dysfunctions, can be mildened by consuming a diet high in PUFAs. For instance, rats fed with fish-oil (n-3 PUFAs) showed longer exploratory behavior while staying in the aversive arms of an elevated plus maze. Further on, these rats also demonstrated a reduction of immobility behavior and an increase in swimming frequency when confronted with a forced swimming test[11]. Regarding locomotor behavior, studies on mice showed an increase in movement when deprived of n-3 FAs[12].

Concerning SFAs, research has tended to focus on the impact on cognitive functions. For example, rats reared with a diet rich in SFAs are impaired in their working memory compared to those groups fed with UFAs or a standard diet.

Furthermore, a decreased level of n-3 FAs was found in the SFA group[13], indicating that PUFAs, in particular n-3 FAs, might positively affect memory retention. Nevertheless, there is evidence of physiological effects. Rats supplemented with a diet enriched in SFAs were more sensitive to pain and poorer in body temperature regulation in a cold environment than those fed with PUFAs[14]. However, effects of SFAs on social behavior have rarely been investigated.

The hypothalamic-pituitary-adrenocortical (HPA) system and free FAs (FFAs) also interplay with each other. The HPA-axis is responsible for an appropriate stress response when confronted with a psychological or physical stressor[15]. Thus, this system can be used to estimate stress load. The activity of the HPA-axis can be measured by analyzing cortisol or corticosterone concentrations in plasma, saliva or their metabolites in feces[16]. Glucocorticoid hormones are essential for coping with stressors and as a result restoring homeostasis. Actions of glucocorticoids involve different physiological systems, including cardiovascular effects, neurobiological effects, and metabolism[17]. Studies report an inhibition of the HPA-axis caused by circulating FFA levels[11,18]. In human females an increased FFA level led to a decrease in cortisol secretion[19]. This might indicate that PUFAs have a regulating effect on cortisol levels in stressful situations. This was demonstrated in a social confrontation test, in which prior isolated guinea pigs fed with n-3 or n-6 PUFAs showed lower cortisol concentrations compared to individuals supplemented with n-9 MUFAs or a control group[20]. This supports previous findings in rats[11] consuming a diet high in n-3 PUFAs. When confronted with a stressor, n-3 supplemented rats were able to maintain a glucocorticoid concentration at a level that was comparable to the one of non-stressed control individuals, whereas the stressed control group showed increased concentrations[11]. Interestingly, Nemeth et al.[20] revealed that cortisol concentrations were continuously higher in male than female guinea pigs regardless of the presence or absence of a stressor.

Few researchers have addressed the effect of UFAs on social behavior in relation to SFAs within one study. They mostly concentrated on one of the two nutritional components. The present study, therefore, investigated the effect of unsaturated and saturated fatty acids on social behavior and the HPA-axis. Due to their complex social system, guinea pigs (*Cavia aperea f. porcellus*) are suitable animal models in this context[21]. This complex social system is clear when comparing

the social organization of guinea pig groups with high and low population densities. When kept in groups with high population numbers, the social organization of guinea pigs is characterized by establishing different territories within a group[22]. Each territory consists of an alpha male (highest ranking male), which forms long-lasting social bondings with females within his territory. Subdominant males are tolerated. Interestingly, territories do not overlap within these groups. However, when kept in groups with low population densities, linear dominance hierarchies are established among males[22,23]. Furthermore, social behavior of guinea pigs was studied in different social environments, including setups testing stress-related events. Dominance relationships and social bondings are known to play an important role in stress management. For instance, individuals in a stable social system are able to predict a conspecific's behavior better than in the absence of an established dominance hierarchy. Although there are clear differences in the behavior of high and low ranking animals, the stress level in low ranking guinea pigs is comparable to the one of high ranking individuals. Hence, lower ranks in the dominance hierarchy are not always negatively affected by social stress. Furthermore, bonding partners provide social support, which also has a positive effect on stress responses[24].

More recent evidence[20] highlights the effects of UFAs on social behavior and physiological stress responses in guinea pigs. In a social confrontation test, prior isolated individuals showed an increase of locomotion and a decrease of saliva cortisol concentration when exposed to a diet rich in n-3 or n-6 PUFAs compared to a diet rich in n-9 MUFAs and untreated control individuals. These results indicated an impact of n-3 and n-6 PUFAs on stress-related behaviors, suggesting positive effects on stress coping. Based on this evidence, the present study was designed to compare the impact of UFAs and SFAs on the social behavior, the dominance hierarchy, and the HPA-axis in guinea pigs. We hypothesized that individuals provided with a diet high in UFAs would show less agonistic behavior and lower cortisol concentrations compared to individuals supplemented with high levels of SFAs or a control group. With this in mind we fed male and female adult guinea pigs, which were constantly kept in social groups, with a diet either rich in UFAs or SFAs. Behavioral interactions (socio-positive and agonistic behaviors) between individuals and hence the dominance hierarchy as well as locomotion and saliva

cortisol concentrations were observed during social settings. During a period of 60 days we investigated FA-based changes in these parameters.

Material and Methods

The experiment was carried out in accordance with the Austrian laws for animal experiments and animal keeping and approved of by the Ethics Committee of the Faculty of Life Sciences, University of Vienna (2014-005), and the Austrian Federal Ministry of Science and Research (BMWF-66.006/0024-II/3b/2013).

Subjects and Housing

In the present study 63 sexually intact adult domestic guinea pigs (*Cavia aperea f. porcellus;* 33 males and 30 females) were used. They were 18.1±10.1 (mean±SD) month old and weighed 797.3±153.7 g (mean±SD) at the onset of the study. The different age classes were evenly distributed within the groups. All subjects were housed in six isosexual groups for a period of 60 days (group housing period). The subjects were assigned to three different treatment groups (11 males and respectively 10 females per group). All groups were already established four months prior to the experiment, meaning that no additional individuals were introduced to or removed from the groups. The subjects were kept in a temperature controlled room (22°C) under a 12 h light-dark cycle (with lights on at 07:00 h) at the University of Vienna, Austria. The enclosures (3.16 m²) were enriched with shelters and various viewing platforms. Woodchips were used as bedding for the enclosures. The standard food supply consisted of 350 g (♀)/400 g (♂) guinea pig pellets (Ssniff V2233, Ssniff Spezialdiäten GmbH, Soest, Germany) and 100 g hay, which were provided each day. Water was provided *ad libitum*. All subjects were familiar to human contact.

Treatment

The subjects were divided into three treatment groups: 1) UFA, 2) SFA, and 3) control group. Throughout the group housing period all subjects received either water (control), unsaturated (UFA: walnut oil, Manako® Premium Speiseöl, Makana

Produktion & Vertrieb GmbH, Offenbach a.d. Qeeich, Germany) or saturated (SFA: virgin coconut oil, Manako® Premium Speiseöl, Makana Produktion & Vertrieb GmbH, Offenbach a.d. Qeeich, Germany) fatty acids in addition to standard rodent pellets. A daily amount of 3 ml water, walnut or coconut oil per kg bodyweight were orally administered to the individuals of the respective treatment groups using a syringe (Henry Schein®, Henry Schein Inc., Melville, New York, USA). The nutritional compositions of the standard food supply and the treatment diets (walnut and coconut oil) are given below (Table 1). In addition, the ratio between n-6 and n-3 PUFAs appears to be relevant. Yehuda[25] states that a ratio of 4:1 is recommended as the most beneficial ratio due to improved cognitive functions, such as learning abilities, sleep patterns and quality of life of Alzheimer's patients.

Table 1. Nutrient composition of diets. Energy content and nutrient components are given in KJ/1 kg (1 l) and g/1 kg (1 l) of diet. In addition, the percentages of the fatty acids with respect to the total amount of UFAs is shown. The total amount of UFA and SFA are marked in bold. UFA – unsaturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, SFA – saturated fatty acid.

	Standard diet		**Treatment diets**			
Nutrient	Ssniff V2233		walnut oil		coconut oil	
		[%]		[%]		[%]
Energy content	13200.00		38744.40		38900.00	
Crude protein	190.00		0.00		0.00	
Carbohydrate	0.00		0.00		0.00	
Crude fat	32.80		996.00		1000.00	
total UFA	**26.10**	**79.57**	**897.00**	**90.06**	**80.00**	**8.00**
total MUFA	6.40	19.51	158.00	15.86	65.00	6.50
total PUFA	19.70	60.06	739.00	74.20	15.00	1.50
total n-3	3.30	10.06	123.17	12.37	0.00	0.00
total n-6	16.40	50.00	615.83	61.83	15.00	1.50
total n-9	6.20	18.90	158.00	15.86	65.00	6.50
n-6:n-3	4.97		5.00			
total SFA	**6.70**	**20.43**	**99.00**	**9.94**	**920.00**	**92.00**
UFA:SFA	3.90		9.06		0.09	

Experimental procedure

Every day at 09:00 AM the subjects were filmed in their respective enclosures for 30 minutes. Prior to filming the houses and viewing platforms were removed from the enclosures to ensure the visibility of the subjects at all times. Two cameras attached to the ceiling (GoPro Hero 3, Woodman Labs, Inc., San Mateo, California, USA) were used, which were aligned in a way that all corners of the enclosures were clearly visible in the recordings. The recordings were done to analyze behavioral changes over the time course. For measuring various parameters and performing the feeding procedure each individual was removed consecutively from its respective enclosure after video recording. To monitor changes in body mass, the subjects were weighed every day using a customary digital kitchen scale. Every tenth day at 09:30 AM saliva samples were taken to measure cortisol. Afterwards 3 ml water (control), walnut (UFA) or coconut oil (SFA) per kg bodyweight were given to the respective groups. After each session the animals were released back into their respective enclosures and received their standard food supply. Blood samples for analyzing plasma fatty acid concentration were taken once per individual at the end of the group housing period (days 63 and 64). These were taken after the individuals were provided with food.

Sample collection

Saliva samples were collected using cotton buds. In order to exclude diurnal variations and thus enabling a standardized analysis of saliva cortisol concentrations, samples were taken every tenth day at 09:30 AM. These were inserted into the guinea pigs' cheek pouch for at least one minute. The cotton bud's tip was put into a test tube and centrifuged (14000 rpm, 17968xg, 10 minutes). Afterwards the samples were stored at -20°C until further analysis. Since cortisol levels of saliva and plasma samples are highly correlated, this non-invasive method can be used to monitor changes in the adrenocortical function[26,27].

Blood samples were collected by puncturing the marginal ear vein using sterile lancets[26,28]. In order to standardize sample collection by daytime, only half of the subjects were handled per day, respectively. Thus, blood samples were taken on two consecutive days beginning at 09:30 AM. With the assistance of heparinized

micropipettes approximately 300 µl of blood were collected in test tubes. To separate the plasma from the blood's hematocrit the test tubes were centrifuged (14000 rpm, 17968xg, 10 minutes). The plasma was then transferred into another test tube by the use of a Hamilton microliter syringe (Hamilton® Company, Nevada, USA). Afterwards the samples were stored at -20°C until further analysis.

Sample Analyses

Saliva samples were analyzed using an enzyme-linked immunoassay (EIA)[29], with a 1:50 dilution and an input of 10 µl Assaybuffer. The antibody used was specific for cortisol (University of Veterinary Medicine, Vienna, Austria[29]). All samples were applied twice on the microtiter plate. The percentages of cross-reactions with relevant steroids in the cortisol analysis were: 4-Pregnene-11β,21-diol-3,20-dione 6.2%; 4-Pregnene-11β,17α,21-triol-3,20-dione 100%; 5α-Pregnane-11β,17α,21-triol-3,20-dione 4.6%; 5α-Pregnane-3α,11β,17α,21-tetrol-20-one 0.8%; 5β-Pregnane-3α,11β,17α,21-tetrol-20-one 0.1%; all other steroids cross-reacted <0.01%. The intra- and interassay coefficients of variance amounted to 11.1% and 9.4%.

To evaluate plasma fatty acid concentrations, blood samples were analyzed using gas chromatography[30]. Methanolic NaOH (1 ml, NaOH contains butylated hydroxytoluene [BHT], which prevents oxidation) was added to 35 µl plasma to obtain transesterificated fatty acids. Fatty acid methyl esters (FAMES) were then produced by adding 1 ml of 14% boran-triflourid-methanol (BF_3). To extract FAMES, 500 µl hexane were used. This step was repeated four times. Afterwards, they were vaporized and re-dissolved in hexane. In order to separate FAMES, a Rtx-2330 30 m x 0.25 mm i.d. silica column was used by operating an Auto-System-Gaschromatograph (Perkin Elmer, USA) with flame ionization detector (FID). Helium served as carrier gas during the detection of fatty acids. For this procedure, 1 µl of prepared samples was injected at a temperature of 250 °C. The detection of fatty acids itself was carried out at 270 °C. A 37 component FAME Mix Standard (Supelco, Bellafonte, USA) was then used for identification, while the peak integration was done by a TotatChrome Workstation 6.3.0 (PE Nelson, Perkin Elmer, USA). The total percentages of n-3, n-6, n-9, MUFAs, PUFAs, UFAs, SFAs

as well as the ratios of n-6:n-3 PUFAs, MUFAs:SFAs, PUFAs:SFAs, and UFAs:SFAs were calculated by the summation of single fatty acids.

Behavioral Measurements

Following Rood[31], three behavioral categories were defined: 1) socio-positive behavior, 2) agonistic behavior and 3) locomotion. Socio-positive behaviors included side by side (huddling), nose-nose contact (kiss), social grooming, naso-anal, and follow, whereas agonistic behaviors were defined as displacement, chasing, biting, fighting, mounting, rumba-rumble, stand-threat, head-thrust, kick-back, and marking. Walking, running and jumping were termed as locomotion. Sexual behavior was not recorded due to isosexual groups. For measuring initiated and received interactions continuous recording was used[32,33]. The frequencies (events) of the above mentioned behavioral variables were recorded as well as the durations (states) of locomotion. Analyzing the dominance hierarchies of the treatment groups on three consecutive days showed that stable rank orders were maintained throughout this timeframe (Kaplan et al., unpublished data). This suggests that stable dominance hierarchies are maintained over a longer period of time in guinea pigs, which was also proposed by Sachser et al.[24]. Due to this fact we decided that coding the videos for every tenth day (day 01, 10, 20, 30, 40, 50, 60) was sufficient to analyze changes in the social behavior during the group housing period. Another reason was to match the behavioral data to the timeframe of saliva cortisol sample collection. For further statistical analyses all behavioral variables were pooled into their respective behavioral categories (socio-positive or agonistic behavior). This was done to calculate the sum of socio-positive and agonistic behaviors for each individual on each day.

To determine the dominance hierarchy within each treatment group, dyadic interactions were analyzed. Initiators and receivers of all agonistic behaviors were recorded. Following Appleby's procedure[34], each subject was recorded with a score of 0.5, if no agonistic interactions were observed between two individuals. This means that there was a fifty percent chance that the agonistic encounter, if an interaction occurred, was initiated by one of the two individuals. Afterwards the proportion (P_a, modified after Singh et al.[34] and Coulon[35]) of initiated agonistic behaviors in pairwise encounters was calculated. This defines how much agonistic

behavior was initiated from one individual in relation to the total amount of initiated and received agonistic behaviors.

$$P_a = \frac{agonistic\ behavior_{initiated}}{\Sigma\ (agonistic\ behavior_{initiated} + agonistic\ behavior_{received})}$$

By using this equation, proportion values were achieved for each individual and each of its possible interaction partners of the same treatment group for every analyzed day of the group housing period. For further analyses, a Hierarchy Index (HI, modified after Singh et al.[34], Table S1) was then established for each individual per day. This was done by calculating the sum of all proportion values (P_a) for every possible interaction partner on each day.

$$HI_a = \sum_{a=1}^{n} P_a$$

In addition, a delta Hierarchy Index (ΔHI) was computed to analyze the stability of the dominance hierarchy. For this purpose the absolute value (non-negative value) of the difference between each day and the following was calculated. Low ΔHI-values indicate stable dominance hierarchies within the treatment groups. The equation below shows an example for the calculation of the ΔHI for day one and ten.

$$\Delta HI = |HI_{day\ 1} - HI_{day\ 10}|$$

Data Analysis

The videos were analyzed using The Observer XT 10 (Version: 10.5, Noldus, Wageningen, the Netherlands). In order to match behavioral with saliva cortisol data, the videos were coded for every tenth day. All statistical tests were performed using R (Version 3.1.1)[36]. Two subjects had to be excluded from the analyses due to health problems during the experiment.

The distribution of the data was analyzed with Shapiro-Wilk test and visual inspection of the fitted values. Non-normally distributed variables were transformed

by applying the second or third root transformation. For the visualization of the results non-transformed data was used. Levene's test was conducted to examine the homogeneity of variance. Furthermore, mean values are given in mean±standard deviation.

Linear Mixed-Effects Model (LME: package 'nlme')[37] with type-III sum of squares was used to analyze changes in the ΔHI, bodyweight, behavioral categories (socio-positive and agonistic behaviors, locomotion) as well as cortisol concentrations. This was done between groups (three treatment groups and all six groups), sexes, and days. Hence, the factors 'treatment' (control, UFA, SFA), 'sex' (male, female), 'day' (always day 01, 10, 20, 30, 40, 50, 60), and 'HI' were included in all models (except for the model with the response variable ΔHI) as predictors and thus as fixed factors. Due to the significant impact of the Hierarchy Index on social behaviors (see Results), the HI was used as predictor in all models. Hence, none of the behavioral parameters was used as predictor. For the response variable ΔHI, 'treatment', 'sex', and 'day' (day 1/10, 10/20, 20/30, 30/40, 40/50, 50/60) were used as predictors. The parameters ΔHI, bodyweight, socio-positive behavior, agonistic behavior, locomotion, and cortisol concentration were used as response variables. The random effect was specified to single individuals. All LMEs were fit by the method of maximum likelihood. The Akaike information criterion (AIC) was used to fit the starting models (three-way interactions, full models). Non-significant terms were excluded in the model through stepwise deletion. Post hoc interaction analyses with Bonferroni correction were conducted using the package 'phia'[38]. In addition, differences in plasma fatty acid concentrations were analyzed using two-way ANOVA (package 'WRS2' – Wilcox' Robust Estimation and Testing[39]) with the interaction 'treatment' and 'sex' as independent variables. Post hoc interaction analyses with Bonferroni correction were done by pairwise comparisons using t tests.

Results

Behavioral Measurements

Regarding agonistic behavior, a significant interaction of treatment and day was detected (Table 2). Agonistic behavior decreased significantly in all treatment groups over the time course (control χ^2=16.530, p=0.011; UFA χ^2=40.127, p=<0.001; SFA χ^2=80.128, p=<0.001; Figure 1A & B). However, the intake of a diet high in SFAs resulted in a more pronounced decline compared to individuals of the control group (control-SFA χ^2=25.948, p=<0.001). No effects of treatment were observed between control and UFA (control-UFA χ^2=13.209, p=0.119) as well as UFA and SFA group (UFA-SFA χ^2=6.271, p=1.000). This result was also supported by the findings on days 40 and 60, on which the SFA group showed significantly lower frequencies in agonistic behavior compared to the control group (control-SFA: day40 χ^2=9.888, p=0.035; day60 χ^2=20.735, p=<0.001). Furthermore, on day 60 there was also a trend towards more agonistic interactions in the control group compared to individuals fed a diet high in UFAs (control-UFA day60 χ^2=8.729, p=0.066). A significant interaction of sex and day was found as well (Table 2). Both sexes displayed less agonistic behavior over time (♂ χ^2=27.110, p=<0.001; ♀ χ^2=88.979, p=<0.001). The decline in the female groups, however, was more pronounced compared to the male groups (♂-♀ χ^2=18.065, p=0.006). Males generally displayed more agonistic behavior compared to females, which was highlighted on single days (♂-♀: day20 χ^2=13.431, p=<0.001; day40 χ^2=8.075, p=0.004; day60 χ^2=9.640, p=0.002).

A significant interaction of treatment and day was also observed when analyzing socio-positive behavior (Table 2). Post hoc analysis revealed a decline in behavior for all treatment groups throughout the experiment (control χ^2=43.915, p=<0.001; UFA χ^2=48.702, p=<0.001; SFA χ^2=57.266, p=<0.001; Figure 1C & D). Dietary manipulation (UFA and SFA) resulted in a continuous decrease in the frequency of displayed behavior compared to the control group (control-UFA χ^2=22.602, p=0.003; control-SFA χ^2=29.654, p=<0.001). There is also a trend towards showing more socio-positive behavior when exposed to a diet high in SFAs

compared to UFAs (UFA-SFA χ^2=14.792, p=0.066). This was also confirmed by significant differences on single days when compared to both UFA and control (UFA-SFA day10 χ^2=18.810, p=<0.001; control-SFA day30 χ^2=13.756, p=0.004). In addition, displayed behaviors significantly decreased for females and males over time (♂ χ^2=71.621, p=<0.001; ♀ χ^2=54.824, p=<0.001), during which the males showed a more pronounced decline compared to the females (♂-♀ χ^2=23.244, p=<0.001). Males generally displayed more socio-positive behavior compared to females, which was emphasized by statistically significant differences on day 01 and 40 (♂-♀: day01 χ^2=5.181, p=0.023; day40 χ^2=5.962, p=0.015). Concerning the interaction effect of treatment and sex, control males generally displayed more socio-positive behavior compared to control females (control ♂-♀ χ^2=8.295, p=0.004). However, no such effect was found when comparing the sexes of fatty acid groups. Furthermore, male individuals of the control group showed significantly more socio-positive behavior than the ones consuming a diet high in UFAs (control-UFA ♂ χ^2=7.262, p=0.042).

Table 2. Results of the LMEs for the seven days of the group housing period using agonistic and socio-positive behaviors as well as locomotion as response variables.

Predictor	Df	agonistic		socio-positive		locomotion	
		F-value	p (>F)	F-value	p (>F)	F-value	p (>F)
HI	1	66.007	**<0.0001**	18.675	**<0.0001**	0.275	0.600
treatment	2	0.172	0.843	1.405	0.254	1.121	0.333
sex	1	3.793	0.056	1.188	0.281	11.228	**0.002**
day	6	0.932	0.472	1.323	0.246	9.596	**<0.0001**
HI:treatment	-	-	-	-	-	-	-
HI:sex	1	7.023	**0.008**	3.622	0.058	19.516	**<0.0001**
treatment:sex	2	-	-	3.945	**0.025**	0.535	0.589
HI:day	-	2.313	**0.034**	2.619	**0.017**	-	-
treatment:day	12	2.519	**0.004**	3.718	**<0.0001**	1.708	0.064
sex:day	6	3.011	**0.007**	3.874	**0.001**	3.435	**0.003**
HI:treatment:sex	-	-	-	-	-	-	-
HI:treatment:day	-	-	-	-	-	-	-
HI:sex:day	-	-	-	-	-	-	-
treatment:sex:day	12	-	-	-	-	2.095	**0.017**

Significant p-values are marked in bold. Predictors in gray indicate interactions which were removed using AIC.

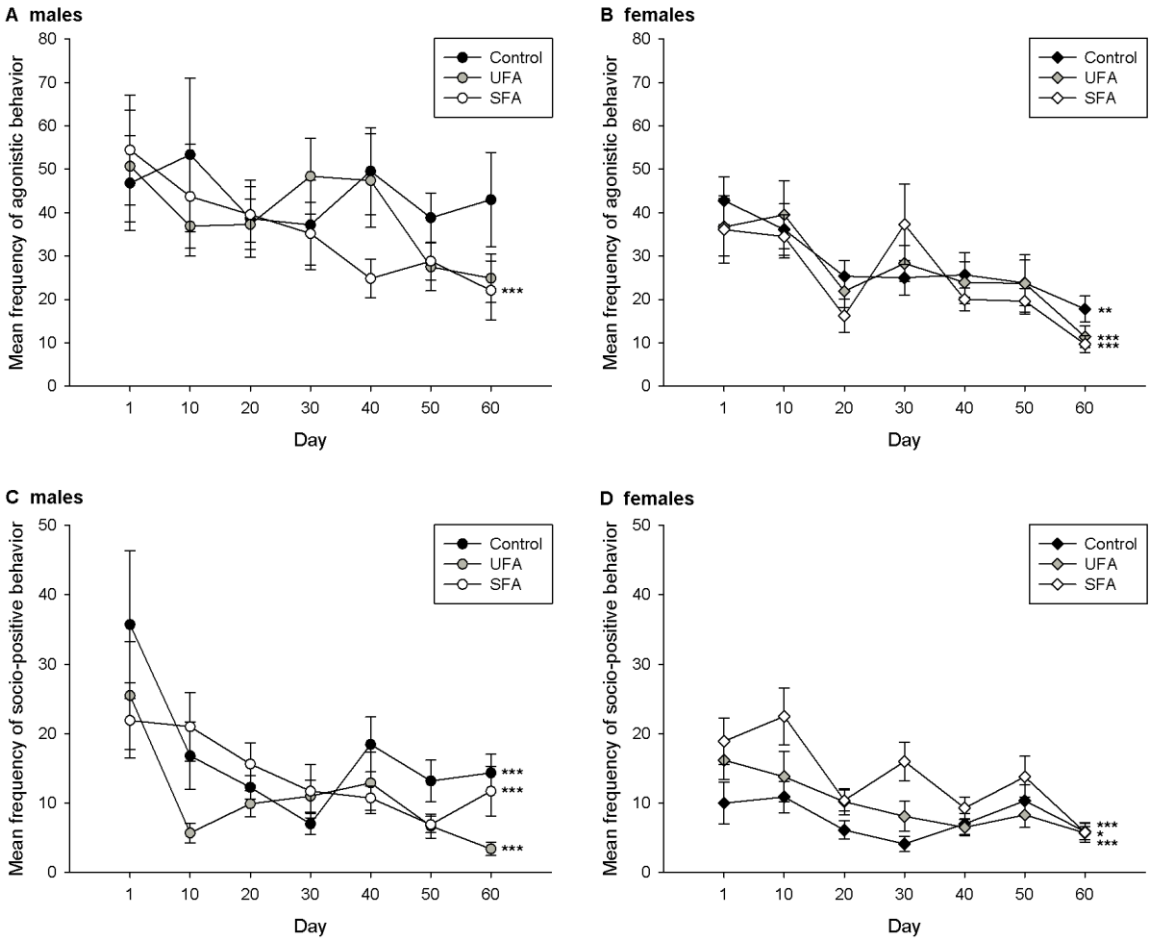

Figure 1. Agonistic and socio-positive behaviors. Mean frequency of agonistic ([A] – males, [B] – females) and socio-positive behaviors ([C] – males, [D] – females) for each day of the group housing period. Error bars indicate standard error. The asterisks mark the significance level (calculated for the full model): * p≤0.05, ** p≤0.01, *** p≤0.001.

Concerning locomotion, a significant interaction of treatment, sex, and day was detected (Table 2). A decline in locomotor behavior was found in all treatment groups and sexes throughout the experiment (control: ♂ χ^2=23.963, p=<0.001; ♀ χ^2=57.575, p=<0.001; UFA: ♂ χ^2=24.982, p=<0.001; ♀ χ^2=82.447, p=<0.001; SFA: ♂ χ^2=26.303, p=<0.001; ♀ χ^2=117.341, p=<0.001; Figure 2A & B). Nevertheless, the exposure of male individuals to a diet high in SFAs led to a significantly more pronounced decrease in movement, while the control males' locomotor activity remained nearly at the same level as on the first day of the group housing period (control-SFA ♂ χ^2=18.558, p=0.030). However, no significant differences were found when comparing the consumption of a diet high in UFAs to the control group. On day 40 decreased locomotor activity was observed in the SFA males, whereas increased durations of movement were detected in the control

males (control-SFA ♂ day40 χ^2=14.059, p=0.007). Further on, control females showed a more pronounced decrease in the duration of locomotor activity over time compared to control males (control ♂-♀ χ^2=20.611, p=0.002). In contrast, the females of the FA groups were more active and showed higher individual variation in the duration of the locomotor behavior compared to males (UFA ♂-♀ χ^2=32.215, p=<0.001; SFA ♂-♀ χ^2=31.673, p=<0.001). These differences were also detected on single days (control ♂-♀: day40 χ^2=8.371, p=0.004; day60 χ^2=4.797, p=0.029; UFA ♂-♀ day10 χ^2=22.209, p=<0.001; SFA ♂-♀: day01 χ^2=4.951, p=0.026; day10 χ^2=7.494, p=0.006). However, on day 50 the SFA males showed significantly more locomotor behavior than SFA females (SFA ♂-♀ day50 χ^2=5.622, p=0.018).

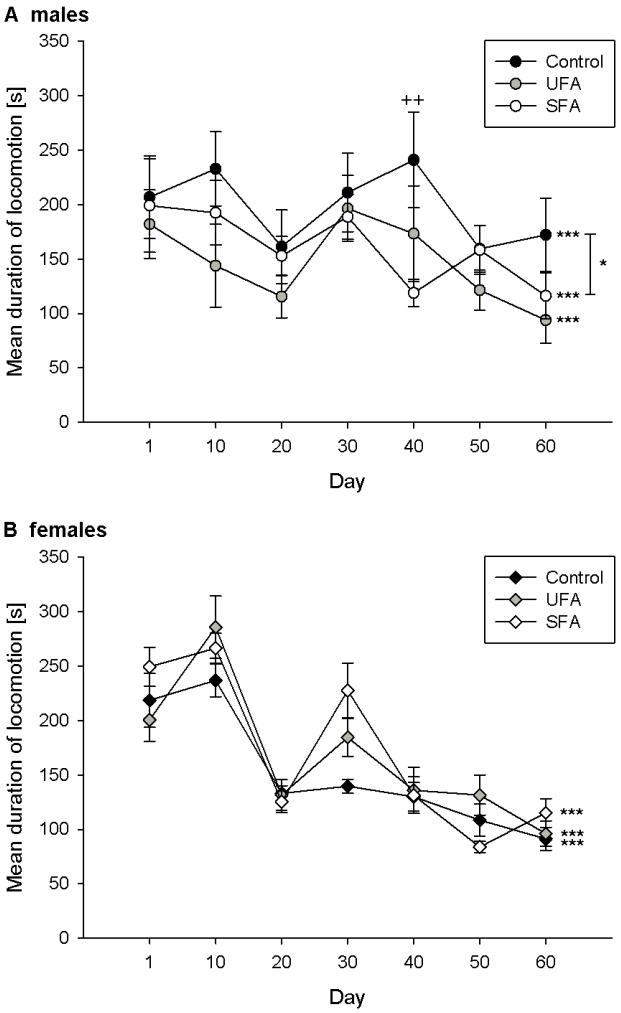

Figure 2. Locomotion. Mean duration of locomotion for males (A) and females (B) for each day of the group housing period. Error bars indicate standard error. The asterisks mark the significance level (calculated for the full model): * p≤0.05, *** p≤0.001. The symbols mark the comparison between treatment groups: ++ p≤0.01 comparing control and SFA group.

Regarding the delta Hierarchy Index (ΔHI), a significant interaction of treatment, sex and day was detected (Table 3). Post hoc interaction analysis revealed no significant changes during the time course in the dominance hierarchy of all treatment groups (control: ♀ χ^2=6.829, p=0.234; ♂ χ^2=5.780, p=0.328; UFA: ♂ χ^2=7.097, p=0.214; ♀ χ^2=9.868, p=0.079; SFA: ♂ χ^2=9.841, p=0.080; ♀ χ^2=4.918, p=0.426; Figure 3A & B). Further analyses did not confirm any significant differences between or within the treatment groups over the time course either within the sexes or between sexes. However, when analyzing single days, the ΔHI of the female control and UFA groups significantly changed less compared to male groups (control ♂-♀: day 20_30 χ^2=4.607, p=0.032; day 30_40 χ^2=6.968, p=0.008; UFA ♂-♀ day 40_50 χ^2=7.189, p=0.007).

Table 3. Results of the LME for the six delta days of the group housing period using the delta Hierarchy Index as response variable.

Predictor	Df	F-value	p (>F)
treatment	2	0.639	0.532
sex	1	0.353	0.555
day	5	1.366	0.237
treatment:sex	2	0.556	0.577
treatment:day	10	1.379	0.190
sex:day	5	1.716	0.131
treatment:sex:day	10	2.098	**0.025**

Significant p-values are marked in bold.

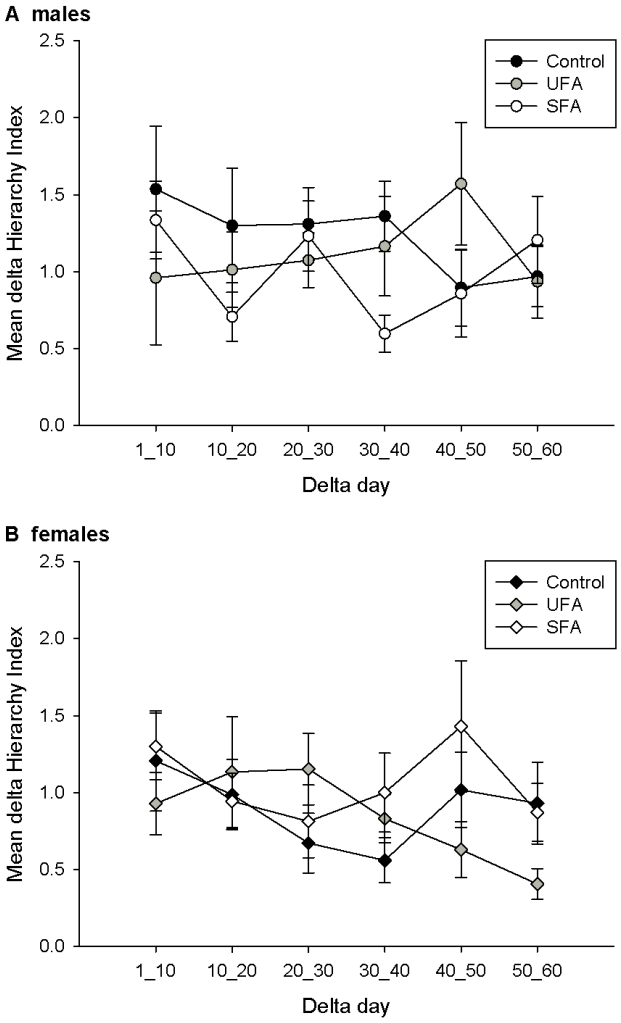

Figure 3. Delta Hierarchy Index. Mean delta Hierarchy Index of males (A) and females (B) for each delta day of the group housing period. Error bars indicate standard error.

Independent of treatment, the Hierarchy Index had a significant impact on agonistic behavior with an interaction of HI and sex as well as HI and day (Table 2). The more dominant males and females displayed more agonistic behavior. Furthermore, this effect was found on all days of the group housing period (Figure S1 & S2). Regarding socio-positive behavior, the tests also identified an interaction effect of HI and day (Table 2). Although this effect weakened throughout the group housing period, it clearly demonstrated that the more dominant individuals displayed more socio-positive behavior compared to lower ranked animals on all days (Figure S3 & S4). Analyzing locomotor behavior revealed a significant interaction of HI and sex (Table 2). The more dominant males showed higher

durations of movement. However, the HI had no effect on the females' locomotor behavior (Figure 4A). Additionally, a significant effect of HI in regard to saliva cortisol concentrations was observed with interaction effects of HI and treatment as well as sex (Table 4). In general, low ranking individuals had higher cortisol concentrations. However, when looking at males and females separately different courses of dietary manipulation were detected in the male groups. Due to the fact that the HI had no effect on females, only the male treatment groups were considered in the graph (Figure 4B). Control and SFA males with a position low in dominance hierarchy generally had high cortisol concentrations. Interestingly, the contrary was the case for males fed with a diet high in UFAs, even though the effect was not as strong as in the control and SFA group. An impact of the Hierarchy Index on the body mass was also found with an interaction between HI and day (Table 7). Generally, dominant individuals weighed more compared to non-dominant subjects (Figure S5 & S6).

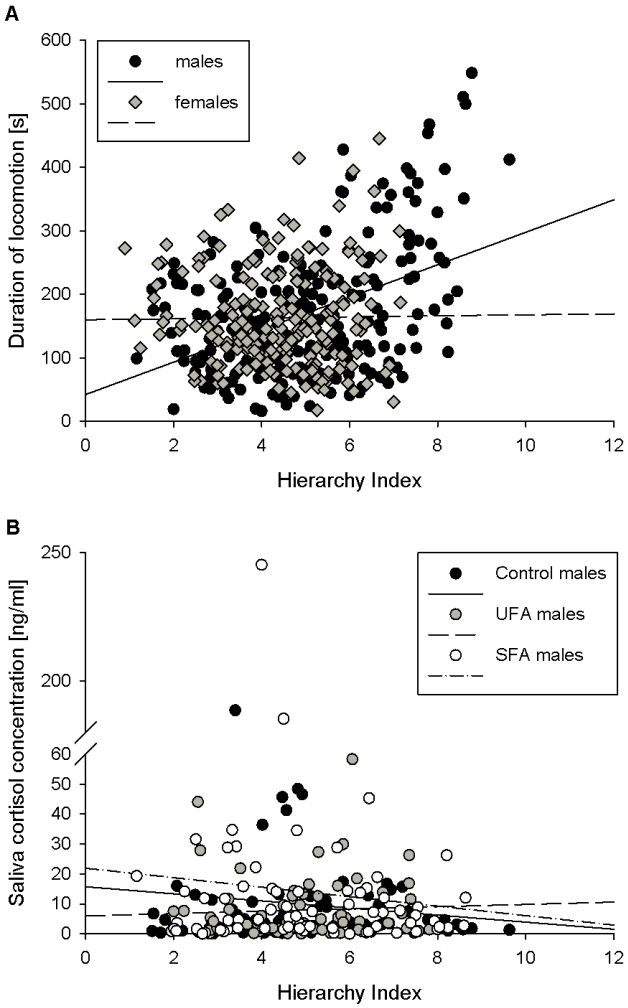

Figure 4. Locomotion and saliva cortisol concentration. (A) The effect of the interaction between the Hierarchy Index and sex on the duration of locomotion is shown. (B) The effect of the interaction between the Hierarchy Index and treatment (male groups) on the saliva cortisol concentration is shown.

Saliva cortisol concentration

The data analysis of saliva cortisol concentrations revealed a significant interaction of treatment and day (Table 4). Post hoc interaction analysis detected significant changes in the concentrations throughout the time course (control χ^2=41.496, p=<0.001; UFA χ^2=67.457, p=<0.001; SFA χ^2=39.245, p=<0.001; Figure 5A & B). Saliva cortisol concentrations were significantly lower in the control group compared to the UFA and SFA group. No clear effect of dietary manipulation was detected in the UFA group compared to the rather stable concentrations of the control and the

decreasing cortisol levels in the SFA group (control-UFA χ^2=42.741, p=<0.001; UFA-SFA χ^2=22.588, p=0.003). In contrast, individuals fed with a diet high in SFAs showed a stronger decline in cortisol concentrations compared to the control (control-SFA χ^2=31.065, p=<0.001). This was partially supported by significant differences on day 10 (control-SFA: day10 χ^2=9.966, p=0.033). On day 50 higher levels were observed in the control compared to the UFA group (control-UFA: day50 χ^2=23.638, p=<0.001).

Table 4. Results of the LME for the seven days of the group housing period using saliva cortisol concentration as response variable.

Predictor	Df	F-value	p (>F)
HI	1	5.320	**0.022**
treatment	2	2.126	0.129
sex	1	0.759	0.387
day	6	5.176	**<0.0001**
HI:treatment	2	3.463	**0.033**
HI:sex	1	5.093	**0.025**
treatment:sex	2	2.422	0.098
HI:day	-	-	-
treatment:day	12	5.335	**<0.0001**
sex:day	6	1.979	0.068
HI:treatment:sex	2	2.413	0.091
HI:treatment:day	-	-	-
HI:sex:day	-	-	-
treatment:sex:day	-	-	-

Significant p-values are marked in bold. Predictors in gray indicate interactions which were removed using AIC.

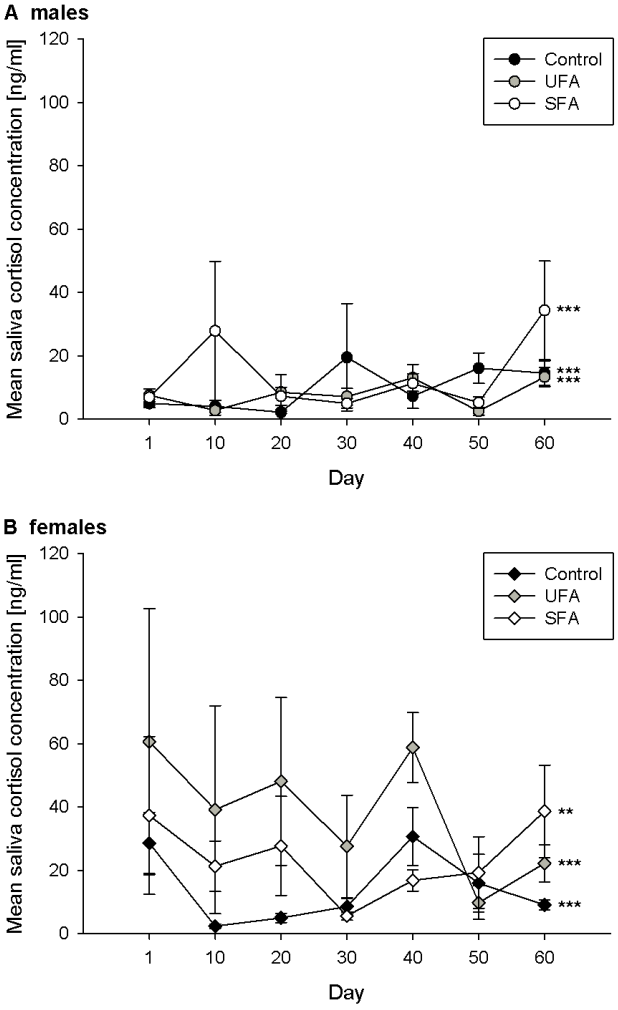

Figure 5. Saliva cortisol concentration. Mean saliva cortisol concentrations of males (A) and females (B) for each day of the group housing period. Error bars indicate standard error. The asterisks mark the significance level (calculated for the full model): *** p≤0.001, ** p≤0.01.

Plasma fatty acid concentration

Plasma analysis revealed significant differences between all treatment groups in the percentages of UFAs and SFAs in relation to the total amount of plasma fatty acid concentration (Table 5 & 6). The intake of a diet high in UFAs led to an increase in the total amount of UFAs and to a decline of SFAs compared to the control and SFA group (total UFA and SFA: control-UFA $F_{2,53}$=47.329, p=<0.001; UFA-SFA $F_{2,53}$=47.329, p=<0.001; Figure 6). However, the percentages of total plasma SFAs were highest when the subjects were exposed to a diet high in SFAs, whereas the amount of UFAs declined in this treatment group (total UFA and SFA: control-SFA

$F_{2,53}$=47.329, p=<0.001; Figure 6). Corresponding to these findings, the plasma UFA:SFA ratio was highest in the UFA and lowest in the SFA treatment group compared to the control (UFA:SFA: all comparisons $F_{2,53}$=51.136, p=<0,001). No interaction effect of treatment and sex was found for the total amount of plasma UFAs, SFAs and UFA:SFA ratio (Table S2).

Furthermore, the total amount of n-3 and n-6 PUFAs was significantly higher in the UFA group compared to the other experimental groups (total n-3: control-UFA and UFA-SFA $F_{2,53}$=26.928, p=<0.001; total n-6: control-UFA and UFA-SFA $F_{2,53}$=15.780, p=<0.001). In contrast, individuals fed with a diet high in SFAs showed a significantly declined amount of n-3 PUFAs compared to the control group (control-SFA $F_{2,53}$=26.928, p=0.004; Table 6). Regarding the ratio of n-6:n-3 PUFAs, post hoc analysis of the interaction effect of treatment and sex revealed significantly higher values for the SFA females compared to control or UFA individuals (control-SFA ♀ $F_{2,53}$=3.731, p=0.006; UFA-SFA ♀ $F_{2,53}$=3.731, p=0.002; Table S2). Interestingly, the recommended ratio of 4:1 was not observed. The total amount of plasma n-9 fatty acids also showed a decline in the SFA treatment group compared to the control and UFA group (control-SFA $F_{2,53}$=6.493, p=<0.001; UFA-SFA $F_{2,53}$=6.493, p=0.004).

MUFAs significantly declined in the SFA group (control-SFA $F_{2,53}$=6.412, p=<0.001; UFA-SFA $F_{2,53}$=6.412, p=0.044), while PUFAs increased when individuals were fed with a diet high in UFAs (control-UFA $F_{2,53}$=27.024, p=<0.001; UFA-SFA $F_{2,53}$=27.024, p=<0.001; Table 6). The highest values for the ratio between MUFA and SFA as well as PUFA and SFA were observed in the UFA group, whereas the SFA had the lowest ratio (MUFA:SFA: control-UFA $F_{2,53}$=23.495, p=0.025; control-SFA and UFA-SFA $F_{2,53}$=23.495, p=<0.001; PUFA:SFA: all comparisons $F_{2,53}$=45.344, p=<0.001).

Table 5. Results of the two-way ANOVA for plasma fatty acid concentrations.

Fatty acid	Predictor	Df	F-value	p (>F)
total n-3	treatment	2	26.928	**<0.001**
	sex	1	3.5406	0.065
	treatment:sex	2	3.1161	0.053
total n-6	treatment	2	15.780	**<0.001**
	sex	1	3.754	0.058
	treatment:sex	2	2.863	0.066
n6:n3	treatment	2	10.853	**<0.001**
	sex	1	5.349	**0.025**
	treatment:sex	2	3.731	**0.030**
total n-9	treatment	2	6.493	**0.003**
	sex	1	1.133	0.292
	treatment:sex	2	0.115	0.892
total MUFA	treatment	2	6.412	**0.003**
	sex	1	2.835	0.098
	treatment:sex	2	0.163	0.850
total PUFA	treatment	2	27.024	**<0.001**
	sex	1	1.981	0.165
	treatment:sex	2	2.143	0.127
total UFA	treatment	2	47.329	**<0.001**
	sex	1	0.441	0.510
	treatment:sex	2	2.712	0.076
total SFA	treatment	2	47.329	**<0.001**
	sex	1	0.441	0.510
	treatment:sex	2	2.712	0.076
MUFA:SFA	treatment	2	23.495	**<0.001**
	sex	1	0.539	0.466
	treatment:sex	2	1.076	0.348
PUFA:SFA	treatment	2	45.344	**<0.001**
	sex	1	0.971	0.329
	treatment:sex	2	2.740	0.074
UFA:SFA	treatment	2	51.136	**<0.001**
	sex	1	0.405	0.527
	treatment:sex	2	2.739	0.074

Significant p-values are marked in bold.

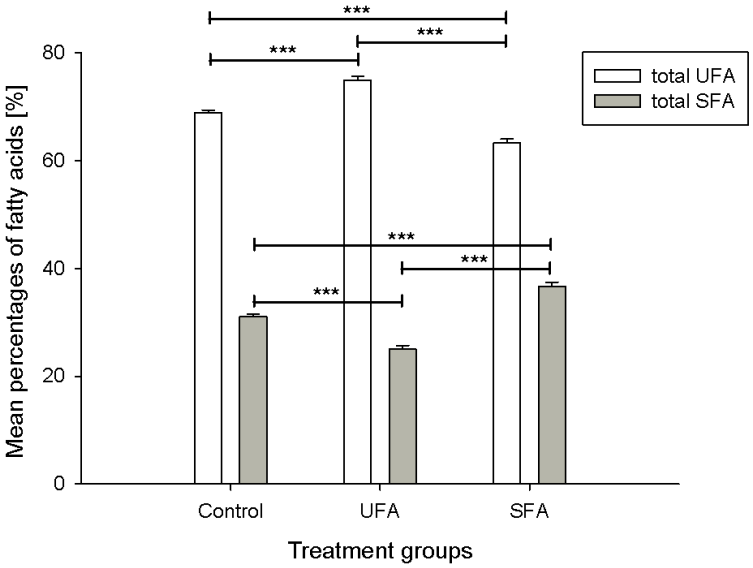

Figure 6. Plasma fatty acid concentration. The mean percentages of UFAs and SFAs on the total amount of plasma fatty acid concentrations are shown for all treatment groups. Error bars indicate standard error. The asterisks mark the significance level: *** $p \leq 0.001$.

Table 6. Plasma fatty acid concentration. The percentages of fatty acids on the total amount of plasma fatty acid concentration are listed below (comparing treatment groups and sexes). The total amount of UFA and SFA are marked in bold. Values are given in mean±SD. Superscript letters indicate significant differences between treatment groups or sexes: a – difference between control and UFA, b – difference between control and SFA, c – difference between UFA and SFA, d – difference between males and females. The asterisks mark the significance level: * p≤0.05, ** p≤0.01, *** p≤0.001.

Fatty acid	Treatment			Sex		Overall mean
	control	UFA	SFA	males	females	
total n-3	6.17±0.99^{a***,b**}	7.47±1.22^{a***,c***}	5.04±0.91^{b**,c***}	5.91±1.00	6.56±1.75	6.23±1.44
total n-6	44.95±4.57^{a***}	51.31±2.92^{a***,c***}	43.55±2.74^{c***}	46.69±5.29	46.57±4.44	46.63±4.85
n6:n3	7.52±1.90^{b*}	7.06±1.32^{c**}	8.99±2.18^{b*,c**}	8.09±1.55^{d*}	7.63±2.36^{d*}	7.86±1.99
total n-9	15.82±3.53^{b***}	14.87±1.31^{c**}	12.86±1.44^{b***,c**}	14.14±3.00	14.86±2.04	14.49±2.58
total MUFA	17.71±3.50^{b***}	16.13±1.41^{c*}	14.58±1.61^{b***,c*}	15.60±2.94	16.65±2.20	16.11±2.63
total PUFA	51.20±4.57^{a***}	58.86±2.94^{a***,c***}	48.70±2.57^{c***}	52.70±5.75	53.21±5.42	52.95±5.55
total UFA	**68.91±2.01^{a***,b***}**	**74.99±3.04^{a***,c***}**	**63.28±3.37^{b***,c***}**	**68.30±5.65**	**69.86±5.59**	**69.06±5.63**
total SFA	**31.09±2.01^{a***,b***}**	**25.01±3.04^{a***,c***}**	**36.72±3.37^{b***,c***}**	**31.70±5.65**	**30.14±5.59**	**30.94±5.63**
MUFA:SFA	0.57±0.11^{a*,b***}	0.66±0.11^{a*,c***}	0.40±0.07^{b***,c***}	0.51±0.13	0.58±0.15	0.54±0.14
PUFA:SFA	1.66±0.23^{a***,b***}	2.40±0.40^{a***,c***}	1.34±0.18^{b***,c***}	1.74±0.47	1.87±0.59	1.80±0.53
UFA:SFA	2.23±0.21^{a***,b***}	3.05±0.49^{a***,c***}	1.74±0.24^{b***,c***}	2.25±0.56	2.44±0.71	2.34±0.64

Bodyweight

Concerning bodyweight, an interaction effect of sex and day was observed (Table 7). Highly significant increases in body mass for both sexes were revealed over the time course (♂ χ^2=249.521, p=<0.001; ♀ χ^2=40.672, p=<0.001; Figure 7A & B). This increase amounted to 19.2±26.3 percent in the males' bodyweight, whereas the females' weight only increased by 8.9±10.4 percent. Moreover, female guinea pigs generally weighed more compared to males (♂-♀ χ^2=37.659, p=<0.001), which was also highlighted on the first day of the experiment (♂-♀ day01 χ^2=4.012, p=0.045). Comparing the bodyweight from days 01 and 60, a significant increase in bodyweight was found for both sexes (♂ day01-day60 χ^2=151.575, p=<0.001; ♀ day01-day60 χ^2=25.564, p=<0.001).

Table 7. Results of the LME for the seven days of the group housing period using bodyweight as response variable.

Predictor	Df	F-value	p (>F)
HI	1	5.216	**0.023**
treatment	2	0.414	0.663
sex	1	4.604	**0.036**
day	6	18.779	**<0.0001**
HI:treatment	2	0.554	0.575
HI:sex	1	0.001	0.977
treatment:sex	2	0.411	0.665
HI:day	6	11.123	**<0.0001**
treatment:day	-	-	-
sex:day	6	6.277	**<0.0001**
HI:treatment:sex	2	2.849	0.059
HI:treatment:day	-	-	-
HI:sex:day	-	-	-
treatment:sex:day	-	-	-

Significant p-values are marked in bold. Predictors in gray indicate interactions which were removed using AIC.

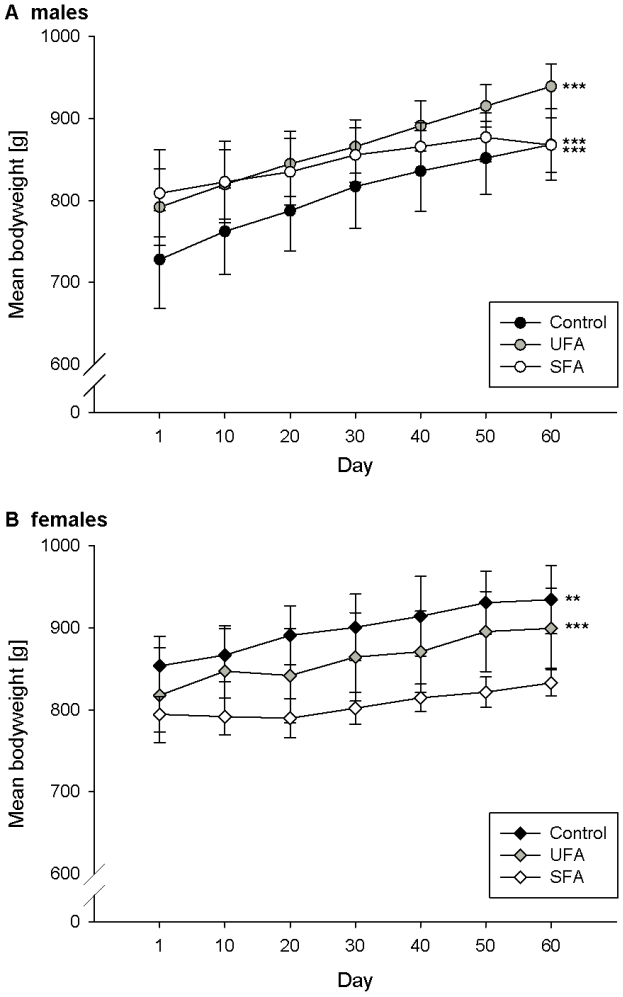

Figure 7. Bodyweight. Mean bodyweight of males (A) and females (B) for each day of the group housing period. Error bars indicate standard error. The asterisks mark the significance level (calculated for the full model): ** p≤0.01, *** p≤0.001.

Discussion

It is well known that nutritional components can influence physiological, behavioral as well as cognitive parameters, such as stress perception and the physiological response[19,20,40], social[8,41–43] or exploratory behavior[44], and learning[45–47], in humans and in animals. The mentioned studies mostly focused on the effects of unsaturated fatty acids. However, a direct comparison between potential effects of unsaturated (UFAs) and saturated fatty acids (SFAs) would give more insight into the overall picture of nutritional influences on certain physiological and behavioral aspects. Especially agonistic behavior would be of interest, because of the high energetic

costs associated with this kind of behavior. In the present study, the effects of UFAs and SFAs on socio-positive and agonistic behaviors, the dominance hierarchy, and the HPA-axis in guinea pigs were investigated and compared to a control group. Based on the findings of previous studies in cynomolgus monkeys[8], dogs[9], and guinea pigs[20] we hypothesized that individuals provided with a diet high in UFAs would show less agonistic behavior and lower cortisol concentrations compared to individuals supplemented with high levels of SFAs or a control group.

Regarding agonistic behavior previous findings demonstrated that male rat pups subjected to an n-3 polyunsaturated FA (PUFA) deprived diet showed increased blocking of an intruder rat (blocking was defined as: pushing an intruder onto its back or into the enclosures' walls and keeping it in that position to hinder escape) in an isolation-induced resident-intruder test for aggression[42]. On the contrary, n-3 supplementation prevented the increase of aggression in students under mental stress such as final exams[48]. This would indicate that a diet high in PUFAs (in particular n-3 PUFAs) might counteract aggressive behavior, whereas PUFA deprived diets strengthen aggression among individuals. In the present study, all treatment groups decreased in agonistic behavior in the course of the group housing period. Contrary to our expectations that a diet rich in UFAs might counteract high rates of agonistic encounters, we found a more pronounced decrease for the SFA compared to the control group. Nevertheless, similar patterns of declining agonistic behavior were found in the UFA group, even though this finding did not differ from the control group. Furthermore, it looked as if there might be a sex-specific influence of dietary manipulation. Males of both the SFA and UFA group showed decreasing conflicts, while no change was detected in the control group. This would suggest that a diet rich in SFAs as well as in UFAs resulted in lower aggression levels compared to the control group, however more pronounced in the SFA group. In contrast to the males, all female groups decreased constantly in agonistic behavior over the time course, without any detectable effect of dietary manipulation. This could be due to the generally low rates of agonistic encounters among female guinea pigs. Furthermore, in the present study males of all groups displayed more agonistic behavior compared to females, which is consistent with previous findings[24]. Independent of the different treatments, the more dominant individuals of both sexes displayed more agonistic behavior. A similar result was

found in subordinate male rats and defeated male tree shrews, which showed reduced aggression compared to dominant conspecifics[16]. This indicates that dominant individuals might have to continuously defend their current rank position, leading to more energetic costs.

Previous findings in cynomolgus macaques showed that individuals displayed more affiliative behavior when they were fed a high-fat diet compared to a low-fat diet[49]. This is in contrast with findings of the present study, in which dietary manipulation affected the decline of socio-positive behavior more strongly compared to the control group. However, socio-positive interactions might be used as a form of appeasement after agonistic encounters. This is reflected in the finding that males in general and the more dominant individuals of both sexes, which were involved in more agonistic interactions compared to subordinate ones, displayed also more socio-positive behavior. Socio-positive behavior in control males remained at rather high levels throughout the study and was in line with the previously mentioned results, paralleled by high levels of agonistic interactions. This suggests that displaying socio-positive behavior might be useful in reconciliation particularly between high-ranking individuals. Both fatty acid groups showed less agonistic encounters between individuals compared to the control group, thus would require less appeasement after or during conflicts. Socio-positive behavior declined steadily in all female groups over the time course, also reflected in the changes in agonistic behavior. Both agonistic and socio-positive behaviors were less frequently displayed in females compared to males supporting the previous assumption.

Moriguchi et al.[45] demonstrated that movement increased in rats supplemented with an n-3 PUFA deficient diet. They concluded that a diet low in n-3 FAs might negatively affect habituation and thus increase locomotion. The authors state that the habituation to a new experiment can occur within only five days, leading to a decline in movement[50,51]. As locomotor activity decreased in all three treatment groups in the present study, habituation to the experiment might be one explanation for this decline. Fedorova and Salem[7] reviewed that locomotor activity increased when rodents were supplemented with a diet low in n-3 PUFAs. For example, mice subjected to an n-3 deficient diet showed an increase in locomotion compared to individuals fed with an n-3 adequate diet. This is supported by other studies, which found that an n-3 adequate diet decreased locomotor activity over

time[7]. Exploratory behavior of rat pups, which were raised on the same diets as the dams, also appeared to be negatively affected by a diet low in n-3 FAs. The rat pups showed less horizontal movements or rearings when confronted with a new environment. Interestingly, these behavioral deficits could not be reversed after weaning when changing the diet to an adequate one[44]. To our knowledge, the effect of saturated fatty acids on locomotor behavior has not been investigated yet. Contrary to the literature, we found no significant decreasing effect of a diet high in UFAs. Interestingly though, the decline in movement was significantly more pronounced in the SFA males, whereas high durations of locomotor behavior were maintained in the control males. Longer durations of movement are likely to be associated with higher frequencies of agonistic and socio-positive behaviors. This is in line with the result that increased locomotor activity was detected in the dominant males, which also displayed more agonistic and socio-positive behaviors. Control males, for instance, showed longer durations of movement possibly due to higher frequencies of both agonistic and socio-positive encounters. By contrast, SFA males displayed less agonistic and socio-positive behaviors, reflected in lower durations of locomotor behavior. Similar relationships were found when individuals were fed with a diet rich in UFAs. Interestingly, this effect was not present in the female treatment groups, which might be due to the fact that the dominance hierarchy of all-female groups is not as pronounced as in all-male groups[21]. Control females, for example, showed less locomotion compared to control males, which could be related to higher frequencies of agonistic encounters in control males. This is also supported by the fact that females generally display less agonistic behavior towards conspecifics[24], resulting in less locomotor activity.

No significant changes in the dominance hierarchies of all treatment groups were found during the time course of the group housing period. This might indicate that dietary manipulation, regardless of whether the subjects were supplemented with unsaturated or saturated FAs, had no influence on the social rank positions of guinea pigs. Despite high frequencies of agonistic behavior during the initial phase of the experiment, the dominance hierarchy of all treatment groups remained stable. Socio-positive interactions might have been used to maintain a stable dominance hierarchy over time. Interestingly, the control individuals, which were more frequently involved in agonistic interactions compared to the FA groups, also

showed stable rank positions throughout the study. However, FA groups were able to maintain their dominance relationships while conflict rates decreased. Nevertheless, this finding generally demonstrates the stability of the dominance hierarchy in guinea pigs, which often maintain stable rank positions over months[24]. A stable dominance hierarchy is important to predict the behaviors of other individuals and hence might decrease social stress. Reduced agonistic encounters between individuals are another effect of this social stability[24], which might result in reduced energetic costs. Furthermore, male guinea pigs were generally more dominant in relation to females, which might be due to the fact that females' agonistic behavior is less pronounced compared to males'[24]. Hierarchies in all-female guinea pig groups are also not as pronounced compared to male groups[21], in which individuals are either frequently involved in conflicts or avoid agonistic interactions with conspecifics[52].

Saliva cortisol concentrations were measured to analyze the effect of different diets on the HPA-axis. Previous studies[11,43] reported that n-3 PUFAs reduced cortisol secretion and stress-related behaviors. This effect would be beneficial due to potential detrimental effects of elevated cortisol levels, such as decreased immune function[53], increased risk of cardiovascular disease[54], enhanced anxiety[54], learning[25], and memory deficits[25,54]. To our knowledge no effects of SFAs with regard to the HPA-axis are known. In the present study our findings suggest that the intake of a diet high in SFAs results in a decreasing effect on cortisol concentrations over the time course, whereas no clear effect of dietary manipulation was detected in the UFA group. Interestingly, individuals of the control group displayed rather stable and low cortisol concentrations, while also showing the highest frequency of agonistic and socio-positive behaviors. This could suggest that stressful situations caused by frequent conflicts could be counteracted by socio-positive behavior. Furthermore, when looking at males and females separately it looked as if the course of cortisol changes differed between the sexes. Cortisol secretion in the males was rather stable and concentrations were maintained at a low level, further supporting the assumption that socio-positive behavior buffers stress. Similar to findings in rodents and humans[55–57], we found greater fluctuations and higher saliva cortisol concentrations in females compared to males. A previous study revealed that cortisol levels varied during the estrous cycle of guinea pigs (16 days), showing

a peak on day 1 of the cycle[58], indicating that the rhythmicity of the pituitary-adrenal function is affected by the cyclic ovarian function[55]. However, no synchronization of the estrous cycle was detected in the present study, which could have explained the consistent recurring peaks in an interval of 20 days. In addition, the more dominant SFA and control males were less stressed than low-ranking individuals, whereas the contrary was the case for males fed with a diet high in UFAs. The more dominant individuals also displayed more socio-positive behavior compared to subordinate ones. At least in the SFA and control males this might further indicate that socio-positive behavior is used as a form of appeasement and thus reduces cortisol secretion. In high-ranking UFA males, however, socio-positive interactions did not succeed in reducing cortisol concentrations. Sachser et al.[24] proposed that lower ranked guinea pigs do not necessarily have a higher cortisol concentration compared to more dominant ones. One of the main reasons could be predictable behavior, due to established social relationships[24]. However, the experimental setup of Sachser et al.[24] was quite different from the present study, mainly because of the mixed sex colonies of the subjects, which can cause a variety of effects on glucocorticoid secretion.

Dietary manipulation with UFAs and SFAs resulted in different plasma fatty acid concentrations in all treatment groups. Increased plasma UFAs and decreased plasma SFAs were found for the intake of a diet rich in UFAs compared to the control group, whereas the exposure to a diet high in SFAs resulted in increased plasma SFAs and decreased plasma UFAs. These findings highlight the successful uptake of UFAs and SFAs from the diet, which is consistent with previous findings[20]. Studies on adult rats, for example, showed that the incorporation from plasma n-3 and n-6 PUFA concentrations into the brain takes about 2-3 weeks, whereas the incorporation into the human brain amounts to 5 weeks[59]. This indicates that small mammals are able to incorporate dietary fatty acids into the brain within a shorter timeframe, suggesting that enough time was provided in the present study. Furthermore, the SFA group showed an increased n-6:n-3 ratio compared to individuals supplemented with UFAs or a control group. Guinea pigs fed with a diet high in UFAs and control individuals had the lowest ratio. This effect was most pronounced in the female groups, with SFA females showing the highest n-6:n-3 ratio. Various studies reported health benefits from low n-6:n-3 ratios like 2:1, such

as reduced cholesterol levels[60] or atherosclerosis[61], indicating that diets high in SFAs might have a negative effect on health. Yehuda[25] further suggests that a ratio of 4:1 is the optimal ratio resulting in improved cognitive functions, such as learning abilities, sleep patterns and quality of life of Alzheimer's patients. The uptake and incorporation of PUFAs into the brain and neuronal membranes is also facilitated by this ratio[25]. However, the ratios in the present study exceeded the recommended ratio of 4:1.

Regarding the bodyweight, no significant differences were observed between the experimental groups, demonstrating that the dietary treatment did not lead to detectable differences in body condition between the groups. There was also no noticeable weight loss in any of the individuals that would indicate chronic stress effects or other health problems. Interestingly, the control males had the lowest bodyweight, which might be associated to the high frequency of agonistic encounters compared to males fed with a diet high in UFAs or SFAs. On the contrary, in the female treatment groups the control group showed the highest body weight gain, while also showing the lowest cortisol levels. Thus, low stress levels might positively affect body condition, whereas stress can cause a permanent reduction in body weight[62]. The heaviest and probably largest animals were also the dominant ones and even though dominant individuals were involved in more agonistic as well as socio-positive interactions and showed longer durations of locomotion, they were able to maintain a higher bodyweight compared to subordinate individuals. A report about the influence of social defeat on bodyweight observed, for example, that the bodyweight gain was suppressed in defeated individuals. Thus, rats showed a decrease in bodyweight when defeated in a social interaction[63].

Although our hypothesis was not fully supported in the present study, our finding of decreased agonistic behavior in the SFA group compared to the control group represents a potentially positive effect of the consumption of a saturated fatty acid-rich diet. This was also supported by the findings of a stable rank hierarchy during the group housing period, which was accompanied by less socio-positive interactions and lower durations of locomotion. The same pattern was also shown in individuals supplemented with a diet rich in UFAs, even though the differences to the control group were more pronounced in the SFA group. In addition, the results

might indicate sex-specific effects of the dietary manipulations. Thus, it is important to focus more closely on sex-specific effects. A recent study by Nemeth et al.[64] revealed sex differences in the effects of the supplementation with different UFAs (n-3 and n-6 PUFAs and n-9 MUFAs) showing elevated stress levels during a maze task in males but not in females. Nevertheless, UFA supplementation appeared to positively affect memory retention in males and spatial learning abilities in females compared to a control group[64]. The n-6:n-3 ratio was the lowest in the UFA group, which could have long term beneficial effects. However, no health benefits or detrimental effects on health could be linked to either unsaturated or saturated fatty acids due to the limited time frame of the present study. The assumption that high SFA intake would lead to increased aggressive behavior could not be supported in this study.

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Supplementary Materials

Table S1. Hierarchy Index. The sum of all calculated proportion values of initiated agonistic behavior towards another conspecific in pairwise encounters for all seven days of the group housing period are listed below. The subjects Cf02 and Um07 are not listed due to health problems during the experiment and were thus not included in the analyses. Cf – control females, Cm – control males, Uf – UFA females, Um – UFA males, Sf – SFA females, Sm – SFA males.

Subjects	Hierarchy Index						
	Day 01	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60
Cm01	1.54	2.08	3.06	1.71	2.20	2.00	1.81
Cm02	2.85	4.06	5.07	4.13	2.87	5.48	6.70
Cm03	2.49	7.43	4.39	3.40	4.47	4.49	4.56
Cm04	7.78	8.16	7.55	7.85	8.77	8.16	6.85
Cm05	4.03	3.58	6.61	8.58	6.45	4.92	6.66
Cm06	7.33	5.85	5.69	6.72	6.03	6.42	6.94
Cm07	8.24	9.63	6.48	8.44	7.35	7.20	5.34
Cm08	7.47	5.12	4.93	4.06	6.34	4.39	3.60
Cm09	5.45	2.91	3.22	5.17	2.84	3.79	3.42
Cm10	3.89	3.61	3.67	3.44	3.86	4.82	4.02
Cm11	3.92	2.57	4.32	1.51	3.82	3.33	5.11
Cf01	4.89	1.62	3.65	3.66	4.15	3.88	3.93
Cf03	3.78	5.88	4.44	5.11	4.09	2.88	5.11
Cf04	5.76	5.09	5.42	5.63	6.49	4.43	4.55
Cf05	5.38	4.98	3.22	4.47	4.47	4.13	2.63
Cf06	2.14	3.75	3.45	3.40	3.56	3.00	2.48
Cf07	5.83	4.70	3.93	5.41	5.78	4.40	3.13
Cf08	4.41	3.70	5.14	4.83	3.47	3.69	3.07
Cf09	4.78	5.07	5.48	4.83	4.57	2.47	4.29
Cf10	3.87	4.51	4.92	3.50	4.02	3.00	2.75
Um01	6.17	6.29	4.21	5.91	2.55	5.28	4.73
Um02	4.95	4.72	6.90	7.35	5.85	5.18	4.70
Um03	7.57	4.47	2.81	4.57	5.74	4.90	6.87
Um04	3.95	7.81	7.18	7.30	7.39	2.82	4.26
Um05	4.42	4.41	5.63	4.36	6.22	5.57	3.25
Um06	3.92	3.82	3.93	2.24	2.00	2.83	3.52
Um08	5.81	5.37	6.06	5.35	5.85	4.95	4.89
Um09	5.73	5.08	6.20	7.35	6.75	5.79	4.87
Um10	4.50	4.59	4.25	3.12	5.06	2.84	2.61

Um11	3.99	2.99	2.90	3.67	3.27	4.59	5.29
Uf01	5.09	5.57	6.90	5.58	4.77	5.46	5.50
Uf02	1.73	2.69	2.97	2.19	1.65	3.15	2.94
Uf03	5.47	5.21	5.21	4.40	4.50	4.57	3.93
Uf04	5.05	5.98	5.24	6.10	5.48	4.35	5.13
Uf05	5.23	3.85	4.96	4.29	5.26	5.96	5.17
Uf06	6.29	6.67	5.29	5.52	7.15	5.65	5.62
Uf07	5.85	4.82	5.03	5.86	5.52	5.27	5.00
Uf08	4.76	2.69	5.13	3.21	4.39	4.21	4.67
Uf09	4.73	4.85	1.25	4.03	3.41	3.59	3.50
Uf10	1.55	3.24	3.01	4.35	2.86	2.79	3.55
Sm01	5.64	7.55	7.51	6.38	5.95	5.62	6.45
Sm02	6.39	7.49	6.78	7.92	7.14	7.07	6.63
Sm03	6.00	4.80	4.24	2.25	3.58	1.16	3.87
Sm04	8.63	8.00	7.38	8.60	8.23	8.04	8.21
Sm05	6.76	4.00	4.87	2.08	3.23	5.98	3.17
Sm06	3.42	5.02	4.64	6.54	6.61	6.43	4.33
Sm07	3.48	5.60	4.96	6.24	5.72	4.61	3.33
Sm08	5.10	4.50	4.78	3.93	4.57	4.21	4.50
Sm09	1.95	2.53	4.61	4.86	4.03	5.15	6.44
Sm10	5.61	3.46	2.51	3.14	3.23	4.07	4.83
Sm11	2.01	2.06	2.72	3.07	2.70	2.66	3.24
Sf01	4.82	6.70	5.46	4.14	4.07	6.16	4.25
Sf02	6.56	7.14	6.25	6.20	5.38	5.15	5.38
Sf03	4.37	6.19	6.10	6.82	6.39	6.05	7.00
Sf04	4.78	5.98	4.57	5.47	5.67	3.87	3.84
Sf05	5.63	4.18	5.83	4.81	4.20	5.43	4.58
Sf06	6.01	4.38	6.15	6.08	4.81	4.55	4.10
Sf07	6.33	3.81	4.68	3.58	6.36	2.86	3.67
Sf08	0.90	1.84	1.69	1.84	3.67	3.00	3.67
Sf09	3.04	2.58	1.77	4.25	3.34	2.99	4.69
Sf10	2.57	3.07	2.50	2.20	1.12	4.95	3.83

Table S2. Plasma fatty acid concentration. The percentages of fatty acids on the total amount of plasma fatty acid concentration are listed below (comparing all six treatment groups). The total amount of UFA and SFA are marked in bold. Values are given in mean±SD. Superscript letters indicate significant differences between treatment groups and sexes: i – difference between control and SFA females, j – difference between UFA and SFA females. The asterisks mark the significance level: ** p≤0.01. MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, UFA – unsaturated fatty acid, SFA – saturated fatty acid.

Fatty acid	Treatment						Overall mean
	control males	control females	UFA males	UFA females	SFA males	SFA females	
total n-3	5.78±1.01	6.61±0.80	6.82±0.42	8.12±1.42	5.14±0.65	4.95±1.15	6.23±1.44
total n-6	46.38±5.37	43.36±3.00	51.22±3.13	51.41±2.86	42.47±2.97	44.62±2.12	46.63±4.85
n6:n3	8.33±2.29	6.62±0.71^{i**}	7.55±0.75	6.57±1.61^{j**}	8.38±1.21	9.59±2.78^{i**,j**}	7.86±1.99
total n-9	15.52±4.75	16.15±1.57	14.31±0.93	15.42±1.45	12.60±0.93	13.13±1.83	14.49±2.58
total MUFA	17.06±4.58	18.43±1.67	15.59±1.05	16.66±1.57	14.14±1.01	15.03±2.01	16.11±2.63
total PUFA	52.24±5.29	50.05±3.55	58.12±3.03	59.60±2.80	47.73±2.96	49.66±1.74	52.95±5.55
total UFA	**69.30±1.59**	**68.48±2.43**	**73.72±2.78**	**76.26±2.86**	**61.87±3.60**	**64.69±2.57**	**69.06±5.63**
total SFA	**30.70±1.59**	**31.52±2.43**	**26.28±2.78**	**23.74±2.86**	**38.13±3.60**	**35.31±2.57**	**30.94±5.63**
MUFA:SFA	0.55±0.14	0.59±0.05	0.60±0.06	0.71±0.12	0.38±0.05	0.43±0.08	0.54±0.14
PUFA:SFA	1.71±0.24	1.60±0.23	2.24±0.32	2.56±0.42	1.27±0.19	1.42±0.13	1.80±0.53
UFA:SFA	2.27±0.17	2.19±0.24	2.84±0.37	3.27±0.53	1.64±0.24	1.85±0.20	2.34±0.64

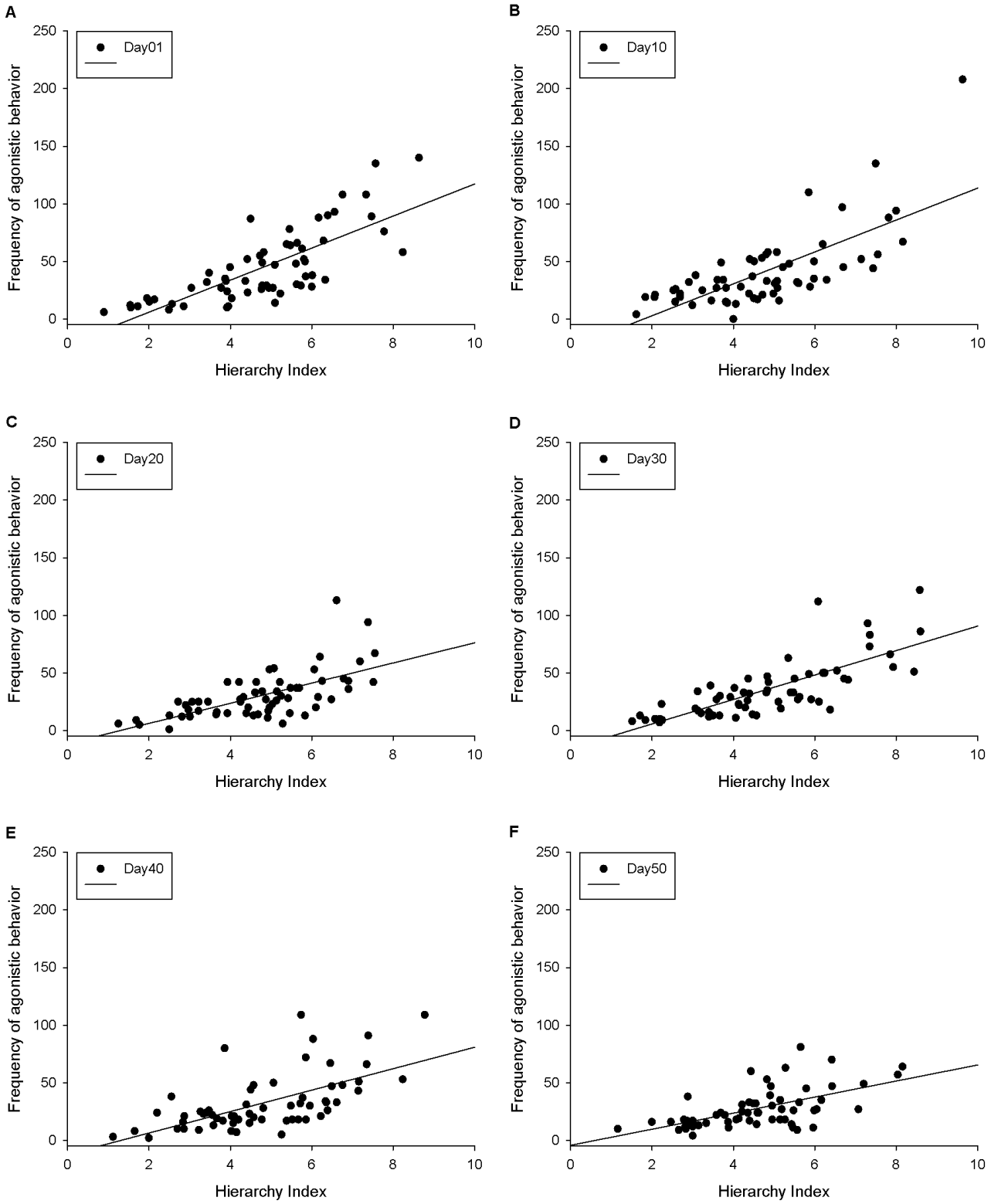

Figure S1. Agonistic behavior. The effect of the interaction between the Hierarchy Index and day on the frequency of agonistic behavior is shown. (A) Day01, (B) Day10, (C) Day20, (D) Day30, (E) Day40, (F) Day50.

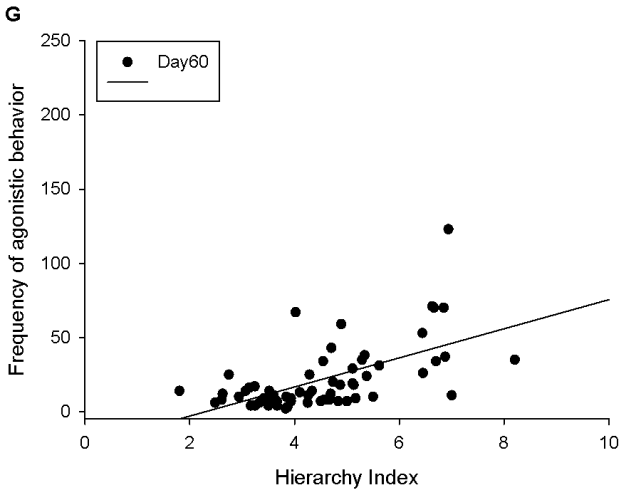

Figure S2. Agonistic behavior. The effect of the interaction between the Hierarchy Index and day on the frequency of agonistic behavior is shown. (G) Day60.

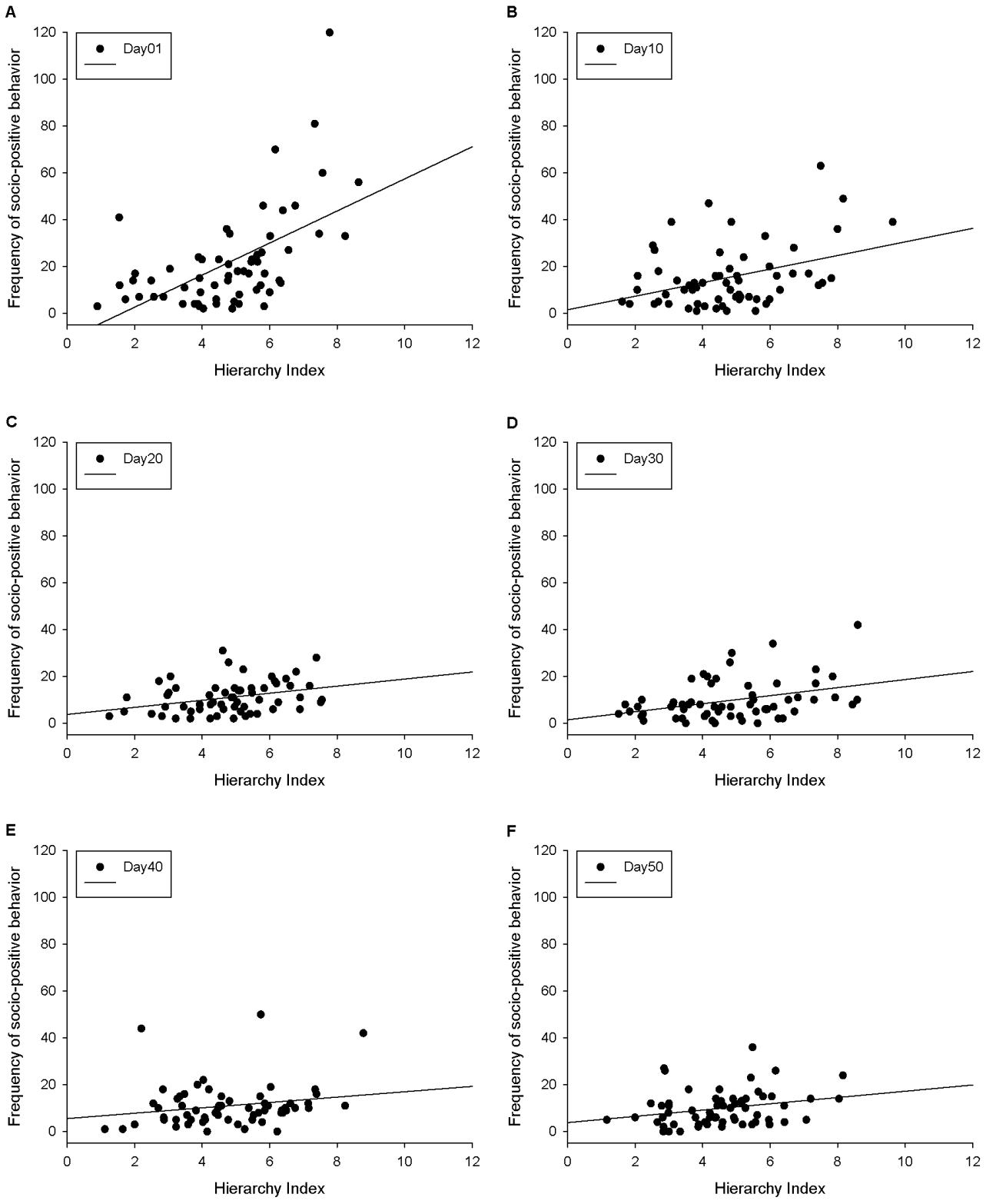

Figure S3. Socio-positive behavior. The effect of the interaction between the Hierarchy Index and day on the frequency of socio-positive behavior is shown. (A) Day01, (B) Day10, (C) Day20, (D) Day30, (E) Day40, (F) Day50.

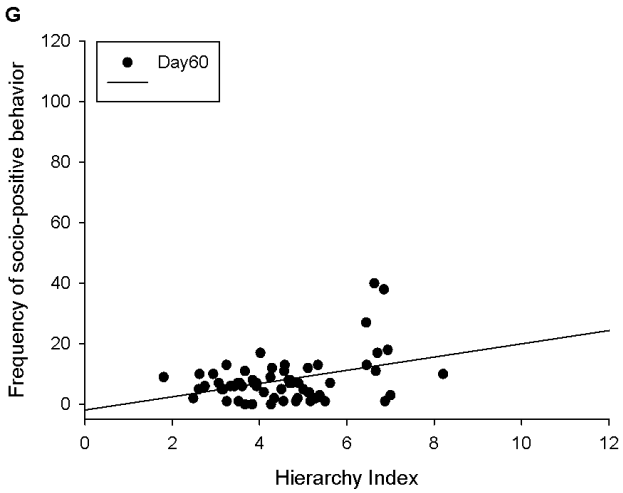

Figure S4. Socio-positive behavior. The effect of the interaction between the Hierarchy Index and day on the frequency of socio-positive behavior is shown. (G) Day60.

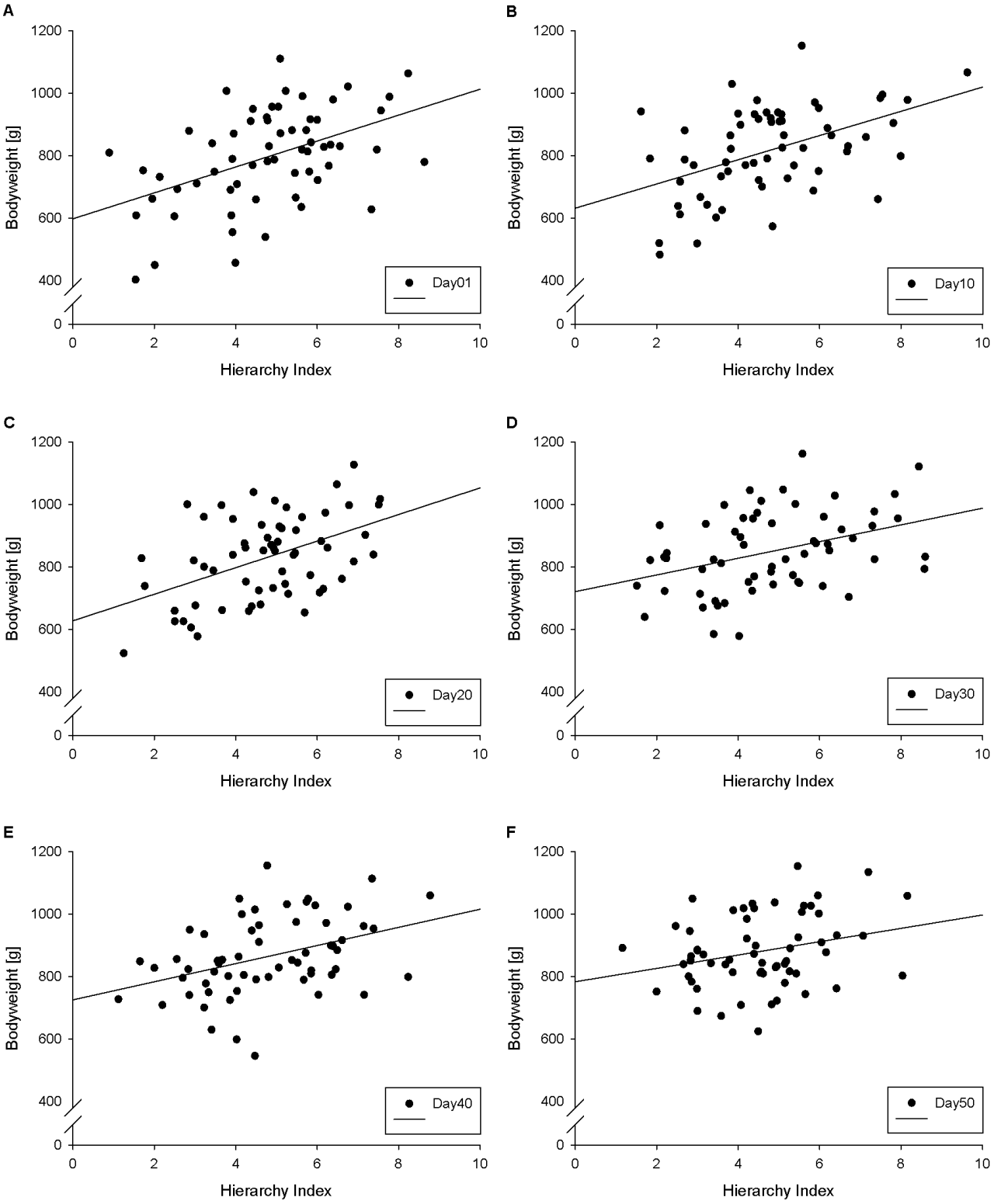

Figure S5. Bodyweight. The effect of the interaction between the Hierarchy Index and day on the bodyweight is shown. (A) Day01, (B) Day10, (C) Day20, (D) Day30, (E) Day40, (F) Day50.

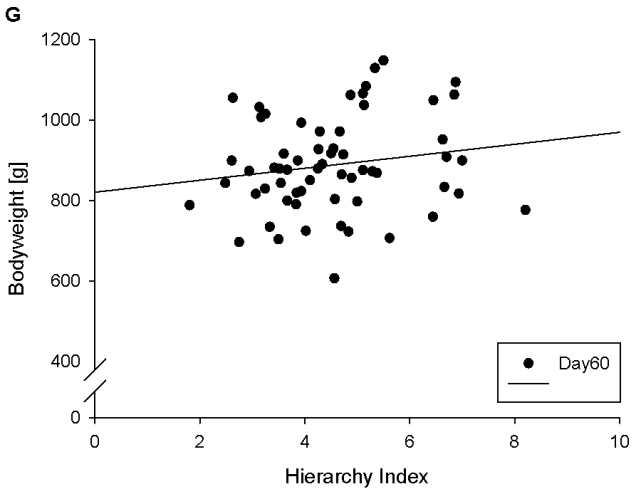

Figure S6. Bodyweight. The effect of the interaction between the Hierarchy Index and day on the bodyweight is shown. (G) Day60.

German Abstract/Deutsche Zusammenfassung

Fettsäuren beeinflussen die Fluidität neuronaler Zellmembranen und spielen somit eine wichtige Rolle in der Struktur und den Funktionen des Gehirns. Dies lässt darauf schließen, dass sich Fettsäuren ebenso auf das Sozialverhalten wie auch die Hypothalamus-Hypophysen-Nebennieren-Achse (HPA-Achse) auswirken können. In dieser Studie wurden die Auswirkungen von einer mit ungesättigten bzw. gesättigten Fettsäuren angereicherten Diät auf das Sozialverhalten und die HPA-Achse von Meerschweinchen (*Cavia aperea f. porcellus*) untersucht und mit einer Kontrollgruppe verglichen. Fortpflanzungsfähige adulte Meerschweinchen (N=63) wurden in gleichgeschlechtlichen Gruppen gehalten (pro Gruppe ♂ N=11, ♀ N=10). Zusätzlich zu ihrem Standardfutter erhielten die Versuchstiere eine oral verabreichte Kost reich an: 1) ungesättigten (UFA) oder 2) gesättigten Fettsäuren (SFA) während einer 60 Tage andauernden Periode. Die Kontrollgruppe erhielt die gleiche Menge an Wasser zusätzlich zum Standardfutter. Die Frequenzen initiierter und erhaltener agonistischer und sozio-positiver Verhaltensweisen wie auch die Dauer der Lokomotion wurden dokumentiert. Veränderungen in der Dominanzhierarchie der einzelnen Gruppen wurden anhand von agonistischen Verhaltensweisen ermittelt. Weiters wurde das Körpergewicht täglich gemessen und die Cortisol-Konzentrationen aus dem Speichel in 10-tägigen Intervallen analysiert. Plasma-Fettsäure-Konzentrationen aus dem Blut wurden am Ende des Experimentes bestimmt. Die Ergebnisse dieser Studie zeigen, dass die Aufnahme einer Diät reich an gesättigten Fettsäuren zu einer ausgeprägteren Abnahme von agonistischem sowie sozio-positivem Verhalten über den Verlauf der Studie führte als bei den Kontrolltieren. Ein ähnlicher, allerdings weniger deutlicher Verlauf konnte auch bei einer mit ungesättigten Fettsäuren ergänzten Nahrung festgestellt werden. Die Kontrollgruppe wies hingegen eine höhere Häufigkeit an agonistischen und sozio-positiven Interaktionen bis zum Ende der Versuchsdauer auf. Hohe Konfliktraten waren mit mehr sozio-positiven Interaktionen verbunden. Dies deutet darauf hin, dass sozio-positives Verhalten als Beschwichtigungshandlung gegenüber Kontrahenten nach einer agonistischen Auseinandersetzung eingesetzt wurde. Vor allem in den Männchen-Gruppen war dieses Verhalten häufiger zu

beobachten als bei den Weibchen. Höhere Frequenzen an agonistischen und sozio-positiven Verhaltensweisen waren mit erhöhter Aktivität, ersichtlich in Lokomotionsdauer, assoziiert. In Bezug auf die Cortisol-Konzentrationen wurde ein Abstieg der Werte während der Studie in der SFA Gruppe gefunden, während kein klarer Effekt von ungesättigten Fettsäuren festgestellt werden konnte. Interessanterweise zeigten die Kontrolltiere verglichen mit den beiden anderen Gruppen von Beginn der Studie an sehr stabile und relativ niedrige Cortisol-Konzentrationen. Dies könnte bedeuten, dass die vermehrten Konfliktraten in dieser Gruppe zu keiner ausgeprägten Stressreaktion führten oder aber, dass sozio-positives Verhalten Stresssituationen entgegenwirken konnte. Des Weiteren konnten keine Effekte der Fettsäurezusätze bezüglich der Dominanzhierarchie sowie des Körpergewichtes gefunden werden. Die Plasma-Fettsäure-Konzentrationen zeigten einen Anstieg der totalen im Plasma zu messenden ungesättigten oder gesättigten Fettsäuren entsprechend der unterschiedlichen Diäten. Das niedrigste Verhältnis der n-6:n-3 mehrfachungesättigten Fettsäuren wurde in der UFA Gruppe nachgewiesen, während in der SFA Gruppe deutlich erhöhte Werte festgestellt wurden. Basierend auf die in vielen Studien nachgewiesenen positiven Effekte eines niederen n-6:n-3 Verhältnisses in der Nahrung auf Gehirnfunktionen und Stoffwechsel könnte dies eine langfristige vorteilhafte Wirkung für die UFA Gruppe bedeuten. Generell zeigen die Ergebnisse, dass die Gruppen mit sehr fettreicher Nahrung ihre Dominanzstrukturen bei sinkenden Konfliktraten aufrecht halten konnten was sich bei limitierten Nahrungsressourcen energetisch günstig auswirken könnte. Überraschend war, dass die Effekte in der SFA Gruppe, trotz ähnlicher Muster, stärker ausgeprägt waren als in der UFA Gruppe. Die Annahme, dass ein hoher Anteil an gesättigten Fettsäuren in der Nahrung aggressives Verhalten steigern würde konnte in dieser Studie nicht bestätigt werden.

Curriculum Vitae

Personal Information

Name	Verena **PÜHRINGER-STURMAYR**
Sex	Female
Nationality	Austria
Date of birth	August 14, 1989
Place of birth	Linz

Work Experience

Since May 2015	Research assistant within the Sparkling Science Project "Sozialer Zusammenhalt und Ausflugsgebiet beim Waldrapp" Konrad Lorenz Research Station, A-4645 Grünau im Almtal, Austria

Education and Training

Since November 2013	Master thesis in the field of Ethology
Since October 2012	Master course "Ethology, Neurobiology and Cognition" University of Vienna, Austria
July 2012	Bachelor degree obtained in "Biology focusing on Zoology" University of Vienna, Austria
2008 – 2012	Bachelor course "Biology focusing on Zoology" University of Vienna, Austria
June 2008	School leaving examination at Higher Secondary School for Economic Professions Landwiedstraße, Linz, Austria Thesis: "Disability – How effective is animal-assisted therapy?"
June 2007	Degree in Service and Cuisine
2003 – 2008	Higher Secondary School for Economic Professions Landwiedstraße, Linz, Austria Focus of education: Social management
1999 – 2003	Musical main school Neuhofen/Krems, Austria
1995 – 1999	Elementary school Pucking, Austria

Additional Information

Research experience

April 2014 — Excursion and special field course “Dessert ecosystem” (Southern Jordan) – Botanical, zoological and pedological studies in different types of desert in Wadi Rum and Wadi Araba

Supervisors: Ao. Univ.-Prof. i.R. Dr. Wolfgang Waitzbauer, Ao. Univ.-Prof. i.R. Dr. Roland Albert, Mag. Dr. Josef Hemetsberger (Core Facility Konrad Lorenz Research Station for Behavior and Cognition, Grünau, Austria), Mag. Margarete Watzka (all University of Vienna, Austria)

Since November 2013 — Master thesis: “Effects of dietary fatty acids on social behavior in guinea pigs (*Cavia aperea f. porcellus*)”

Supervisor: Ao. Univ.-Prof. Dr. Eva Millesi (University of Vienna, Austria)

2013 — Practical work: “Individual differences in the choice of feeding sites of common ravens (*Corvus corax*)”

Supervisors: Univ.-Prof. Mag. Dr. Kurt Kotrschal, Mag. Dr. Josef Hemetsberger, Matthias-Claudio Loretto, MSc (all University of Vienna, Austria)

2013 — Practical work: “Individual Recognition of Heterospecifics in Pigeons (Columba livia)”

Supervisors: Univ.-Prof. Mag. Dr. Thomas Bugnyar (University of Vienna, Austria), Dr. Claudia Stephan (University of Neuchâtel, Switzerland)

2012 — Practical work: “Visually controlled attack behavior of the Central American hunting spider *Cupiennius salei*”

Supervisors: Ao. Univ.-Prof. Dipl.-Biol. Dr. Axel Schmid, Mag. Dr. Lisa Fenk (both University of Vienna, Austria)

2011 – 2012 — Bachelor thesis: ”Pair bond and resource monopolization in African Gray Parrots (*Psittacus erithacus erithacus*)”

Supervisor: Ao. Univ.-Prof. Dr. Eva Millesi (University of Vienna, Austria)

Lab experience

2014 — Research Training in Zoology
DNA Barcoding

2014 — Laboratory work as part of my master thesis: Analysis of saliva and blood samples using enzyme-linked immunoassay

Posters — Puehringer-Sturmayr V, Wascher CAF, Loretto M-C, Kotrschal K, Frigerio D (2015) Affiliative interactions between pair partners during the breeding season in a free flying colony of Northern Bald Ibis (*Geronticus eremita*). Graduate Meeting – Studying Animal Behaviour in the Field 2015, Grünau im Almtal, Austria

Seminars

March 2014 Behavioral Ecology Seminar, Riegersburg, Austria
"Cognition and decision making in animals"

April 2013 Practical Animal Training Workshop, Vienna/Bad Vöslau, Austria
"Fine tuning techniques with mammals, birds, reptiles and fish"

Workshops

September 2015 Introduction to R
Graduate Meeting – Studying Animal Behaviour in the Field 2015, Grünau im Almtal, Austria
Supervisor: Matthias-Claudio Loretto, MSc (University of Vienna, Austria)

September 2015 Non-invasive methods to study hormones and parasites
Graduate Meeting – Studying Animal Behaviour in the Field 2015, Grünau im Almtal, Austria
Supervisors: Alex Munteanu DVM, Dr^{in} rer.nat. Mareike Stöwe

Honors and awards Performance Scholarship for the academic year 2013/2014 of the University of Vienna, Austria