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Effects of dietary polyunsaturated fatty acids on
cognition, social behavior, and saliva cortisol in
guinea pigs (*Cavia aperea f. porcellus*)

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Preface

The current work has already been submitted as a manuscript, entitled “Effects of dietary polyunsaturated fatty acids on cognition, social behavior, and saliva cortisol in guinea pigs (*Cavia aperea f. porcellus*)”, to the peer-review journal *Neuropharmacology* (Elsevier), on the March 31, 2012. The applicant is the first author of this manuscript, which is co-authored by Eva Millesi (Department of Behavioral Biology, University of Vienna), Karl-Heinz Wagner (Department of Nutritional Sciences, University of Vienna), and Bernard Wallner (Department of Anthropology, University of Vienna; Cognitive Science Research Platform, University of Vienna).

All fatty acid analyses in plasma were carried out in the lab of Univ.-Prof. Mag. Dr. Karl-Heinz Wagner, Department of Nutritional Sciences, University of Vienna.

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Abstract

Polyunsaturated fatty acids (PUFAs) are essential nutritional factors to be ingested with diet, and they play a key role in maintaining the soundness of neuromembranes. Several studies have documented that some PUFAs, such as n-3 and n-6 fatty acids can modulate cognitive and social behavior, respectively, physiological stress response. In this study the effects of dietary supplemented natural foods (chia seeds, walnut and peanuts), differing in concentrations of n-3 and n-6 fatty acids, were investigated in socially living guinea pigs. To examine the influence of these nutrients on cognition a maze task was carried out and effects on social behavior were determined in a social confrontation test. Both tests were analyzed in relation to saliva cortisol excretion rates, which is a common marker for physiological stress. Three groups of animals according to the supplemented PUFA diets plus a control group were established (20 males and 20 females). During the learning phase of the maze task (radial arm maze) latency to bait retrieval and error-rates decreased in individuals that were fed with peanuts (high in n-6) while the walnut group (high in n-3 and n-6) showed only a reduction of errors. In the subsequent retention test, experimental groups did not differ in their cognitive performances compared to the learning phase. However, changes in saliva cortisol concentrations in the retention test were negatively related to latency to bait retrieval and mainly positive to the percentage of movement in PUFA supplemented groups in contrast to the control group. During the social confrontation test PUFAs did not affect socio-positive or agonistic behavior. During the first day of this test the peanut group showed fewer sexual interactions compared to the other groups. Furthermore, the peanut and the chia group had decreased cortisol changes in relation to sexual interactions. In summary the results show the most positive effects on cognition and reduced cortisol excretion rates for the peanut group. This seems to be in contrast to former studies carried out in mice or rats, where nutrients high in n-3 fatty acids, such as chia seeds or walnuts, had the mentioned positive behavioral and physiological effects but not peanuts – high on n-6 fatty acids. Therefore, we conclude that possibly the brain metabolism of fatty acids could differ between various rodent species resulting in species dependent cognitive, behavioral and physiological stress related effects to PUFA containing nutrients.

1 Introduction

Nutritional effects on brain developmental processes are well known and of great interest, because numerous studies have documented behavioral and cognitive influences (for reviews see: Benton, 2007; Rogers, 2001; Wallner and Machatschke, 2009). Especially polyunsaturated fatty acids (PUFAs) apparently have a strong effect on structural and functional aspects of certain brain areas during ontogeny (Bourre, 2004; Yehuda et al., 2005).

PUFAs are important components of neuromembrane phospholipids and some cannot be synthesized in most mammalian species *de novo*. Therefore, these essential nutritional factors must be ingested via diet (Hulbert et al., 2005; Simopoulos, 1991). Metabolically, the short-chain-PUFAs α -linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6), which are mainly found in plant products, are the precursors of long-chain-PUFAs, e.g. eicosapentaenoic acid (EPA; 20:5 n-3), docosahexaenoic acid (DHA; 22:6 n-3) and arachidonic acid (AA; 20:4 n-6). The latter three PUFAs are the most frequently detected in neuromembrane phospholipids (Svennerholm, 1968) and play an important role in maintaining membrane functions (Stillwell and Wassall, 2003).

A lack in dietary PUFAs can have negative behavioral and cognitive consequences. In rodents, n-3 fatty acids have a positive effect on cognition and behavior, resulting in improved learning and memory abilities and reduced aggressiveness (Fedorova and Salem Jr, 2006). After daily feeding of walnuts, which contain high concentrations of ALA, rats showed improved cognitive skills in both, the elevated plus maze and the radial arm maze (Haider et al., 2011). In contrast, an ALA-free diet caused learning deficits in mice (Carrié et al., 2000). These deficiencies were fully restored after n-3 PUFA supplementation. The same study also revealed a significantly decreased percentage of DHA in the frontal cortex and the hippocampus brain areas of ALA-deficient mice. In this context it is especially noted that the hippocampus area plays a key role in memory achievement (Squire, 1992), including processing of spatial information (Saab et al., 2009).

In addition to an adequate PUFA intake, the ratio of n-3 and n-6 fatty acids is important as well. A dietary n-6 : n-3 ratio of 4 : 1 has been proved to have strongest effects on cognitive abilities in rats (Yehuda and Carasso, 1993; Yehuda et al., 1998). Furthermore, an optimal n-6 : n-3 ratio boosted growth and development processes in

mice (Santillán et al., 2010). A shift of this ratio towards higher concentrations of n-6 fatty acids can also alter behavioral expressions. In the German Shepherd dog significantly decreased DHA concentrations, correlated with increased n-6 : n-3 ratios, caused pathological aggressive individuals (Re et al., 2008). A similarly increased ratio, caused by elevated n-6 and lowered n-3 concentrations, in brain phospholipids were also associated with increased aggressive behavior and even with depression in rats (DeMar Jr et al., 2006).

PUFAs also have modulatory effects on the hypothalamic-pituitary-adrenal (HPA) axis, although the results remain controversial. After administration of free fatty acids, women showed decreased ACTH secretion rates and lower cortisol concentrations (Lanfranco et al., 2004), whereas no such effects occurred in men (Mai et al., 2006). Even the administration of DHA during final exams - a marker for mental stress - did not alter cortisol plasma concentrations in either sex (Hamazaki et al., 2000). In a recent study, the application of stressors caused increased activity of the HPA axis, correlated with decreased cognitive abilities in rats. Such negative consequences were prevented by administering PUFAs (Ferraz et al., 2011).

The current study was designed to establish a social mammal model, to study the effects of n-6 and n-3 fatty acids, administered as dietary supplements, on spatial abilities, social behavior, and on the reactivity of the HPA axis. For this purpose the guinea pig model was used.

Guinea pigs are polygynous, with dominant males trying to monopolize females. At low density, guinea pig males form linear rank hierarchies in which only the alpha male can monopolize females. In high-density conditions dominant males and their harems peacefully coexist. Lower-ranking males can form close social but not sexual relationships with a female. These are tolerated by the respective harem owner (Sachser et al., 1998).

A number of studies have investigated the influences of different social environments on guinea pig behavior and physiological stress reactions (Machatschke et al., 2004; Wallner and Dittami, 2003; Wallner et al., 2006). Previous studies on the effects of PUFAs showed relations between dietary intake and tissue composition (Abedin et al., 1999; Fu and Sinclair, 2000; Weisinger et al., 1995). Furthermore, some studies clearly documented, that guinea pigs are able to encode spatial information (Dringenberg et al., 2001; Lewejohann et al., 2010; Machatschke et al., 2011). This predestines guinea pigs for a comprehensive overview on the effects of PUFAs. To

investigate the influences of different PUFAs on cognition, a maze task was used, developed for guinea pigs (Machatschke et al., 2011), for behavioral interactions a confrontation test was conducted (Wallner and Dittami, 2003).

We predict enhanced cognitive performance, less aggressive behavior but increased social interactions linked to decreased physiological stress after the supplementation of n-3 rich foods.

2 Methods

2.1 Animals

For this study, 40 domestic guinea pigs *Cavia aperea f. porcellus*, (20 males and 20 females) were used. They were 27.5 ± 13.3 months old and weighed 895.6 ± 150.9 g. All animals were sexually intact, socially skilled and accustomed to daily contact with humans. Prior to the experiments animals were kept in isosexual groups. Both enclosures (each about 4×4 m) for males and females were environmentally enriched with wooden shelters, viewing platforms etc. The floor was covered with woodchip bedding material. The daily food supply contained guinea pig pellets (Altromin 3020, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany), different vegetables and/or fruits and hay. Water was available *ad libitum*. A light-dark cycle (12 hours each with lights on at 07:00 h) and a temperature of 25 °C were maintained during experiments. Experiments were conducted in accordance with the European Communities Council Directive 86/609/EEC and complied with the current animal protection laws decreed by the Austrian Federal Ministry of Education, Science and Culture.

2.2 Experimental design

Experiments were performed in an adapted test room. Animals were singly transferred to cages (85×48×38 cm) and were visually and socially isolated from each other. The floor of each cage was covered with woodchip bedding material and a shelter was provided. Experiments were carried out in five consecutive runs. Each run lasted 23 days, starting with 16 days of isolation, where animals remained in their cages, followed by three days of cognition test (the learning phase), three days of social confrontation test and an additional day of cognition test (the retention test). Afterwards animals were

returned to their isosexual groups. Eight different individuals, four males and four females, were tested per run. Males and females were randomly allocated to four different groups; three groups differed in daily supplementation of PUFAs to their standard food, and a control group. Thus, a total number of 10 animals per experimental group were used.

Every day at 10:00 h, saliva samples were taken to measure cortisol, and the animals were weighed. During the first 16 days of isolation additional PUFA supplementation was carried out at 11:00 h. Thereafter they received their daily standard food, consisting of 20 g Altromin 3020, 10 g cucumber and 10 g carrot; water was provided *ad libitum*. During the test periods feeding procedures were applied after each test. Blood samples were collected to determine PUFA-composition in plasma. Four samples per animal were collected during the isolation period, starting with the first sample on day one and continuing on every fifth day. Additionally, samples were collected after the third day of the learning phase and after the third day of the social confrontation test.

2.2.1 PUFA sources

To examine the effects of PUFAs, three natural sources high in LA and/or ALA with different n-6 : n-3 ratios were chosen: chia seeds (*Salvia hispanica*), walnuts (*Juglans regia*) and peanuts (*Arachis hypogaea*). Chia seeds are one of the best sources for ALA (5.835 g LA and 17.830 g ALA per 100 g; n-6 : n-3 $\approx$ 1: 3), walnuts contain very high amounts of PUFAs in general (38.093 g LA and 9.080 g ALA per 100 g; n-6 : n-3 $\approx$ 4 : 1) and peanuts contain almost only LA (15.555 g LA and 0.003 g ALA per 100 g; n-6 : n-3 $\approx$ 5185 : 1). The stated amounts of LA and ALA in these foods are based on the US Department of Agriculture National Nutrient Database. A daily amount of 500 mg of crushed chia seeds (chia group), walnuts (walnut group) or peanuts (peanut group) per kg body weight were dissolved in 1 ml water and administered orally, using 1 ml syringes. The control group received only 1 ml water during this procedure.

2.2.2 Cognition test

After the first 16 days of isolation the cognition test started to test spatial abilities. The test paradigm consisted of a three-day learning phase and a retention test, performed after the three days of social confrontation. The experiment started at 11:00 h

with the first animal of the current run. For this, a hybrid of a Y- and radial-arm-maze was used, a design that has successfully been used in the past (Machatschke et al., 2011; Millesi et al., 2001). The maze consisted of four arms, arranged in the form of a plus, each splitting like a Y into two distal arms at their end. This yielded eight different arms for a possible decision to access bait. The maze was built of laminated fiberboard, positioned on the floor of the test room. For each animal, one of these eight arms was randomly baited with a 10 g piece of cucumber; this arm remained the same throughout the cognition test for each individual. The piece of cucumber was not visible for an animal until the distal arm was reached. An animal was placed singly and unfed in the center of the maze, with its head pointing into the opposite direction of the baited arm. Each trial lasted 10 minutes and, following Machatschke et al. (2011), each animal had to stay the full time period in the maze, even if the cucumber had been found earlier. Afterwards the animal was removed from the maze, another saliva sample was taken, feeding-procedures were carried out, including PUFA supplementation, and the animal was returned to its cage. After every single animal's performance, the maze was cleaned with a 5% acetic acid solution and paper towels to remove olfactory influences, and a new piece of cucumber was placed in the maze for the next animal. Performances on each of the four days (learning phase and retention test) were recorded with a video camera located above the maze.

2.2.3 Social confrontation test

The social confrontation test took place after the learning phase of the cognition test. This was developed to test male competition for females but also female aggression towards males; other social interactions such as socio-positive and sexual behaviors were also recorded (for details see Wallner et al., 2006; Wallner and Dittami, 2003). In brief, starting at 11:00 h, each single animal of the current run (one male and one female of the four experimental groups) was transferred to a square arena, with a side length of 1.6 m, built of wooden panels. The floor of the arena was covered with woodchip bedding material. Animals remained together in the arena for three days. On each of the three days the behavior of animals was recorded for 30 minutes with a video camera located above the arena, always starting at 11:00 h. After the recordings, saliva samples were taken and feeding procedures were carried out. Afterwards all animals were returned into the arena. After saliva sampling on the third day of the test, all animals were returned to their cages.

2.3 Behavioral measures

Performances in the maze and arena were analyzed using The Observer XT 10 (Version: 10.5.572, Noldus, Wageningen, the Netherlands).

Following Machatschke et al. (2011), three different parameters were measured in the cognition test: (1) Latency to bait: the period of time from release into the maze until the bait was reached. If an animal failed to find the bait, 10 minutes were noted. (2) Error-rate: the number of wrong arm-entries until the bait was found or, if it was not found, until the maximum time of 10 minutes elapsed. Whenever an animal's forelegs passed into a non-baited arm, this was counted as an error. (3) Percentage of movement: the percentage of time an animal spent moving until the bait was found or, if it was not found, until the maximum time of 10 minutes elapsed, with movement being defined as a change in position of at least one body length.

In the social confrontation test, frequencies of initiated and received behaviors were measured by using continuous recording (Altmann, 1974). Definition of behavioral categories and their included behaviors mainly followed Rood (1972): (1) Socio-positive behavior: side by side (huddling), social grooming and nose-nose. (2) Agonistic behavior: displacement, chasing, fighting, biting, teeth chatter, head-thrust, stand-threat, kick-back, riding, rumba-rumble. (3) Sexual behavior: marking, naso-anal, chin-rump follow, rumba-rumble, riding, copulation. Riding and rumba-rumble are two types of behavior that male individuals show towards other males, in case of aggressive or dominant encounters, or towards females, in case of a sexual approach. These were therefore noted either as aggressive or sexual behavior, depending on the sex of the opponent. Behavioral variables (initiated and received behavior) were aggregated to the three behavioral categories to calculate the number of socio-positive, agonistic and sexual interactions.

2.4 Saliva sampling and analysis

Saliva samples were collected by inserting a cotton bud (Q-tip) into the guinea pigs cheek pouch for at least 1 minute (Fenske, 1997). After centrifugation (2500 rpm, 1,006×g, 4 minutes) saliva was stored at -20 °C until further analysis. Saliva cortisol was measured, using an enzyme-linked immunoassay (EIA), as described by Palme and Möstl (1997), with an input of 10 µl, after 1:50 dilution of samples. Cross-reactions with relevant steroids were: 4-pregnene-11 β ,21-diol-3,20-dione 6.2%; 4-pregnene-

11 β ,17 α ,21-triol-3,20,dione 100%; 5 α -pregnane-11 β ,17 α ,21-triol-3,20,dione 4.6%; 5 α -pregnane-3 α ,11 β ,17 α ,21-tetrol-20-one 0.8%; 5 β -pregnane 3 α ,11 β ,17 α ,21-tetrol-20-one 0.1%; all other steroids cross-reacted < 0.01%. Intra- and interassay coefficients of variance were 13.1% and 17%.

2.5 Blood sampling and PUFA analysis

Blood samples were collected by punctuating the marginal ear vein (Sachser and Pröve, 1984) with sterile lancets. Approximately 100 μ l blood was collected in heparinized micropipettes. Samples were immediately put on ice. Plasma was separated by centrifugation (4000 rpm, 2,775 $\times$ g, 10 minutes) and stored at -20 °C until further analysis. Determination of PUFA in plasma was carried out using gas chromatography, following Wagner et al. (2000). Fatty acids were transesterificated by adding 1 ml methanolic NaOH (containing butylated hydroxytoluene BHT to prevent oxidation) to 100 μ l plasma, thereafter, 1 ml 14% BF₃ (Boran-Triflourid-Methanol) was added to obtain fatty acid methyl esters (FAMES). After the FAMES were extracted into 500 μ l hexane four times they were vaporized and re-dissolved in hexane. Using an auto-system gaschromatograph (Perkin Elmer) with flame ionization detector (FID), FAMES were separated by a Rtx-2330 30 m $\times$ 0.25 mm i.d. silica column. 1 μ l of prepared samples were injected at a temperature of 250 °C and detected at 270 °C. Helium was used as carrier gas. Identification of fatty acids was done by a 37 component FAME Mix Standard (Supelco, Bellafonte, USA). For peak integration, TotatChrom Workstation 6.3.0 (PE Nelson, Perkin Elmer, USA) was used.

2.6 Statistical analysis

All statistics were performed by using R (Version 2.14.0; R Development Core Team, 2011). Shapiro-Wilk tests were used to examine the distribution of the data, and Levene's test for homogeneity was carried out as well. To attain normal distribution, some data sets were transformed by applying the natural logarithm or square root. Linear mixed effect models (LME; package 'nlme', Pinheiro et al., 2012) were performed for each group (control and three experimental groups) to analyze changes in the time courses of the learning phase, changes in behavioral interactions, or changes in single fatty acids and in the n-6 : n-3 ratio. In these models the experimental days were defined as predictor variable. To detect performance and behavioral differences between groups, LMEs were calculated using experimental groups and days as predictors.

Cortisol changes during the cognition and social confrontation tests were analyzed using the equation: $((\text{cortisol after the test}) / (\text{cortisol before the test} + \text{cortisol after the test}) - 0.5) * 2$). Because of daily decreasing frequencies of behavior and no differences in cortisol between groups on the second and third day of the social confrontation test, a general linear model (GLM) was calculated for the first day, using the cortisol changes as response variable and experimental groups and behavior (either socio-positive, agonistic or sexual interactions) as predictor variables (only 2-way interactions). To analyze the performance in the retention test, GLMs were applied using either latency to bait, error-rate or percentage of movement as response variables and two-way interactions between experimental groups and one of following parameters as predictors: cortisol change, the performance for the parameter on the last day of the learning phase, socio-positive interactions, agonistic interactions, and sexual interactions. The Akaike information criterion (AIC) was used to fit the GLMs. Significant effects were plotted (package ‘effects’, Fox, 2003) to detect different “effect displays” for the experimental groups (see appendix). One-way analyses of variance (ANOVAs) were used to detect differences in cortisol levels and fatty acids for single days of the social confrontation test between experimental groups. Tukey HSD was performed as post-hoc test (automatically corrected). Paired Student’s t-test was applied to compare cortisol levels before and after the tests. Significance was set at a level of $p \leq 0.05$.

3 Results

The daily body weight revealed no differences between experimental groups based on a daily measurement (group*day: $F_{3,35} = 0.467$, $p = 0.707$). The weight of individuals was in general negatively affected by day (day: $F_{22,770} = 65.898$, $p < 0.001$), but was not affected by group (group: $F_{66, 770} = 0.364$, $p = 1.000$).

The groups did not differ in their daily cortisol-levels throughout the first 16 days of isolation (group*day: $F_{45,475} = 0.636$, $p = 0.969$) and there was no experimental group or day effect (group: $F_{3,35} = 0.714$, $p = 0.550$; day: $F_{15,475} = 0.1.496$, $p = 0.102$).

3.1 Cognition test

After the 16 days of isolation, the animals were tested in the maze over three consecutive days, the learning phase. A significant decrease in the latency to bait during the learning phase was found in the peanut group ($F_{2,18} = 6.751, p = 0.007$). No significant changes in latency during this time course occurred in the remaining three groups (chia: $F_{2,18} = 2.872, p = 0.083$; walnut: $F_{2,18} = 0.469, p = 0.633$; control: $F_{2,16} = 1.773, p = 0.202$) (Fig. 1A). With regard to the experimental groups, the latency was not affected by experimental days or the cortisol change (group*day: $F_{6,58} = 1.622, p = 0.158$; group*cortisol: $F_{3,58} = 0.159, p = 0.924$; group: $F_{3,34} = 0.741, p = 0.535$; day: $F_{2,58} = 1.510, p = 0.229$; cortisol: $F_{1,58} = 0.286, p = 0.595$).

The error-rate was in general significantly affected by the experimental day (day: $F_{2,58} = 8.745, p < 0.001$), whereas the cortisol change had a marginal effect (cortisol: $F_{1,58} = 3.520, p = 0.066$); experimental group showed no effects at all (group*day: $F_{6,58} = 0.836, p = 0.548$; group*cortisol: $F_{3,58} = 1.181, p = 0.325$; group: $F_{3,34} = 0.717, p = 0.549$). Individuals of the peanut ($F_{2,18} = 4.275, p = 0.030$) and walnut group ($F_{2,18} = 4.002, p = 0.037$) showed a decreasing number of errors (wrong arm entries) during the learning phase. The chia and control group showed no change in their error rates (chia: $F_{2,18} = 0.864, p = 0.438$; control: $F_{2,16} = 1.376, p = 0.281$) (Fig. 1B).

The percentage of movement until the bait was found was also affected by day (day: $F_{2,58} = 5.609, p = 0.006$), but no other effects were detected (group*day: $F_{6,58} = 1.242, p = 0.299$; group*cortisol: $F_{3,58} = 0.614, p = 0.609$; group: $F_{3,34} = 1.295, p = 0.292$; cortisol: $F_{1,58} = 0.416, p = 0.522$). The peanut group exhibited a significant increase in the percentage of movement during the learning phase ($F_{2,18} = 4.632, p = 0.024$), while no significant effects occurred in the remaining groups (chia: $F_{2,18} = 2.085, p = 0.153$; walnut: $F_{2,18} = 0.062, p = 0.940$; control: $F_{2,16} = 2.915, p = 0.083$) (Fig. 1C).

Saliva cortisol measured before the cognition test started differed between the groups (pre Day 1: $F_{3,32} = 2.965, p = 0.047$) and was significantly higher in the peanut versus control group (Tukey: $t = 1.242, p = 0.028$). These differences, however, diminished after the first day of the learning phase (post Day 1: $F_{3,35} = 0.062, p = 0.979$). Cortisol concentrations did not differ between experimental groups on the second day of the learning phase (pre Day 2: $F_{3,33} = 0.678, p = 0.572$; post Day 2: $F_{3,34}$

= 0.341, $p = 0.796$) or before the test on the third day (pre Day 3: $F_{3,34} = 0.105$, $p = 0.957$). After the learning phase cortisol differed between groups (post Day 3: $F_{3,34} = 2.958$, $p = 0.050$) and was significantly elevated in the control versus chia group (*Tukey*: $t = 2.830$, $p = 0.050$). Cortisol levels before and after the tests differed in the chia group on day two ($t_9 = 2.691$, $p = 0.025$) and in the peanut group on day three of the learning phase ($t_9 = 2.414$, $p = 0.039$). In both cases, cortisol was higher after the performance in the maze (Table 1).

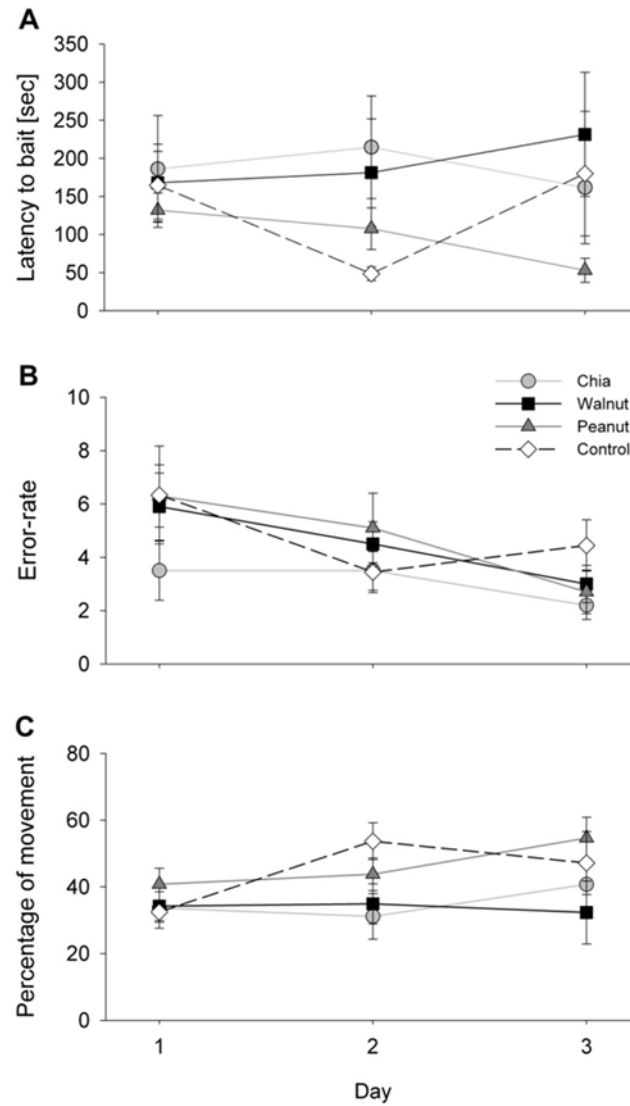


Fig. 1: Behaviors of the chia, walnut, peanut, and control group per day of the learning phase. Values are mean $\pm$ SEM. (A) latency time to bait [sec], (B) number of errors until retrieval of the bait, (C) percentage of movement until retrieval of the bait.

Table 1: Cortisol concentrations [ng/ml] per day of the learning phase for each experimental group. Values are mean $\pm$ SEM. (pre = concentration before the test, post = concentration after the test)

Group	Day 1		Day 2		Day 3	
	pre	post	pre	post	pre	post
Chia	44.21 $\pm$ 16.55	41.97 $\pm$ 8.65	18.44 $\pm$ 4.73 *	35.17 $\pm$ 7.11	22.22 $\pm$ 4.91	34.57 $\pm$ 6.16 ^a
Walnut	42.8 $\pm$ 11.41	47.53 $\pm$ 14.56	31.06 $\pm$ 5.53	37.79 $\pm$ 6.76	18.29 $\pm$ 4.27	47.14 $\pm$ 10.96
Peanut	53.32 $\pm$ 10.41 ^a	56.42 $\pm$ 21.49	31.74 $\pm$ 9.76	43.03 $\pm$ 7.35	27.04 $\pm$ 7.3 *	50.03 $\pm$ 6.41
Control	16.78 $\pm$ 3.33 ^a	45.82 $\pm$ 12.95	29.5 $\pm$ 13.11	46.89 $\pm$ 11.53	34.19 $\pm$ 13.71	96.4 $\pm$ 38.18 ^a

Note: Superscript letters indicate significant differences between groups ($p \leq 0.05$; Tukey post-Hoc test);

* $p \leq 0.05$ (paired t-test for pre and post).

3.2 Social confrontation test

The learning phase of the cognition test was followed by three days of the social confrontation test. During this period, socio-positive interactions increased significantly from day to day (day: $F_{2,70} = 18.616$, $p < 0.001$), while experimental groups did not differ (group: $F_{3,35} = 0.843$, $p = 0.480$) and without a significant interaction between experimental group and experimental day (group*day: $F_{6,70} = 1.860$, $p = 0.100$). Animals of the chia, peanut, and control group exhibited a significant increase in socio-positive behavior during the experimental days (chia: $F_{2,18} = 15.046$, $p < 0.001$; peanut: $F_{2,18} = 4.477$, $p = 0.026$; control: $F_{2,16} = 7.870$, $p = 0.004$), while the walnut group remained constant (walnut: $F_{2,18} = 2.796$, $p = 0.088$) (Fig. 2A).

In contrast to the former results, agonistic interactions decreased significantly in all experimental groups ($F_{2,10} = 27.001$, $p < 0.001$; chia: $F_{2,18} = 9.409$, $p = 0.002$; walnut: $F_{2,18} = 10.148$, $p = 0.001$; peanut: $F_{2,18} = 7.339$, $p = 0.005$; control: $F_{2,16} = 9.836$, $p = 0.002$), but with no differences between groups ($F_{3,35} = 0.274$, $p = 0.844$) and no significant interaction between experimental groups and experimental days ($F_{6,70} = 0.313$, $p = 0.928$) (Fig. 2B).

Sexual interactions decreased significantly in the chia, walnut, and control group (chia: $F_{2,18} = 5.657$, $p = 0.012$; walnut: $F_{2,18} = 11.628$, $p < 0.001$; control: $F_{2,16} = 8.294$, $p = 0.003$), but not in the peanut group ($F_{2,18} = 2.061$, $p = 0.367$). The interaction between experimental groups and experimental days missed the criterion of significance marginally (group*day: $F_{6,70} = 2.069$, $p = 0.068$). This provides evidence for differences caused by fewer sexual interactions in the peanut group on the first day and no change in sexual interactions during the remaining experimental days (Fig. 2C).

Cortisol levels previous to the social confrontation test did not differ between experimental groups ($F_{3,34} = 1.650$, $p = 0.196$), but differed afterwards ($F_{3,35} = 3.966$, $p = 0.016$). Cortisol was higher in the control group compared to the chia (*Tukey*: $t = 2.963$, $p = 0.027$) and walnut group (*Tukey*: $t = 3.062$, $p = 0.021$) (Fig. 3). On the second day of social confrontation, cortisol after the observation periods did not differ between groups ($F_{3,32} = 0.837$, $p = 0.484$; chia: 88.87 ± 25.7 , walnut: 154.29 ± 37.87 , peanut: 129.37 ± 19.2 , control: 152.5 ± 44.36) and marginally differed on day three ($F_{3,33} = 2.699$, $p = 0.062$; chia: 62.46 ± 8.19 , walnut: 82.73 ± 18.87 , peanut: 136.21 ± 30.67 , control: 139.85 ± 27.4).

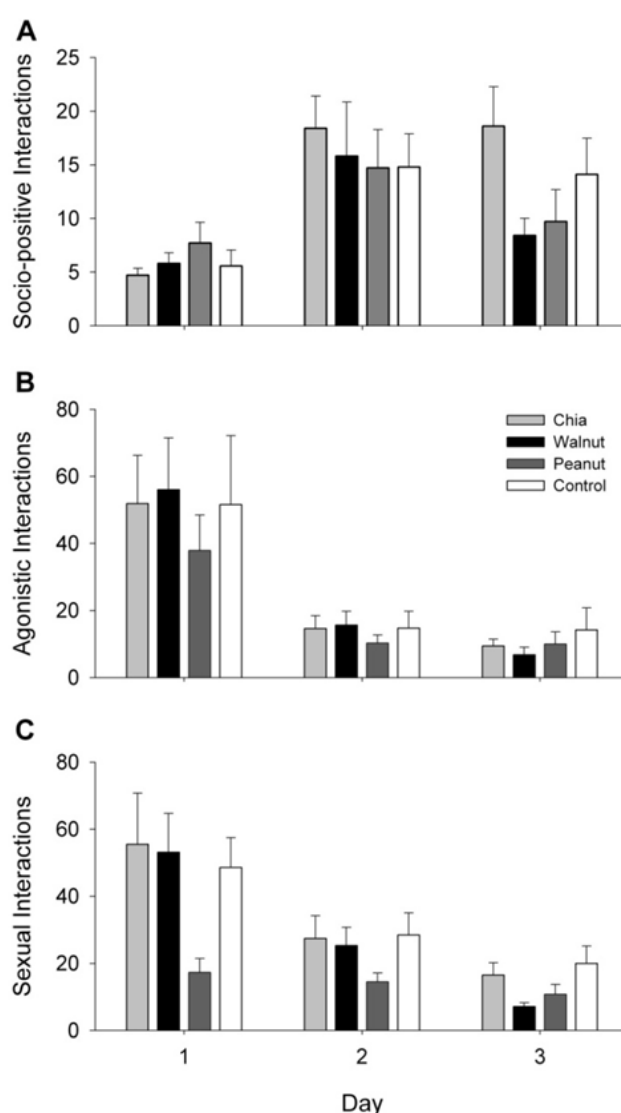


Fig. 2: Number of behavioral interactions for chia, walnut, peanut, and control group on each day of social confrontation. Values are mean $\pm$ SEM. (A) socio-positive interactions, (B) agonistic interactions, (C) sexual interactions.

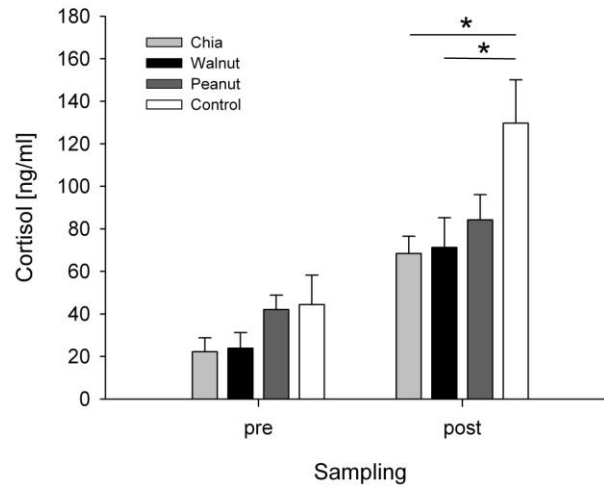


Fig. 3: Salivary cortisol concentrations [ng/ml] before (pre) and after (post) the observation period on the first day of social confrontation. Post concentrations are significantly increased for all groups compared to pre values (paired t-test; chia: $t_9 = 5.349$, $p < 0.001$; walnut: $t_9 = 3.175$, $p = 0.011$; peanut: $t_8 = 3.247$, $p = 0.012$; control: $t_8 = 4.592$, $p = 0.002$). Values are mean $\pm$ SEM. * $p \leq 0.05$.

With regard to the first day of the social confrontation test, social interactions had no effect on cortisol changes (Table 2). In general agonistic interactions were positively correlated with cortisol changes, but did not differ between the groups. Sexual interactions also affected cortisol, but this was differed in the groups. A positive relationship between sexual interactions and an increased cortisol change was found in the control and walnut group, a negative relationship in the chia and peanut group (Fig. 4).

Table 2: Results of the GLM for the first day of the social confrontation test, using the cortisol change as response variable.

Predictor	Df	F value	p (>F)
Group	3	1.307	0.297
Socio-positive Interactions	1	1.876	0.185
Agonistic Interactions	1	11.251	0.003
Sexual Interactions	1	1.123	0.301
Group $\times$ Socio-positive Interactions	3	0.391	0.761
Group $\times$ Agonistic Interactions	3	2.703	0.07
Group $\times$ Sexual Interactions	3	4.699	0.011
Residuals	22		

Note: Bold numbers indicate statistical significance.

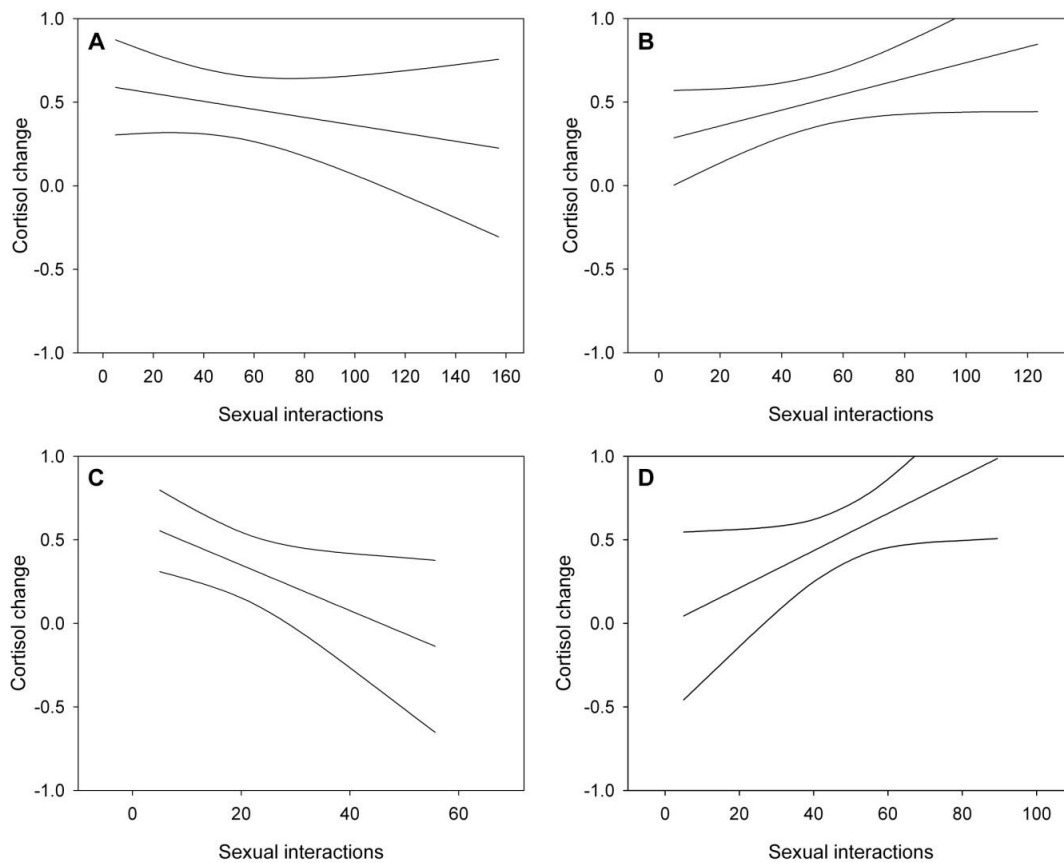


Fig. 4: Effect size plots for the effects of sexual interactions on the cortisol change for each group and the 5 % and 95 % confidence interval. (A) chia, (B) walnut, (C) peanut, (D) control. (Cortisol change of 0: no change)

3.3 Retention test

In the retention test the latency to bait was positively related to the time the animals needed to pass the test on the third day of the learning phase, with no differences between groups (Table 3). The more time individuals needed to find the bait on the third day of the learning phase, the more time they needed in the retention test. However, groups were differently affected by their cortisol changes during the retention test. Individuals of the chia, walnut and peanut group were slightly but positively affected by cortisol. In contrast, individuals of the control group needed more time to reach the bait, the stronger cortisol increased. In addition control animals were also strongly affected by socio-positive and agonistic interactions during the social confrontation test. The latency to bait in control animals increased with more socio-positive interactions and decreased with more agonistic interactions. Individuals of the

chia, walnut and peanut group were only weakly affected in the latency to bait (in the retention test) by their socio-positive and agonistic interactions (in the social confrontation test). In contrast, agonistic interactions were positively related to the latency to bait in the walnut group. The latency to bait did not differ between the third day of the learning phase and the retention test in any group (chia: $t_9 = 0.6136$, $p = 0.555$; walnut: $t_9 = 0.741$, $p = 0.477$; peanut: $t_9 = 0.381$, $p = 0.712$; control: $t_8 = 0.112$, $p = 0.914$).

The number of errors in the retention test was not affected by any of the parameters. Comparing the number of errors on the third day of the learning phase and the number of errors in the retention test, revealed that the number of errors did not differ between the tests (chia: $t_9 = 1.071$, $p = 0.312$; walnut: $t_9 = 1.299$, $p = 0.226$; peanut: $t_9 = 1.300$, $p = 0.226$; control: $t_8 = 0.612$, $p = 0.557$).

Regarding the percentage of movement during the retention test, a negative relationship between the number of socio-positive interactions in the social confrontation test and the percentage of movement was detected. Animals of all groups were negatively affected and showed less movement when socio-positive interactions increased. Animals of the chia and control group exhibited less movement during the retention test, the higher their change in cortisol was. In contrast, animals of the walnut and peanut group were positively affected by a higher increase in cortisol and showed a higher percentage of movement. Movement in the retention test in general was positively related to the movement of the third day of the learning phase, but the slope was steepest for the control group. No differences in the percentage of movement were detected between the third day of the learning phase and the retention test for any group (chia: $t_9 = 1.395$, $p = 0.197$; walnut: $t_9 = 0.714$, $p = 0.493$; peanut: $t_9 = 1.388$, $p = 0.199$; control: $t_8 = 1.194$, $p = 0.267$). (For effect plots of significant interactions of the GLMs see Appendix)

Table 3: Results of the GLMs for the retention test using latency to bait, error-rate, and percentage of movement as response variables. Not stated predictors were removed using the AIC. (Parameter on day 3 = day 3 of learning phase)

Predictor	Latency to bait			Error-rate			Percentage of movement		
	Df	F value	p (>F)	Df	F value	p (>F)	Df	F value	p (>F)
Group	3	2.457	0.098	3	0.576	0.636	3	1.83	0.188
Cortisol Change	1	0.427	0.522	1	1.86	0.184	1	3.988	0.066
Parameter on day 3	1	14.196	0.002				1	35.89	< 0.001
Socio-positive interactions	1	0.507	0.486	1	0.218	0.644	1	19.074	< 0.001
Agonsitic interactions	1	0.052	0.822				1	0.192	0.668
Sexual interactions	1	2.696	0.119				1	1.894	0.19
Group × Cortisol Change	3	5.438	0.008				3	7.946	0.002
Group × Parameter on day 3							3	3.365	0.049
Group × Socio-pos. interactions	3	3.871	0.028	3	1.739	0.184			
Group × Agonistic interactions	3	5.32	0.009				3	2.495	0.102
Group × Sexual interactions	3						3	1.609	0.232
Residuals	17			26			14		

Note: Bold numbers indicate statistical significance.

3.4 PUFAs

Plasma analysis revealed pronounced differences between groups in short chain PUFAs. Feeding on chia seeds led to higher proportions of ALA (18:3 n-3), compared to all the other groups. ALA increased significantly in the chia and in the walnut group. LA (18:2 n-6c) did not change over time in any of the groups and was significantly higher in the walnut versus chia group. A significant increase with time in the long chain PUFAs AA (20:4 n-6) and DHA (22:6 n-3) was detected mainly in the peanut and control groups (Table 4).

Regarding the n-6 : n-3 ratio, no differences between the experimental groups were detected on the first sample point ($F_{3,35} = 2.413$, $p = 0.083$). At the second sample point, on day 6 of the isolation, the ratio was significantly lower in the chia than in the peanut group ($t = 3.660$, $p = 0.004$). At the subsequent sample points, on days 11, 16 (both isolation), 19 (after learning phase) and 22 (after social confrontation test), the ratio was always significantly lower in the chia group than any other groups (ANOVA; day 11: $F_{3,35} = 8.001$, $p < 0.001$; day 16: $F_{3,35} = 12.121$, $p < 0.001$; day 19: $F_{3,35} = 13.453$, $p < 0.001$; day 22: $F_{3,35} = 4.781$, $p = 0.007$). A significant decrease in the n-6 : n-3 ratio, according to the time course of sampling, was detected in the chia and walnut groups

(chia: $F_{5,45} = 4.685$, $p = 0.002$; walnut: $F_{5,44} = 2.826$, $p = 0.027$), while no such differences were detected for the remaining two groups (peanut: $F_{5,45} = 0.521$, $p = 0.759$; control: $F_{5,40} = 0.957$, $p = 0.455$) (Fig. 5).

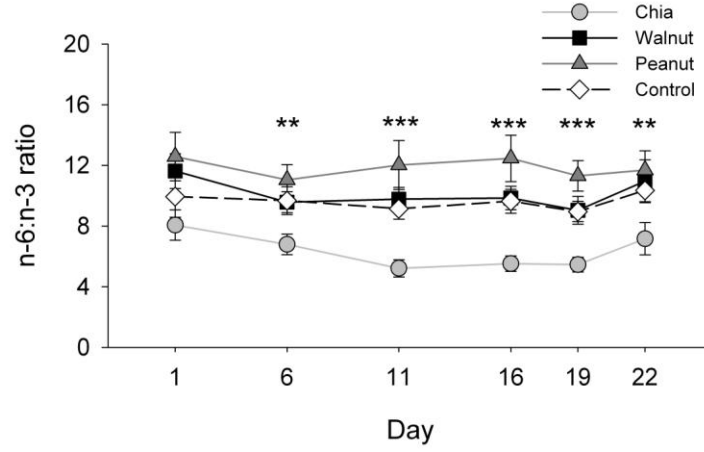


Fig. 5: n-6:n-3 ratios for groups, throughout the study. Sample points 1, 6, 11, 16 occurred during the isolation period, sample point 19 after the learning phase of the cognition test, and sample point 22 after the social confrontation test. ** $p < 0.01$, *** $p < 0.001$ (day 6: difference between chia and peanut group; days 11, 16, 19, 22: differences between chia and all other experimental groups).

Table 4: Plasma n-3 and n-6 fatty acid patterns (in percent of total detected plasma fatty acids) for the groups before and during intervention. Values are mean $\pm$ SEM. Results of LMEs, with day as predictor, for each group and PUFA, are stated.

PUFA	Group	Day 1	Day 6	Day 11	Day 16	Day 19	Day 22	Df	F value	p (> F)
18:2 n-6t	Chia	0.3 $\pm$ 0.05	0.23 $\pm$ 0.02	0.27 $\pm$ 0.03 ^a	0.21 $\pm$ 0.03	0.26 $\pm$ 0.02	0.26 $\pm$ 0.02	5,45	1.885	0.116
	Walnut	0.26 $\pm$ 0.03	0.16 $\pm$ 0.01	0.14 $\pm$ 0.02 ^a	0.14 $\pm$ 0.02 ^a	0.16 $\pm$ 0.03	0.18 $\pm$ 0.03 ^a	5,44	4.999	0.001
	Peanut	0.29 $\pm$ 0.03	0.16 $\pm$ 0.02	0.21 $\pm$ 0.01	0.19 $\pm$ 0.01	0.19 $\pm$ 0.02	0.21 $\pm$ 0.02 ^b	5,45	5.413	< 0.001
	Control	0.32 $\pm$ 0.05	0.2 $\pm$ 0.03	0.2 $\pm$ 0.04	0.26 $\pm$ 0.02 ^a	0.24 $\pm$ 0.03	0.32 $\pm$ 0.03 ^{ab}	5,40	2.496	0.047
18:2 n-6c (LA)	Chia	46.46 $\pm$ 2.1	46.55 $\pm$ 1.47	46.46 $\pm$ 1.06 ^a	46.26 $\pm$ 1.47 ^a	45.93 $\pm$ 1.05 ^a	47.1 $\pm$ 1.47	5,45	0.253	0.936
	Walnut	49.8 $\pm$ 1.13	50.51 $\pm$ 0.73	51.77 $\pm$ 1 ^{ab}	51.29 $\pm$ 0.91 ^a	50.56 $\pm$ 1.14 ^a	50.2 $\pm$ 1.05	5,44	1.642	0.169
	Peanut	46.64 $\pm$ 1.37	48.13 $\pm$ 1.23	46.18 $\pm$ 1.21 ^b	47.44 $\pm$ 0.91	47.35 $\pm$ 0.73	47.32 $\pm$ 1.53	5,45	0.755	0.587
	Control	46.17 $\pm$ 1.37	47.99 $\pm$ 1.13	47.8 $\pm$ 1.15	47.58 $\pm$ 1.14	47.7 $\pm$ 0.87	47.92 $\pm$ 0.92	5,40	1.387	0.25
18:3 n-6 (GLA)	Chia	0.01 $\pm$ 0.01	n.d. ^a	n.d. ^{ab,c}	0.02 $\pm$ 0.02 ^{ab}	0.02 $\pm$ 0.02 ^a	0.02 $\pm$ 0.01 ^{ab}	5,45	0.529	0.753
	Walnut	0.09 $\pm$ 0.03	0.11 $\pm$ 0.02 ^a	0.11 $\pm$ 0.02 ^a	0.11 $\pm$ 0.02 ^a	0.13 $\pm$ 0.01 ^a	0.11 $\pm$ 0.01 ^a	5,44	0.496	0.777
	Peanut	0.07 $\pm$ 0.03	0.08 $\pm$ 0.03	0.08 $\pm$ 0.03 ^b	0.09 $\pm$ 0.02	0.07 $\pm$ 0.02	0.08 $\pm$ 0.02	5,45	0.363	0.871
	Control	0.04 $\pm$ 0.03	0.07 $\pm$ 0.03	0.1 $\pm$ 0.03 ^c	0.12 $\pm$ 0.03 ^b	0.09 $\pm$ 0.03	0.12 $\pm$ 0.02 ^b	5,40	2.66	0.036
18:3 n-3 (ALA)	Chia	6.28 $\pm$ 0.8 ^a	7.15 $\pm$ 0.56 ^{ab,c}	9.71 $\pm$ 1.05 ^{ab,c}	8.95 $\pm$ 0.93 ^{ab,c}	8.91 $\pm$ 0.85 ^{ab,c}	7.3 $\pm$ 0.83 ^{ab,c}	5,45	3.933	0.005
	Walnut	4.41 $\pm$ 0.38	5.04 $\pm$ 0.39 ^a	5.31 $\pm$ 0.41 ^a	5.17 $\pm$ 0.38 ^a	5.69 $\pm$ 0.44 ^a	4.71 $\pm$ 0.44 ^a	5,44	2.986	0.021
	Peanut	4 $\pm$ 0.47 ^a	4.3 $\pm$ 0.37 ^b	4.08 $\pm$ 0.46 ^b	3.94 $\pm$ 0.44 ^b	4.12 $\pm$ 0.31 ^b	3.96 $\pm$ 0.3 ^b	5,45	0.249	0.938
	Control	5.17 $\pm$ 0.64	4.92 $\pm$ 0.36 ^c	5.16 $\pm$ 0.34 ^c	4.93 $\pm$ 0.43 ^c	5.2 $\pm$ 0.36 ^c	4.57 $\pm$ 0.37 ^c	5,40	0.852	0.521
20:2 n-6 (EDA)	Chia	0.35 $\pm$ 0.04	0.29 $\pm$ 0.03	0.24 $\pm$ 0.03	0.23 $\pm$ 0.02	0.26 $\pm$ 0.02	0.24 $\pm$ 0.02	5,45	5.951	< 0.001
	Walnut	0.3 $\pm$ 0.03	0.23 $\pm$ 0.02	0.23 $\pm$ 0.02	0.2 $\pm$ 0.04	0.25 $\pm$ 0.01	0.23 $\pm$ 0.02	5,44	1.875	0.118
	Peanut	0.35 $\pm$ 0.04	0.24 $\pm$ 0.02	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03	5,45	4.039	0.004
	Control	0.33 $\pm$ 0.03	0.25 $\pm$ 0.03	0.28 $\pm$ 0.02	0.3 $\pm$ 0.03	0.35 $\pm$ 0.05	0.34 $\pm$ 0.04	5,40	2.213	0.072

20:4 n-6 (AA)	Chia	2.16 ± 0.31	2.32 ± 0.29	2.17 ± 0.27	2.12 ± 0.2	2.29 ± 0.14	2.61 ± 0.24	5,45	0.997	0.431
	Walnut	1.65 ± 0.19	2.33 ± 0.2	2.14 ± 0.23	2.21 ± 0.21	2.15 ± 0.16	2.48 ± 0.25	5,44	3.703	0.007
	Peanut	2 ± 0.19	2.59 ± 0.25	2.59 ± 0.29	2.82 ± 0.24	2.73 ± 0.21	3.06 ± 0.3	5,45	4.344	0.003
	Control	1.91 ± 0.24	2.53 ± 0.26	2.28 ± 0.22	2.79 ± 0.37	2.8 ± 0.27	3.07 ± 0.21	5,40	4.726	0.002
20:5 n-3 (EPA)	Chia	0.12 ± 0.09	n.d.	n.d.	n.d.	0.03 ± 0.02	0.02 ± 0.02	5,45	1.437	0.229
	Walnut	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	n.d.	n.d.	0.02 ± 0.01	5,44	1.261	0.298
	Peanut	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.02	5,45	0.968	0.448
	Control	0.08 ± 0.04	0.04 ± 0.03	0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	n.d.	5,40	1.882	0.119
22:5 n-3 (DPA)	Chia	0.19 ± 0.04	0.25 ± 0.08	0.37 ± 0.09	0.33 ± 0.08	0.36 ± 0.06	0.4 ± 0.07	5,45	1.946	0.105
	Walnut	0.19 ± 0.03	0.29 ± 0.03	0.22 ± 0.02	0.27 ± 0.03	0.26 ± 0.04	0.3 ± 0.05	5,44	2.986	0.021
	Peanut	0.31 ± 0.08	0.42 ± 0.11	0.39 ± 0.11	0.37 ± 0.1	0.36 ± 0.08	0.41 ± 0.11	5,45	1.62	0.174
	Control	0.25 ± 0.03	0.39 ± 0.08	0.3 ± 0.04	0.32 ± 0.04	0.37 ± 0.05	0.36 ± 0.06	5,40	1.79	0.137
22:6 n-3 (DHA)	Chia	0.33 ± 0.1	0.41 ± 0.12	0.26 ± 0.08	0.23 ± 0.1	0.24 ± 0.09	0.33 ± 0.09	5,45	2.208	0.07
	Walnut	0.21 ± 0.04	0.46 ± 0.13	0.28 ± 0.09	0.28 ± 0.06	0.25 ± 0.05	0.29 ± 0.04	5,44	1.878	0.118
	Peanut	0.17 ± 0.06	0.32 ± 0.12	0.29 ± 0.13	0.3 ± 0.11	0.28 ± 0.1	0.32 ± 0.11	5,45	3.37	0.011
	Control	0.1 ± 0.03	0.24 ± 0.03	0.25 ± 0.03	0.3 ± 0.07	0.33 ± 0.07	0.25 ± 0.04	5,40	2.956	0.023

Note: Superscript letters indicate significant differences between groups ($p \leq 0.05$; Tukey post-Hoc test). Bold numbers indicate statistical significance for the LMEs. n.d.: not detectable.

4 Discussion

The present study was designed to examine the effects of dietary PUFAs in a socially living species, the guinea pig. Our investigations revealed positive effects of n-6 fatty acids and a high n-6 : n-3 ratio on cognitive abilities. Animals fed on peanuts showed an improvement in all three cognitive parameters (latency to bait, error-rate, percentage of movement). Positive effects of n-3 fatty acids were only detected by a significant reduction of errors in the walnut group. With regard to the latency to bait in the retention test, PUFA supplemented groups were only slightly affected by agonistic and socio-positive interactions during preceded social challenge test and generally less affected in comparison to the control group. During the retention test cortisol had a negative effect on the latency to bait and the percentage of movement in the control group, while in PUFA supplemented groups cortisol positively affected these parameters. Animals fed on peanuts were less frequently involved in sexual interactions during the social challenge test. Sexual interactions led to a stronger increase in cortisol in the walnut and control groups, while in the peanut group cortisol decreased at a higher frequency of sexual interactions.

Yehuda and Carasso (1993) determined the overall positive effects of a n-6 : n-3 ratio with approximately 4 : 1 in rats. In our study this ratio was nearly detected in the plasma of the chia group. According to the rat study a better performance during the cognition test would have been expected in the chia group, compared to the remaining ones. However, this was not the case. Surprisingly the peanut group, which revealed a high n-6 : n-3 ratio in plasma, performed best in all three defined cognitive parameters. Previous studies showed that the long-chain n-3 fatty acid DHA significantly increases cognitive abilities, mainly determined by a gradual reduction of errors (e.g., Gamoh et al., 2001). DHA was not used in our study, due to low amounts in natural sources compared to the amounts of ALA and LA in our chosen foods. However, the only positive effect of n-3 fatty acids was the reduction of errors in the walnut group. Whether these differences of cognitive performances between the walnut and peanut group reflect comparable cognitive brain processes are unclear, because the walnut group did not change in their latency to bait and percentage of movement during the learning phase. However, cognitive enhancement in our study was not caused by the lowest n-6 : n-3 ratio, detected in the chia group. This is in contrast to our prediction

and previous findings, where increased cognitive abilities were related to lower ratios in general, especially to a ratio of 4 : 1 (Yehuda and Carasso, 1993).

During the retention test the experimental groups acted different in relation to cortisol changes. Stressors, or elevated levels of glucocorticoids in general, are known to have negative impacts on the hippocampus (Sapolsky et al., 1990), resulting in impaired cognitive abilities (McEwen and Sapolsky, 1995), which can be reversed by PUFA supplementation (Ferraz et al., 2011). Our results showed that individuals from the control group needed more time to reach the bait and showed a decreased percentage of movement in relation to increasing saliva cortisol concentrations. Machatschke et al. (2004) showed that reduced movement was associated with physiological stress symptoms in guinea pig. However, PUFA fed groups were able to perform better in this test compared with the control group by an expression of elevated cortisol titers. According to this, our results suggest a diminishing effect for cortisol dependent impairments in cognition.

Moreover, our study documents influences of displayed behavior in the social confrontation test on the performance in the subsequently retention test. Socio-positive interactions influenced the latency to bait negatively whereas agonistic encounters showed positive effects in the control group. With regard to these results inconsistent findings among PUFA treated groups were found, namely, no influence of socio-positive and agonistic behaviors in the peanut group but slight positive and negative influences for these behavioral categories were described for the remaining groups. These results are partially consistent with the arguments of van Praag et al. (2000) about the positive effects of social environments on cognition, but are also in line with former results on guinea pig cognition where the social environment can harm cognition (Machatschke et al., 2011).

The change in behavioral interactions during the social confrontation test revealed subtle differences between the groups, which were not necessarily corroborated with PUFA treatments. The most obvious difference was shown for the sexual behavior: it stayed constant for the peanut group for the whole social confrontation test but decreased in all other groups during the time course. This effect may be due to an elevated n-6 : n-3 ratio in the peanut group, that could affect the serotonergic system in the brain, which influences sexual behavior (Hull et al., 2004). This ratio could ultimately cause a down regulation in sexuality. No differences between the groups were detected for agonistic interactions. Again, several studies

documented positive effects of PUFAs on the serotonergic system, resulting in lowered aggression (De Vriese et al., 2004; Haider et al., 2011). However, with respect to agonistic interactions our control group did not differ to the PUFA fed groups. So our results do not provide such conclusions.

The first day of confrontation seemed to be important, because groups were different affected in cortisol changes by the number of sexual interactions. Animals of the control group exhibited pronounced increases in cortisol concentrations in relation to sexual interactions. In the peanut group initial low sexual interactions were related to increased cortisol concentration whereas increased sexuality led to decreased saliva cortisol excretions (see Fig. 3 C). These results suggest that n-6 fatty acids in the context with sexual behavior can have modulating effects on the HPA-axis.

In summary some results of this study do not correspond with previous findings on rodents. The highest controversy is represented by the high n-6 : n-3 ratio of approximately 12 : 1 of the peanut and 10 : 1 of the walnut group and their effects on increased cognitive abilities, whereas the lower n-6 : n-3 ratio of approximately 6 : 1 of the chia group showed no influence on cognition. Nevertheless, positive effects of PUFAs in relation to saliva cortisol excretion rates were found. However; PUFAs had marginal impact on social interactions. In conclusion the authors suggest that different brain metabolism in relation to the unsolved and complex blood brain barrier crossing mechanism for PUFAs (Hamilton and Brunaldi, 2007) could be responsible for these deviating results in guinea pigs.

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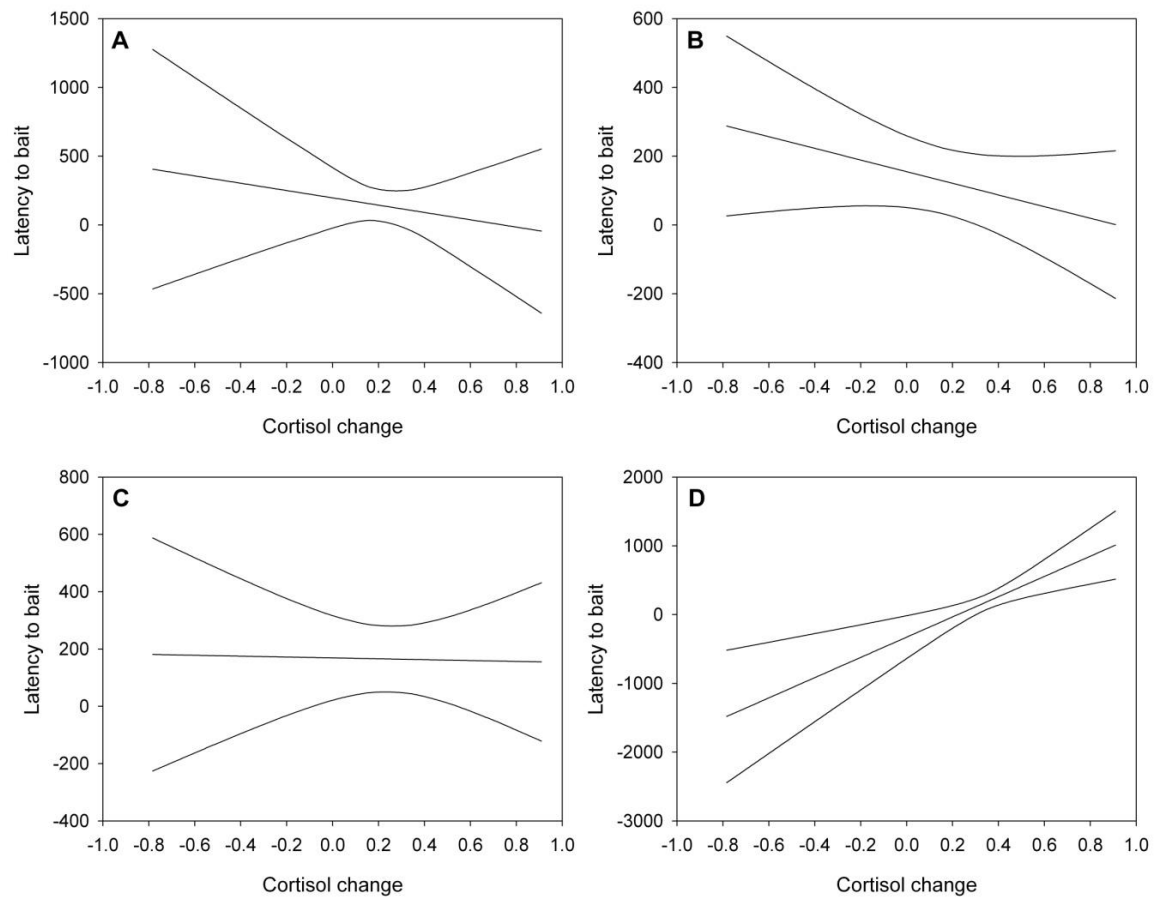
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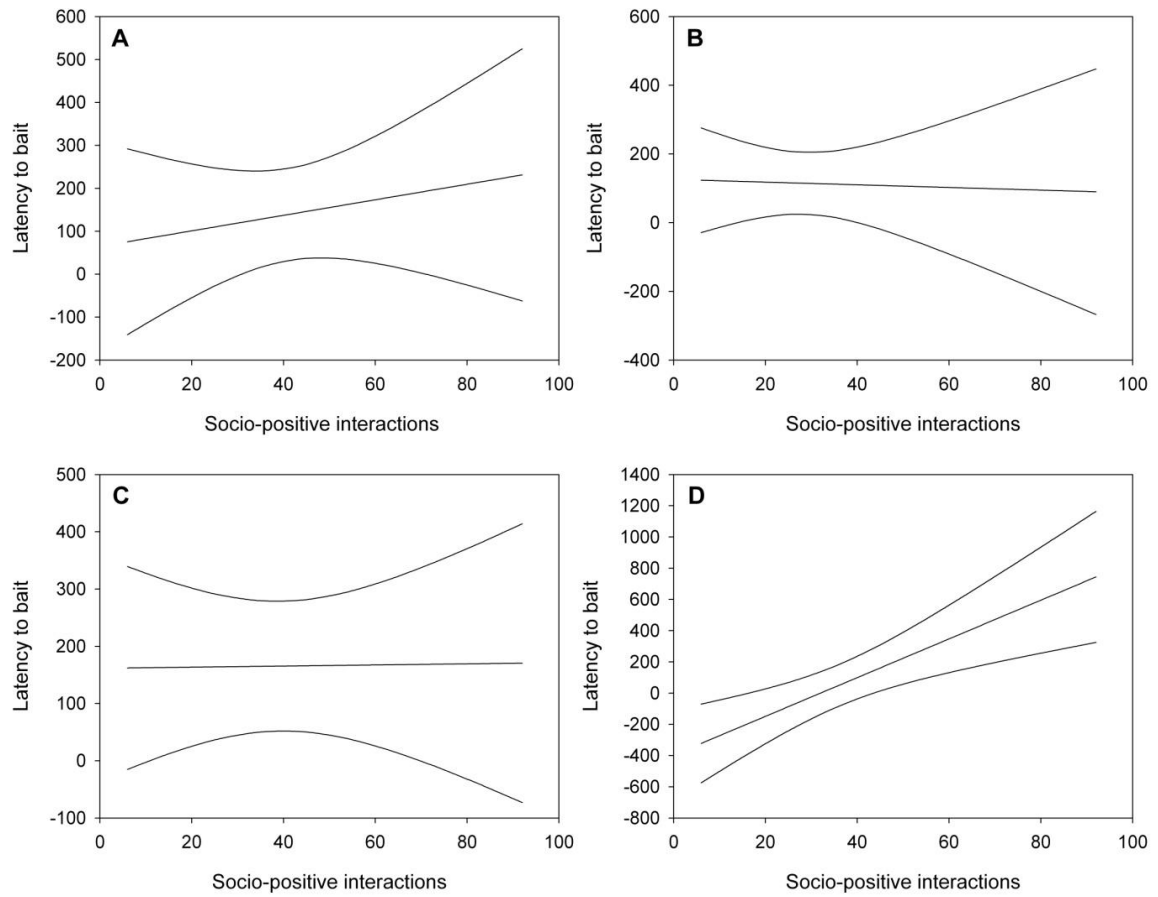
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Appendix

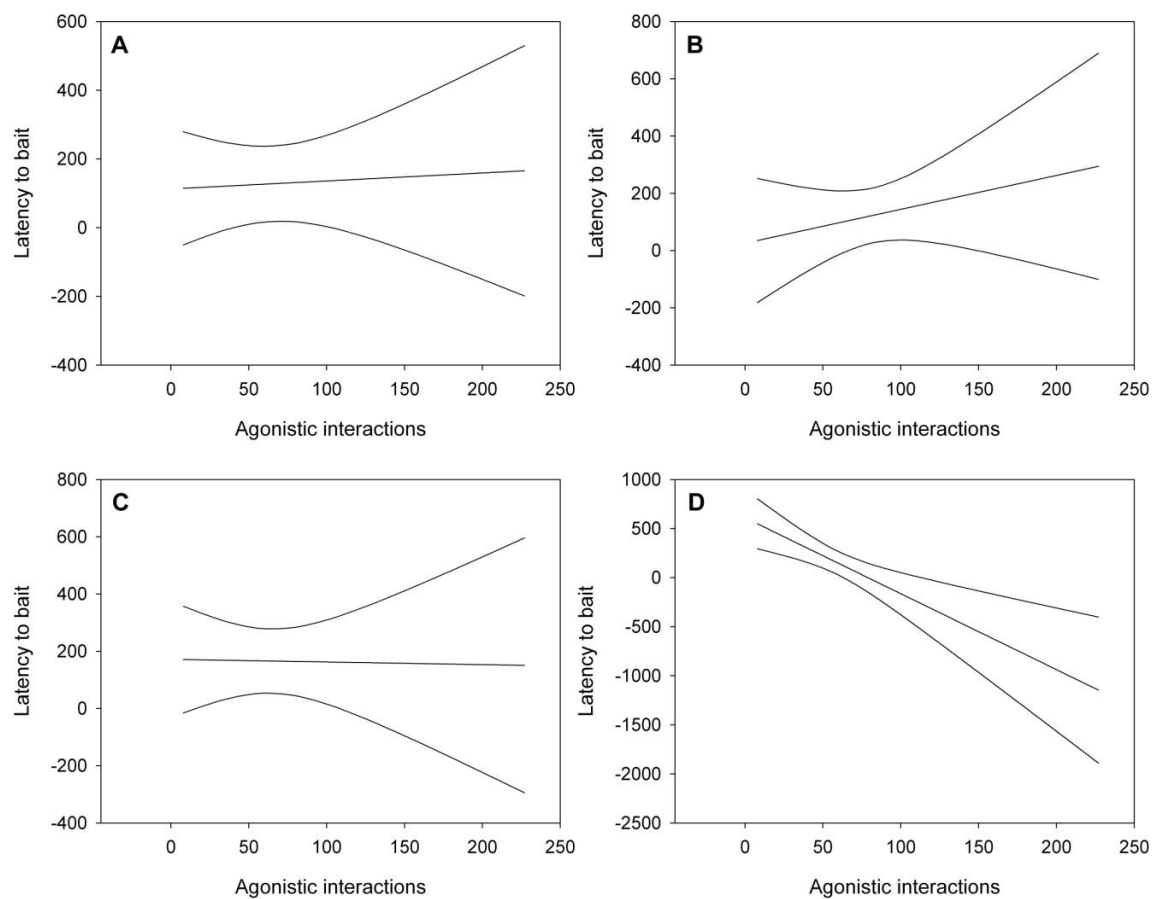
Appendix 1. Effect plots showing the significant interaction between experimental groups and cortisol change in relation to the latency to bait during the retention test. (A) Chia, (B) walnut, (C) peanut, (D) control.



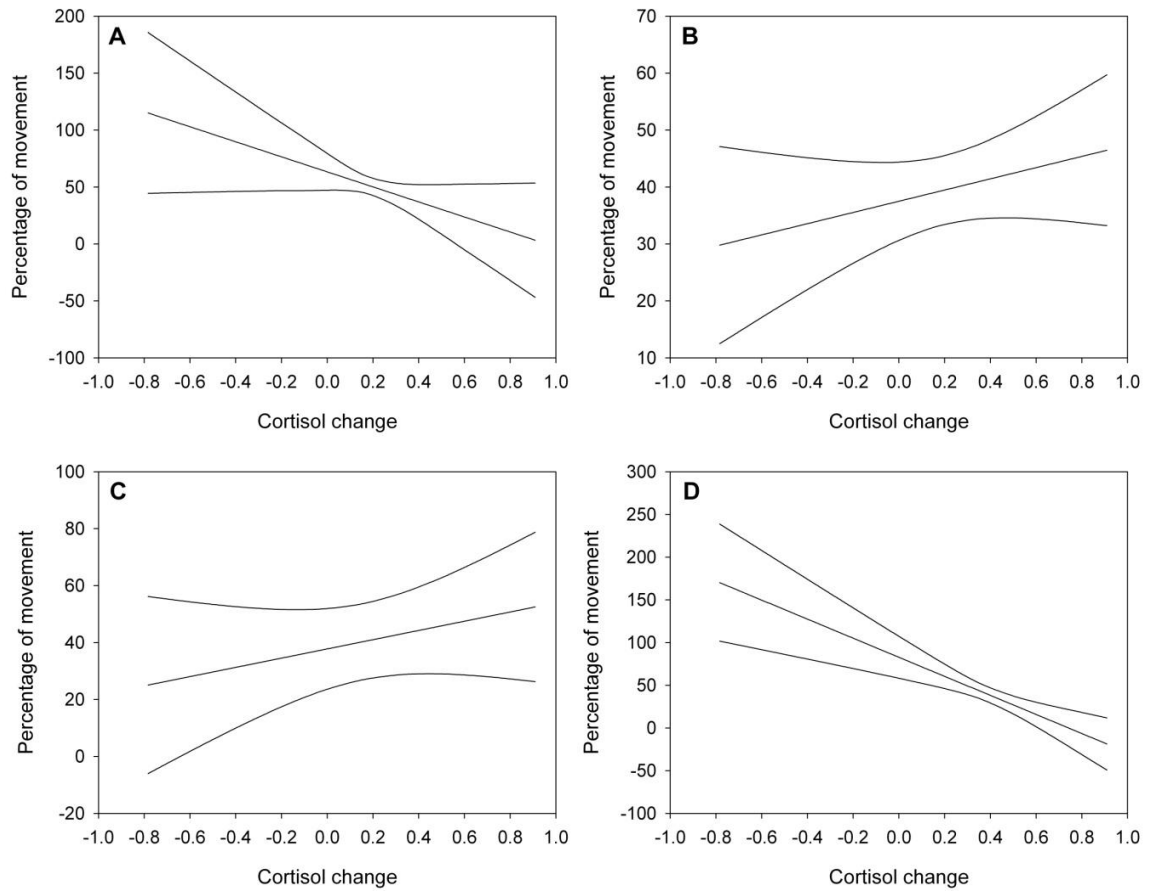
Appendix 2. Effect plots showing the significant interaction between experimental groups and socio-positive interactions in relation to the latency to bait during the retention test. (A) Chia, (B) walnut, (C) peanut, (D) control.



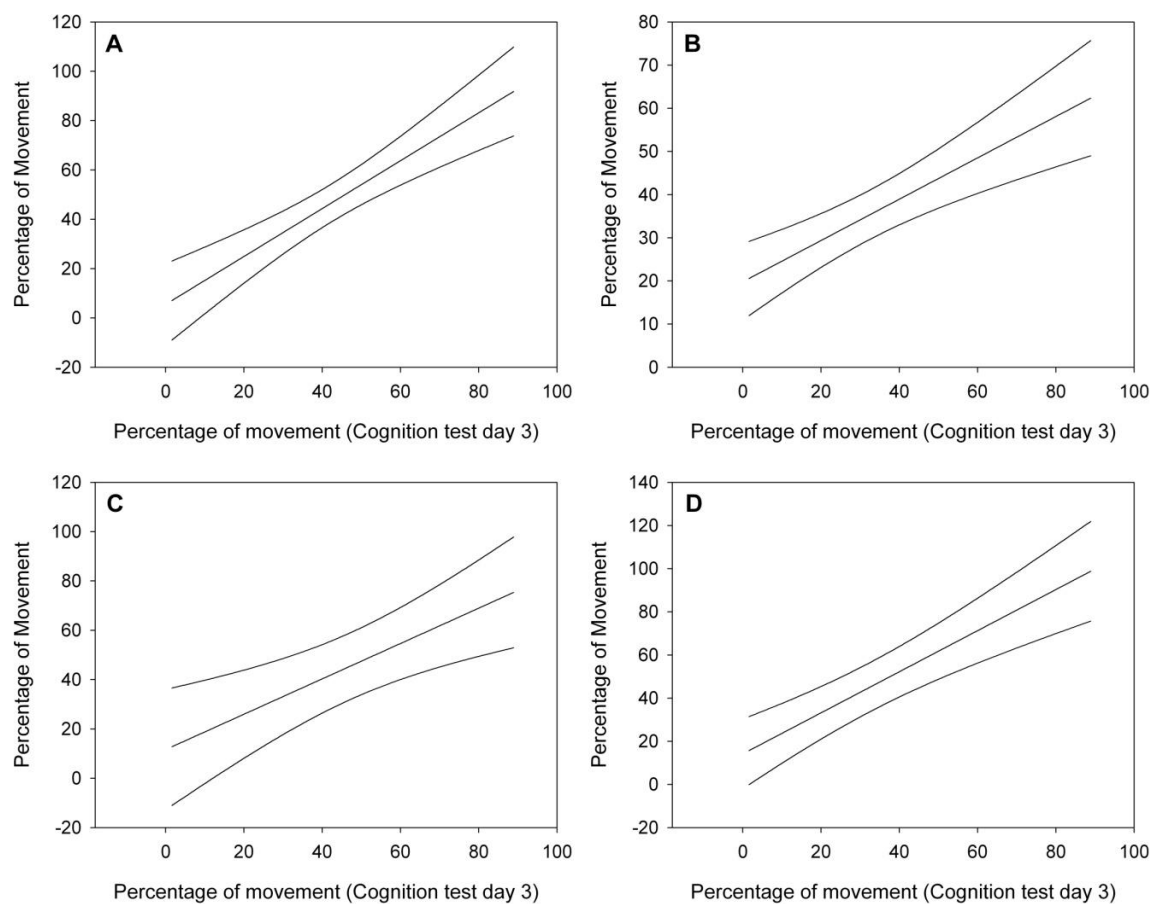
Appendix 3. Effect plots showing the significant interaction between experimental groups and agonistic interactions in relation to the latency to bait during the retention test. (A) Chia, (B) walnut, (C) peanut, (D) control.



Appendix 4. Effect plots showing the significant interaction between experimental groups and cortisol change in relation to the percentage of movement during the retention test. (A) Chia, (B) walnut, (C) peanut, (D) control.



Appendix 5. Effect plots showing the significant interaction between experimental groups and the percentage of movement on the third day of the learning phase in relation to the percentage of movement during the retention test. (A) Chia, (B) walnut, (C) peanut, (D) control.



German abstract / deutsche Zusammenfassung

Mehrfach ungesättigte Fettsäuren (PUFAs) sind essentielle Nährstoffe, die mit der Nahrung aufgenommen werden müssen, und eine wichtige Rolle für die Aufrechterhaltung der Stabilität von neuronalen Zellmembranen spielen. Studien haben gezeigt, dass n-3 und n-6 Fettsäuren modulierend auf kognitive Fähigkeiten, Sozialverhalten und physiologischen Stress wirken können. In dieser Studie wurden die Auswirkungen unterschiedlicher Konzentrationen von n-3 und n-6 Fettsäuren, anhand von mit der Nahrung verabreichten natürlichen Quellen (Chia Samen, Walnüsse, Erdnüsse), auf sozial lebende Meerschweinchen untersucht. Die Einflüsse dieser Nahrungsmittel auf die kognitiven Fähigkeiten wurden mittels eines Labyrinth-Tests überprüft und Auswirkungen auf das Sozialverhalten mittels eines Konfrontationstests. Beide Tests wurden in Abhängigkeit von Cortisol analysiert, ein Marker für physiologischen Stress. Hierfür wurden drei Gruppen, entsprechend den PUFA-Quellen, und eine Kontrollgruppe etabliert (insgesamt 20 Männchen und 20 Weibchen). Während der Lernphase des Kognitionstests konnte eine Reduktion in der Latenzzeit und in der Anzahl der Fehler bei Tieren festgestellt werden, welchen Erdnüsse verabreicht wurden (reich an n-6), und eine sinkende Fehlerrate in der Walnussgruppe (reich an n-6 und n-3). Im nachfolgenden Gedächtnistest zeigte keine der Gruppen eine Veränderung in ihren kognitiven Leistungen, im Vergleich zur Lernphase. Jedoch gab es im Vergleich zur Kontrollgruppe einen negativen Zusammenhang zwischen den Veränderungen der Cortisol Konzentrationen und der Latenzzeit und einen hauptsächlich positiven Zusammenhang zwischen Cortisol und der prozentuellen Dauer der Fortbewegung in den PUFA ergänzten Gruppen. Während des Konfrontationstests zeigten sich keine Auswirkungen der PUFAs auf soziopositives oder agonistisches Verhalten. Am ersten Tag des Tests waren jedoch jene Tiere, denen Erdnüsse verabreicht wurden, im Vergleich zu den anderen Gruppen, seltener in sexuelle Interaktionen verwickelt. Des Weiteren konnten in der Erdnuss- und Chiagruppe verringerte Veränderungen im Cortisol in Relation zu sexuellen Interaktionen festgestellt werden. Zusammenfassend zeigen die vorliegenden Resultate die stärksten positiven Effekte auf kognitive Fähigkeiten und reduzierte Cortisol-Konzentrationen für die Erdnussgruppe. Dies steht im klaren Gegensatz zu früheren Studien an Mäusen und Ratten, welche die erwähnten positiven Effekte auf Verhalten und Physiologie für eine n-3-reiche Ernährung aufzeigten, jedoch nicht für n-6 Fettsäuren. Daraus kann

möglicherweise geschlossen werden, dass sich der Metabolismus von Fettsäuren und deren Aufnahme in das Gehirn zwischen Nagetiergruppen unterscheidet, was in speziesabhängigen Effekten von PUFA-reicher Ernährung auf Kognition, Verhalten und physiologischen Stress resultieren würde.

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Curriculum Vitae

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