universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Effects of polyunsaturated fatty acids on spatial orientation in rats“

verfasst von / submitted by

Thomas Jost, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2020 / Vienna, 2020

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 066 878
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Verhaltens-, Neuro- und Kognitionsbiologie
Betreut von / Supervisor:	ao. Univ.-Prof. Dr. Eva Millesi

Table of Contents

Abstract

Polyunsaturated fatty acids (PUFAs) are essential diet components in mammals and have been shown to improve neuronal function, spatial orientation, and memory. The aim of this study was to investigate the effects of PUFAs on spatial cognition in relation to corticosterone (CORT), as CORT concentrations seem to be negatively linked to spatial orientation performance. It was hypothesized that PUFA-related effects differ between males and females due to sex-specific neurophysiological functions. Male and female rats were maintained on a high PUFA diet (10% walnut oil) or a control diet. In the initial five-week feeding phase, body mass was measured and fecal samples were collected to analyze fecal corticosterone metabolite (FCM) concentrations. In the subsequent T-maze and multiple T-maze experiments, rats were tested for spatial learning abilities and short- and long-term memory. Performance in these experiments was statistically corrected for individual FCM concentrations. PUFA males became heaviest and showed highest FCM concentrations during the initial feeding phase. In the T-maze, PUFAs resulted in a lower predicted number of trials to pass the test when considering individual FCM concentrations. However, PUFA males tended to perform worse than other groups in the multiple T-maze learning phase. Nevertheless, PUFA animals performed better in the multiple T-maze long-term memory test than control groups, although PUFA females showed impaired short-term memory. These results suggest that PUFAs affect learning and memory performance, but CORT concentrations and sex obviously play an important role. This highlights the consideration of physiological conditions with regard to cognitive influences of dietary PUFAs.

1. Introduction

Polyunsaturated fatty acids (PUFAs) are important structural components of neuronal cell membranes and have well-known protective and regulatory effects (Ferreri et al., 2017). Certain PUFAs represent essential nutrients in mammals since they cannot be synthesized *de novo*. Thus, PUFAs need to be consumed with the diet to maintain the neuromembrane PUFA-status and to prevent serious developmental dysfunctions (Brenna, 2011). The most frequent omega-3 (n-3) and omega-6 (n-6) PUFAs in the mammalian brain are eicosapentaenoic acid (EPA, 20:5, n-3), docosahexaenoic acid (DHA, 22:6, n-3) and arachidonic acid (AA, 20:4, n-6) (Sinclair, 1975; Svennerholm, 1968). These fatty acids are mainly synthesized from dietary essential PUFAs alpha-linolenic acid (ALA,18:3, n-3) and linoleic acid (LA, 18:2, n-6)(Sprecher et al., 1995). The PUFA content of the neuronal membrane influences membrane fluidity, physiological functions of the brain, and neurotransmission, which ultimately affects synaptic plasticity and cognitive functions (Fukaya et al., 2007; Mazza et al., 2007; Okaichi et al., 2005). This includes a facilitation of long-term potentiation (LTP, the persistent strengthening of synapses based on recent patterns of activity) and memory formation (Das, 2003).

DHA is the most important n-3 PUFA in neuronal membranes with regard to cognitive performance. It has been shown that brain levels of DHA are positively related to performance in spatial tasks in rats (Moriguchi & Salem Jr, 2003). For example, a deficiency of brain DHA is associated with reduced learning abilities and poor spatial reference memory performance (Fedorova et al., 2009; Lim et al., 2005). Recovery from brain DHA deficiency, however, significantly improves spatial reference memory and working memory performance (Chung et al., 2008). This might be linked to a faster development of neurons in the brain, which has been shown in response to n-3 PUFA supplementation in rats (Hajjar et al., 2013). The effects of n-6 fatty acids on cognitive functions is less studied to date. N-6 fatty acids seem to have opposite effects, namely impaired learning abilities associated with higher n-6 PUFA concentrations in the brain (Ikemoto et al., 2001). However, this highlights the importance of the dietary n-6:n-3 fatty acid ratio with regard to brain and cognitive functions (Yehuda, 2003). For example, decreasing the dietary n-6:n-3 ratio from 65:1 to 4.5:1 is associated with an increase in the number and size of neurons, resulting in an increased expression of pre-synaptic proteins such as synaptophysin in the CA1 hippocampal subregion in the rat brain (Hajjar et al., 2013). The hippocampus (with its subregions CA1, CA2 and CA3) is the main brain region associated with spatial information and memory (Kesner et al., 2004; Martin &

Clark, 2007). Fatty acid composition in this area is correlated with cognitive and behavioral changes in rats (Kelly et al., 2011; de Souza et al., 2012).

The importance of the hippocampus with regard to cognitive functions is also underlined by a high density of glucocorticoid receptors in this brain region. Hippocampus-dependent cognitive functions are strongly affected by physiological stress responses and the related increase in glucocorticoid concentrations, like corticosterone (McEwen, 2007). Corticosterone (CORT) is a steroid hormone produced in the cortex of the adrenal glands as a result of the hypothalamic-pituitary-adrenal (HPA)-axis activity. It is an important stress mediator which allows an organism to cope with prevalent stressors and restores body homeostasis (McEwen, 2007). Long term PUFA supplementation leads to a reduction in stress-related CORT increase and cognitive impairment (Ferraz et al., 2011). N-3 fatty acid deficiency has been shown to increase the vulnerability to stress in several animal species, which also impaired their learning ability under stressful conditions (Lim et al., 2005).

Furthermore, the HPA-axis and related CORT concentrations play an important role with regard to sex-specific differences in cognition. CORT concentrations seem to impact female cognition more than male, because male rats outperform females in cognitive tasks under acute stress situations (Harris et al., 2008). This may ultimately contribute to different effects of PUFAs on cognitive functions in male and female rodents, as demonstrated in guinea pigs (Nemeth et al., 2015). However, sex-specific effects of PUFAs on cognitive performance remain understudied, especially in relation to CORT secretion.

Considering sex-specific effects regarding a potential interplay between PUFAs and CORT concentrations on cognitive performance may help to better understand the physiological role of these nutrients. Therefore, the aim of this study was to test the effects of PUFAs on cognitive performance in spatial tasks with respect to sex and CORT concentrations in rats (*Rattus norvegicus*). We used walnut oil as a PUFA source, because of its n-6 to n-3 ratio of 4:1 – 5:1, which has been shown to be most beneficial in rats (Yehuda, 2003). We measured changes in CORT concentrations by analyzing fecal corticosterone metabolites (FCM) and body mass over time. The analysis of CORT through FCM concentrations is non-invasive as it involves no handling stress in contrast to measuring blood or saliva CORT and has been validated in rats (Lepschy et al., 2007). Moreover, we were interested in long-term HPA-axis activity and its effects on cognition, hence, FCM measurements were of adequate use here,

because they accumulate over several hours in the feces independent of acute stress responses. To determine effects on cognitive abilities, two different tests were performed: a T-maze task to test immediate learning performance and a more complex multiple T-maze task to test for learning as well as short- and long-term memory.

2. Methods

2.1. **Animals and housing conditions**

A total number of 28 rats of the species *Rattus norvegicus* from 5 different litters with a mean age of 6 ± 2 months were used in this experiment. All animals were adult and therefore at the same developmental level. The rats were born and reared in the Department of Behavioral and Cognitive Biology at the University of Vienna and accustomed to daily contact with humans. Eight weeks prior to the start of the experiments, rats were randomly allocated to same-sex groups (PUFA females n = 7, PUFA males n = 6, control females n = 8, control males n = 7) to ensure the manifestation of hierarchies and general familiarity between animals. The groups were kept in 2-level cages (75 x 80 x 160 cm) with standard woodchip bedding material covering the floors. The cages were also environmentally enriched with shelters and platforms and *ad libitum* water access. A light-dark cycle of 12 hours per phase (lights on at 0700 hours) and a temperature of 22 ± 2 °C were maintained throughout the study. All cages were located in the same room. All experimental groups exclusively received standard pellet food for rodents (deukanin standard, Deutsche Tiernahrung Cremer GmbH & Co. KG, Düsseldorf, Germany; 950 kJ/100 g). The amount of pellet food for each group per day was calculated with 35 g per male and 25 g per female. The animals were fed between 10 a.m. and 12 p.m. each day. The food was provided in two bowls per cage (one bowl/floor) to ensure that each animal had access to the food.

2.2. **Experimental diets**

With the start of the experiment, the pellet food for PUFA animals was enriched with 10 % (w/w) walnut oil (Makana Produktion und Vertrieb GmbH, Offenbach a. d. Queich, Germany; per 100 g: 99.6 g total fat, 73.9 g total PUFA, 15.8 g total MUFA, 9.9 g total SFA, 3615 kJ). Control animals continued to receive the pure pellet food. Due to the higher caloric density of

walnut oil, the food per animal in the PUFA groups was adjusted (27.3 g/male, 19.5 g/female), resulting in isocaloric amounts in male and female groups, respectively. Both control and PUFA pellets were well accepted by all animals and rejections were never observed. The food was usually completely consumed by early evening.

2.3. **Experimental regime**

The experimental regime consisted of 4 phases: the initial feeding phase, the T-maze experiment, the multiple T-maze experiment with learning and memory phase. The experimental timeline is outlined in Fig. 1.

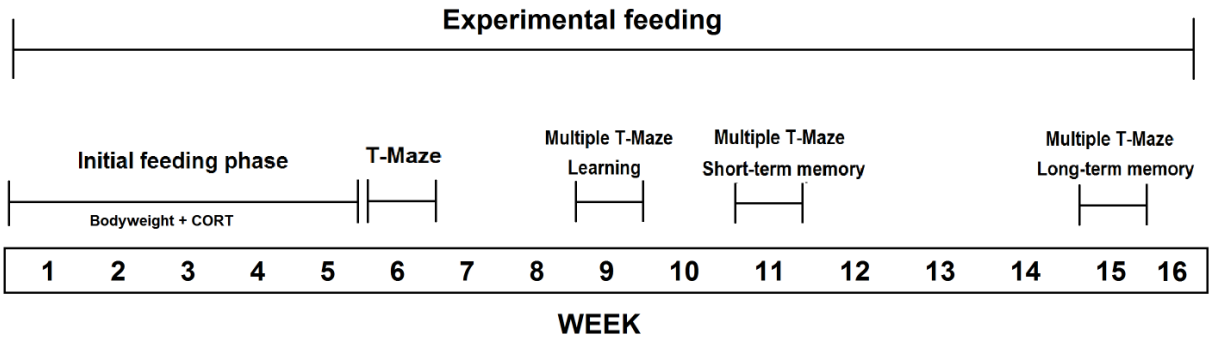

Fig. 1. Experimental timeline for the whole experimental procedure.

2.4. **Initial feeding phase**

In the initial feeding phase, which lasted five weeks, the group-specific feeding regime started. Body mass was recorded and fecal samples for FCM analysis were collected in all animals in weekly intervals (Mondays and Tuesdays 09.00 a.m. - 12.00 a.m.). Body mass was measured with an electronic scale. For fecal sample collection, all animals of a group were temporarily transferred to single cages to ensure individual sample collection. The floor in these cages was covered with standard woodchip bedding material to prevent urine contamination of fecal samples. The animals were moved to the single cages in a transport box to which they had been habituated prior to the experiment onset. To minimize handling stress, this procedure was also applied in both maze experiments. CORT metabolites accumulate over several hours in the feces and the hormonal response to acute stress can be detected after about 14.8 ± 2.4

h in the feces in rats (Lepschy et al., 2007). Therefore, the fecal samples were always collected in the morning between 08:00 a.m. and 11:00 a.m. in order to eliminate diurnal fluctuations and obtain valid FCM values for individual rats. The whole procedure lasted no longer than one hour per animal. Thereafter, the animals were returned to their respective group and received their food.

2.5. **T-Maze Experiment**

In week six after the start of the experiment, the T-maze test was carried out. The T-maze (Fig. 2) was built of acrylic glass covered with black film to ensure that the rats were not able to see through the walls. Each arm of the T-maze measured 50 x 15 x 40 cm (length x width x height). The floor was covered with standard woodchip bedding material. At the end of the left and the right arm a dish was placed, but only the dish in the left arm contained a food reward. It was also ensured that the animals inside the T-maze did not have any external cues they could use for orientation. After each trial the T-maze was cleaned to prevent the presence of possible orientation cues (i.e. scent marks) from previous trials. The test was performed in a separate test room.

A rat was placed in the start arm and had to make one left/right decision when approaching the intersection. As soon as the animal reached the dish in either the left or the right arm, this was immediately recorded as correct (left dish) or wrong (right dish) decision. The animal was then temporarily removed from the maze and kept in a small box to allow cleaning of the maze (walls, floor, dishes). A new food reward was placed in the left arm and the animal was returned to the start arm for the next trial. The maximum number of trials per animal was 40 and the rats had to make 8 correct decisions out of 10 trials to be successful. The number of trials was documented during testing. In addition, all trials were videotaped from the bird's eye view with a GoPro HERO6 camera to later analyze the time needed for each trial. Experiments were performed between 8:30 a.m. and 12:30 p.m.

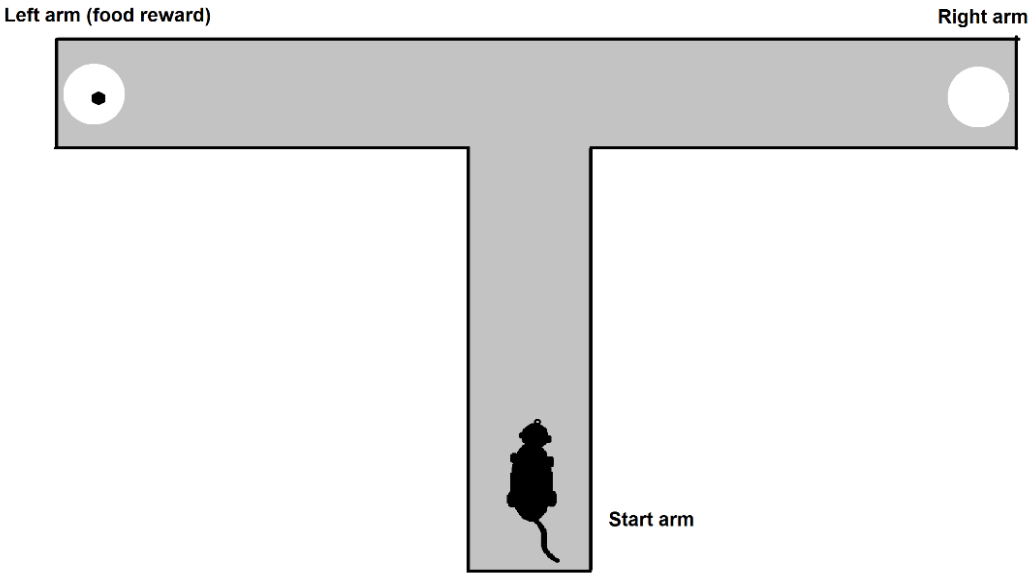

Fig. 2. Schematic illustration of the T-maze setup.

2.6. **Multiple T-Maze Experiment**

The multiple T-maze experiment started two weeks after the T-maze experiment. The multiple T-maze consisted of 3 T-maze parts connected in a way that the rats had to make three left/right decisions (correct: left-right-left) to get to the goal (food reward) (Fig. 3). At the intersections of the maze the rats were not able to see which arm was correct or wrong, because each wrong arm ended in a dead branch which the rats had to visit first before realizing that this arm was wrong. The walls and the floor were made of black coated wooden boards. Each arm of the multiple T-maze measured 50 x 15 x 30 cm (length x width x height). The dead branches measured 15 cm in length. Between each trial the maze was cleaned to prevent the presence of possible orientation cues (i.e. scent marks). Like in the T-maze experiment, it was also ensured that the animals inside the multiple T-maze did not have any external cues they could use for orientation.

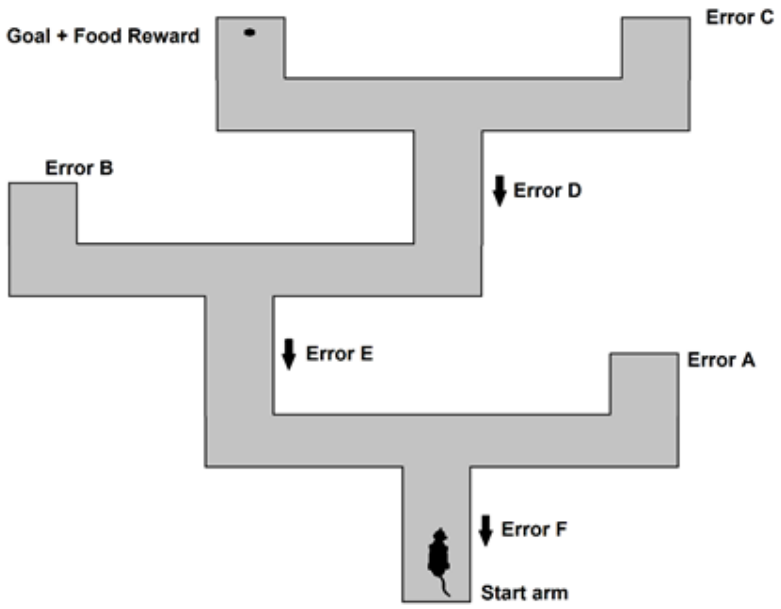

Fig. 3. Schematic illustration of the multiple T-maze setup. Errors were categorized into forward (Errors A, B, C) and backward (Errors D, E, F) errors.

In the multiple T-maze, the rats had a time limit of 3 minutes to reach the goal. If they did not reach the goal within the time limit, the trial was unsuccessful. Entering a wrong arm was counted as an error. All errors were categorized into forward (Errors A, B, C) and backward (Errors D, E, F) errors. Each animal was tested once a day for six consecutive days in the learning phase. For short-term memory, the animals were tested in a single trial one week after the learning phase; for long-term memory, a single trial was carried out four weeks after the short-term memory test. In addition, all trials were videotaped from the bird's eye view with a GoPro HERO6 camera to later analyze the time needed for each trial and detailed error analysis. Experiments were performed between 8:30 a.m. and 12:30 p.m.

2.7. **Video Analysis**

Videos recorded during the experiments were analyzed using the Solomon Coder(version beta 19.08.02) (Péter, 2011). For the T-maze experiment, the time each animal spent in the maze per trial was analyzed. In the multiple T-maze task, the time each animal spent in the maze per trial and the total number of errors made per trial were analyzed. For a detailed error analysis, single errors (A, B, C, D, E, F) were considered to analyze the total number of single errors (number of Error A, number of Error B, etc.), the number of forward errors (A+B+C), the

number of backward errors (D+E+F), and the number of errors per T-maze part (Errors A+F, Errors B+E, Errors C+D).

2.8. **FCM Analysis**

Analysis of FCM in a total of 177 fecal samples was performed by enzyme immunoassay (EIA) using an antibody against 5α-pregnane-3β,11β,21-triol-20-one (Lepschy et al., 2007). First, the samples were air dried at 75 °C in a drying chamber for 24 hours. Then the samples were homogenized, weighed (0.02 – 0.1 g) into test tubes, mixed with methanol (80 %), and vortexed for 30 minutes. After centrifugation (2500 rpm, 10 min.) the samples were diluted 1:200 and analyzed in 10µl duplicates. Coefficient of variation for intra- and interassay were 12.99 % and 1.61 %, respectively.

2.9. **Statistical Analysis**

Statistical analysis was done using the statistical software package R version 3.6.1 (R Core Team, 2019). Changes in body mass and FCM concentrations were analyzed using linear mixed effect (LME) models using library “nlme” (Pinheiro et al., 2017). Diet (experimental groups), sex, and week, as well as their interactions, were included as fixed effects, individual ID was included as random effect to correct for repeated measurements.

Analysis of the T-maze test (number of trials, mean time) was done using linear models with experimental groups and sex and their interaction as predictors. The number of trials was additionally corrected for FCM concentrations measured in week five.

Performance in the multiple T-maze was analyzed using LMEs. The time needed to finish the maze (only successful individuals) and number of errors were the response variables. Group, sex, day, as well as their interactions, were used as predictors, individual ID was included as random effect. To determine if multiple T-maze performance was affected by FCM concentrations or related to T-maze performance (number of trials), both parameters were

included as covariates in the analysis of conducted errors on the last day of the learning phase but showed no effects at all.

The detailed error analysis of the multiple T-maze was carried out by performing LMEs with the same fixed and random effects for total number of single errors, forward and backward errors, and the number of errors per T-maze part.

Short- and long-term memory performance was analyzed in comparison to the performance at the end of the learning phase (day 6). These LMEs included experimental groups, sex, day (day 6 of the learning phase, day of short-term memory, day of long-term memory) as well as their interactions as predictors.

The Akaike information criterion (AIC) was used for model reduction (stepwise removal of non-significant interactions and main effects). Post hoc interaction analyses with Bonferroni corrections were applied on significant effects using library "phia" (De Rosario-Martinez, 2015). Model assumptions were checked by performing Shapiro-Wilk normality tests and Levene's test for homogeneity of variance as well as by plotting model residuals and fitted values. Model statistics are based on type 3 sum of squares. The level of significance was set at $p \leq 0.05$. Results are presented as mean ± standard error.

3. Results

3.1. **Body mass**

Body mass analyses revealed a significant three-way interaction of diet, sex and week during the initial feeding phase (Fig. 4, Table 1). In general, males were heavier than females (p < 0.001 in all cases). Body mass changes across the five weeks differed between PUFA and control females (χ^2 = 49.372, p < 0.001) as well as between PUFA and control males (χ^2 = 226.958, p < 0.001), because PUFA animals showed an initial decline in body mass followed by an increase towards the end of the initial feeding phase. Comparing body mass changes across the five weeks in males and females, control animals showed no differences (χ^2 = 11.206, p = 0.095), whereas in PUFA animals only males showed an increase in body mass during this phase due to a stronger pronounced U-shaped pattern compared to females (χ^2 = 101.692, p < 0.001). When comparing body mass per week, a difference was found between PUFA and control males only in week five (χ^2 = 20.101, p < 0.001) with PUFA males being heavier than the control males. Between PUFA females and control females no significant differences in body mass were found during any of the five weeks (p > 0.05 in all cases).

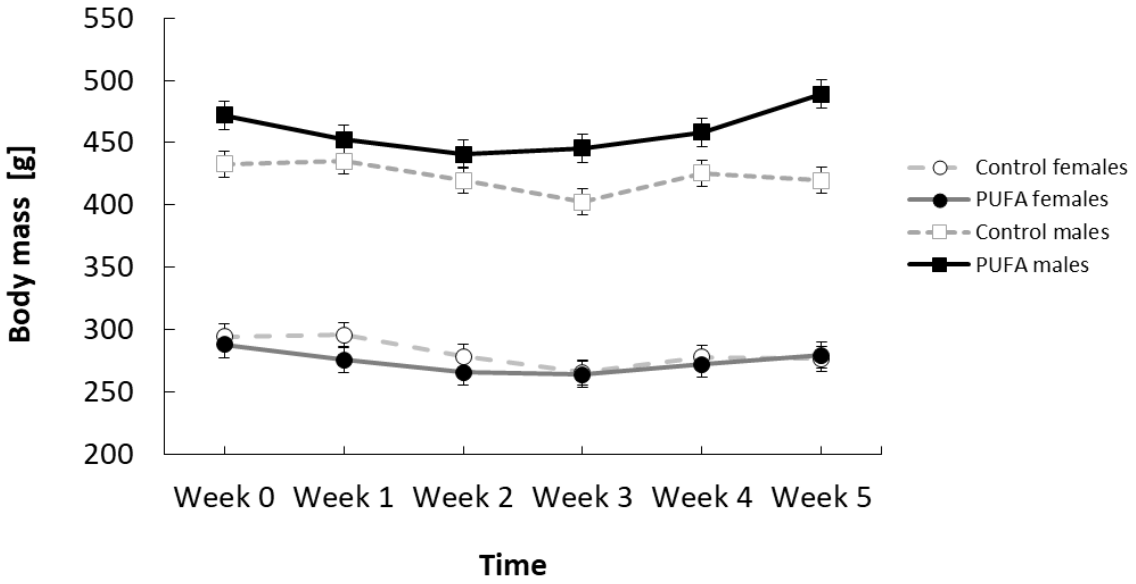

Fig. 4. Body mass changes across the initial feeding phase. Interaction of Diet:Sex:Week p < 0.001 (for details see text and Table 1).

Table 1. ANOVA (Type 3) results of full and fitted linear mixed model (LME) analysis of body mass change and FCM concentration change over the five-week feeding phase.

Response	Full model Predictor	Statistics			Fitted model Predictor	Statistics		
		df	**F-value**	**p-value**		**Df**	**F-value**	**p-value**
Body mass	Diet	1,24	0.161	0.692	Diet	1,24	0.161	0.692
	Sex	**1,24**	**78.864**	**< 0.001**	**Sex**	**1,24**	**78.864**	**< 0.001**
	Week	**5,120**	**36.546**	**< 0.001**	**Week**	**5,120**	**36.546**	**< 0.001**
	Diet:Sex	1,24	3.928	0.059	Diet:Sex	1,24	3.928	0.059
	Diet:Week	**5,120**	**8.464**	**< 0.001**	**Diet:Week**	**5,120**	**8.464**	**< 0.001**
	Sex:Week	5,120	1.921	0.096	Sex:Week	5,120	1.921	0.096
	Diet:Sex:Week	**5,120**	**8.232**	**< 0.001**	**Diet:Sex:Week**	**5,120**	**8.232**	**< 0.001**
FCM	**Diet**	**1,24**	**4.898**	**0.037**	**Diet**	**1,24**	**10.879**	**0.003**
	Sex	1,24	3.077	0.092	Sex	1,24	2.894	0.102
	Week	5,112	1.480	0.202	**Week**	**5,117**	**3.013**	**0.014**
	Diet:Sex	1,24	0.065	0.802	Diet:Sex	1,24	2.342	0.139
	Diet:Week	**5,112**	**4.359**	**0.001**	**Diet:Week**	**5,117**	**9.507**	**< 0.001**
	Sex:Week	5,112	1.621	0.16	**Sex:Week**	**5,117**	**4.193**	**0.002**
	Diet:Sex:Week	5,112	0.670	0.647	Diet:Sex:Week	n.a.	n.a.	n.a.

n.a.: not available due to model reduction based on the AIC.

3.2. **Fecal corticosterone metabolites**

For changes in fecal corticosterone metabolite concentrations (FCM concentrations) during the initial feeding phase, significant interactions between diet and week as well as sex and week were found (Table 1, Fig. 5). Comparing PUFA and control animals, significant differences in FCM concentrations were found at the onset of the feeding phase (week 0) as well as in week 2 and week 5 with PUFA animals initially (week 0) showing lower (χ^2 = 9.505, p = 0.012) and later (weeks 2 and 5) higher FCM concentrations (week 2: χ^2 = 27.966, p < 0.001; week 5: χ^2 = 15.831, p < 0.001) than control animals. No differences were found in week 1 and week 3 (p > 0.05 in both cases). In general, males showed higher FCM concentrations than females (p < 0.05 in all cases), except for week two (χ^2 = 0.288, p = 1.000).

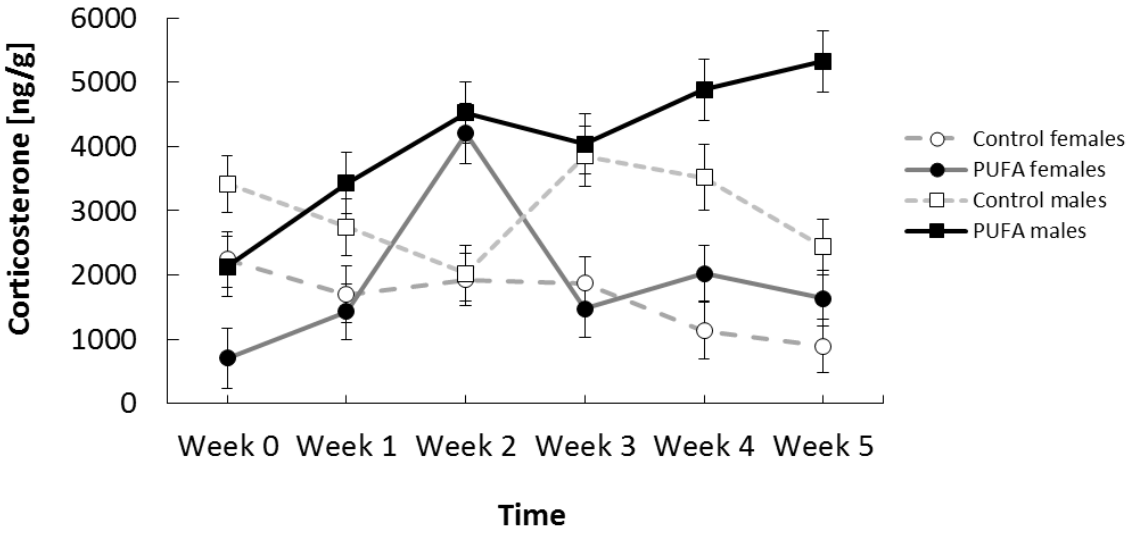

Fig. 5. FCM concentrations across the initial feeding phase. Interactions of Diet:Week $p < 0.001$; Sex:Week $p = 0.002$ (for details see text and Table 1).

3.3. **T-Maze Experiment**

The number of trials needed to complete the T-maze task did not differ between the four groups (Fig. 6, Table 2).

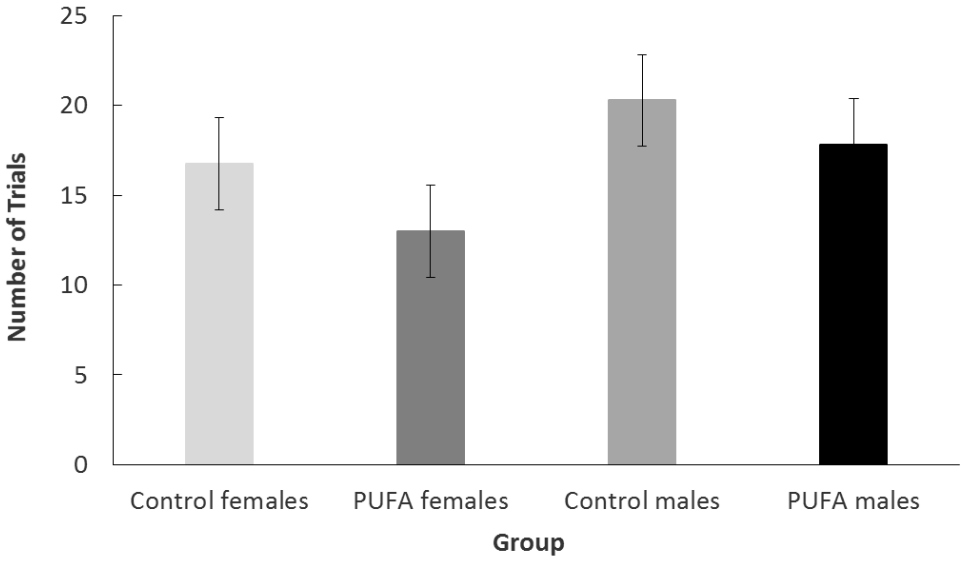

Fig. 6. Number of trials needed to complete the T-maze task. No effects of diet or sex were found ($p > 0.05$; for statistical details see Table 2)

When T-maze performance was adjusted for FCM concentrations of week 5 (i.e. the last day of the initial feeding phase), the fitted model revealed significant effects of diet and CORT concentrations on the number of trials (Table 2). Animals with higher FCM levels showed an increased number of trials to complete the T-maze task compared to animals with lower FCM levels (Fig. 7a). The number of trials was lower in PUFA compared to control animals (Fig. 7b).

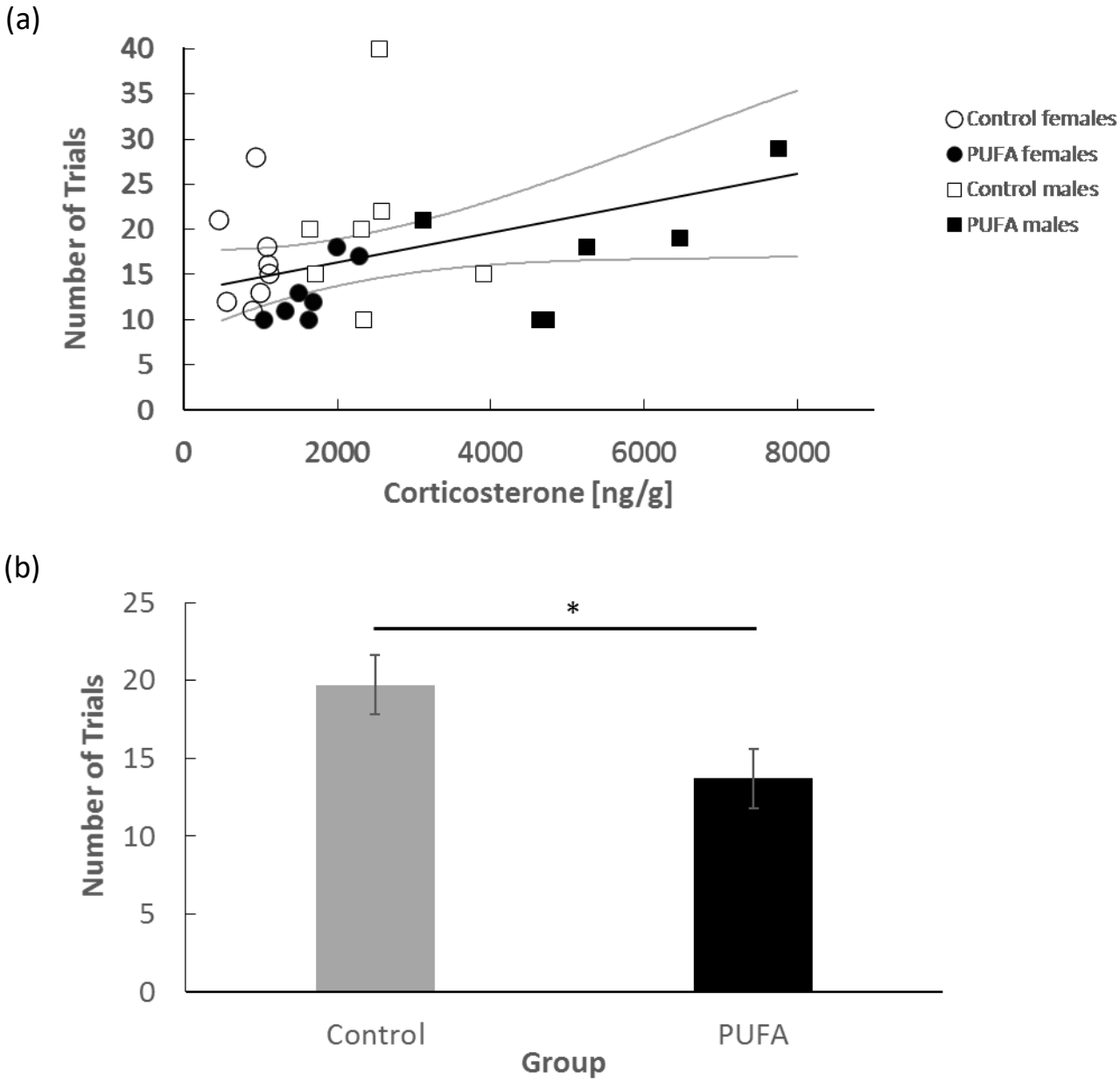

Fig. 7. Performance in T-maze linked to FCM concentrations. (a) Effects of FCM concentrations of week 5 on the number of trials needed to complete the T-maze task (p = 0.042). (b) Number of trials needed to complete the T-maze task in control and PUFA individuals when corrected for individual FCM concentrations (p = 0.04).

Table 2. ANOVA results of full and fitted linear model analysis of T-maze trials (A) and T-maze trials corrected for FCM concentrations (B).

	Full model				**Fitted model**			
	Predictor	**Statistics**			**Predictor**	**Statistics**		
		df	**F-value**	**p-value**		**df**	**F-value**	**p-value**
(A)	Diet	1,24	1.150	0.294	Diet	n.a.	n.a.	n.a.
	Sex	1,24	1.022	0.322	Sex	1,26	2.676	0.114
	Diet:Sex	1,24	0.064	0.803	Diet:Sex	n.a.	n.a.	n.a.
(B)	Diet	1,20	1.022	0.324	**Diet**	**1,25**	**4.702**	**0.040**
	Sex	1,20	0.051	0.824	Sex	n.a.	n.a.	n.a.
	FCM_D6	1,20	0.005	0.943	**FCM_D6**	**1,25**	**4.600**	**0.042**
	Diet:Sex	1,20	0.000	0.985	Diet:Sex	n.a.	n.a.	n.a.
	Diet: FCM_D6	1,20	0.349	0.561	Diet: FCM_D6	n.a.	n.a.	n.a.
	Sex: FCM_D6	1,20	0.004	0.953	Sex: FCM_D6	n.a.	n.a.	n.a.
	Diet:Sex: FCM _D6	1,20	0.135	0.717	Diet:Sex:FCM _D6	n.a.	n.a.	n.a.

n.a.: not available due to model reduction based on the AIC.

3.4. **Multiple T-Maze Experiment – Learning Phase**

All groups except the PUFA males reached a 100 % success rate by day six of the learning phase (Fig. 8). PUFA males started with a success rate of 100 % but decreased over the learning phase to 66.7 % at the end as some individuals did not finish in time. In general, each animal succeeded at least twice out of 6 times in the learning phase, the overall mean success rate was 5 out of 6 times.

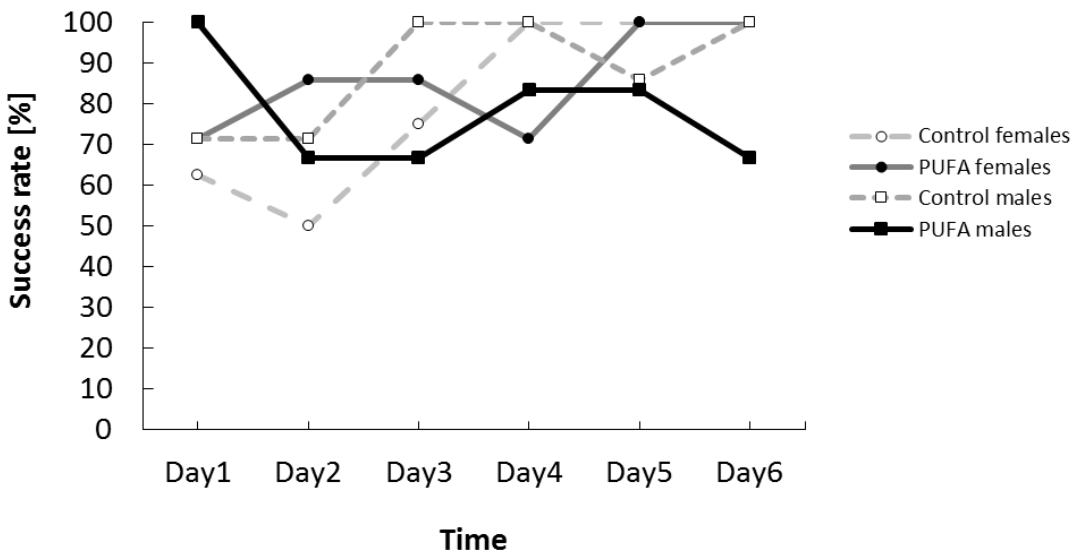

Fig. 8. Success rate (percentage of animals per group) in the multiple T-maze experiment during the six-day learning phase.

During the learning phase of the multiple T-maze, the time for completing the task decreased in all groups (Fig. 9, Table 3). PUFA animals tended to need more time to finish the task (Table 3).

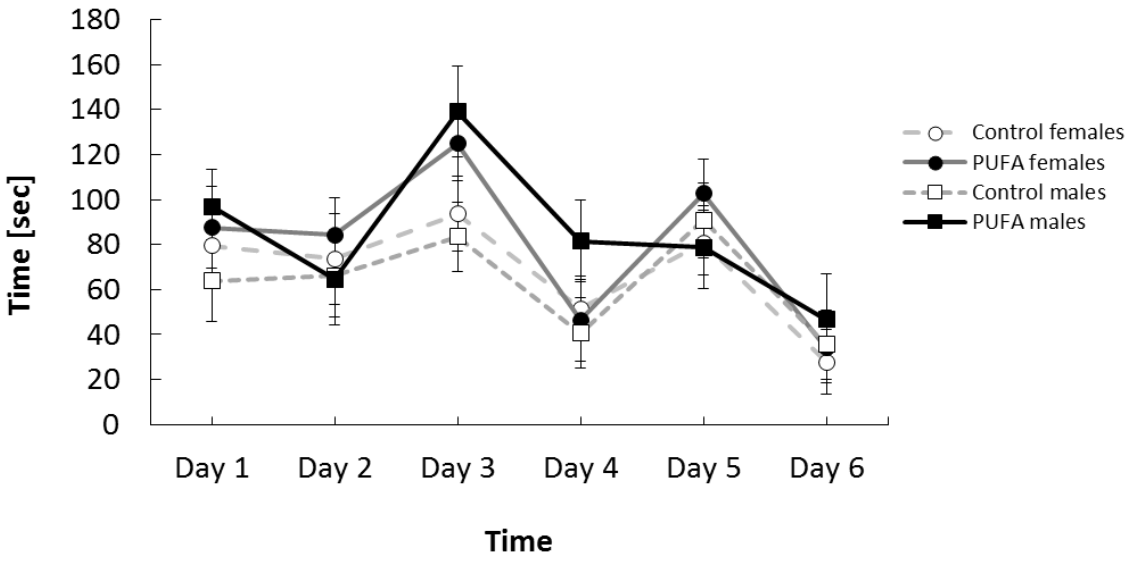

Fig. 9. Time needed to complete the multiple T-maze task during the learning phase (for statistical details see text and Table 3).

The number of errors made in the multiple T-maze decreased during the learning phase. However, the number of errors tended to be higher in PUFA animals (Fig. 10, Table 3). When only comparing individuals that finished the multiple T-maze in time, the effect of day remained significant ($F_{5,107} = 7.988$, $p < 0.001$), but PUFA animals now made significantly more errors than control animals ($F_{1.26} = 4.726$, $p < 0.039$). On day six, the end of the learning phase, a significant interaction of diet and sex was detected ($F_{1,24} = 4.412$, $p = 0.046$), which was caused by a higher number of errors in PUFA males compared to the other groups.

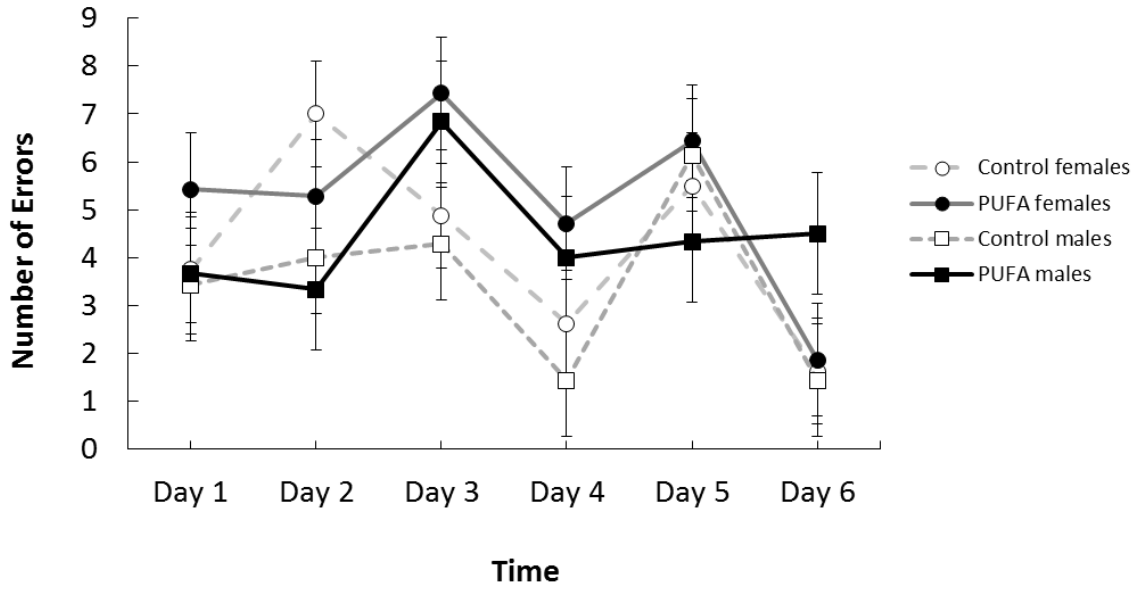

Fig. 10. Number of errors (including unsuccessful individuals) in the multiple T-maze task during the learning phase (for statistical details see text and Table 3).

Table 3. ANOVA (Type 3) results of full and fitted linear mixed model (LME) analysis for time needed and errors made in the multiple T-maze during the learning phase.

	Full model				**Fitted model**			
Response	**Predictor**	**Statistics**			**Predictor**	**Statistics**		
		df	**F-value**	**p-value**		**df**	**F-value**	**p-value**
MTM Time	Diet	1,24	0.082	0.777	Diet	1,26	4.095	0.053
	Sex	1,24	0.311	0.583	Sex	n.a.	n.a.	n.a.
	Day	5,92	2.298	0.051	**Day**	**5,107**	**9.277**	**< 0.001**
	Diet:Sex	1,24	0.415	0.526	Diet:Sex	n.a.	n.a.	n.a.
	Diet:Day	5,92	0.279	0.924	Diet:Day	n.a.	n.a.	n.a.
	Sex:Day	5,92	0.204	0.96	Sex:Day	n.a.	n.a.	n.a.
	Diet:Sex:Day	5,92	0.675	0.643	Diet:Sex:Day	n.a.	n.a.	n.a.
MTM Errors	Diet	1,24	0.931	0.344	Diet	1,25	3.566	0.071
	Sex	1,24	0.034	0.855	Sex	1,25	2.179	0.152
	Day	**5,120**	**2.733**	**0.023**	**Day**	**5,135**	**5.093**	**0.003**
	Diet:Sex	1,24	0.318	0.578	Diet:Sex	n.a.	n.a.	n.a.
	Diet:Day	5,120	0.794	0.556	Diet:Day	n.a.	n.a.	n.a.
	Sex:Day	5,120	0.510	0.768	Sex:Day	n.a.	n.a.	n.a.
	Diet:Sex:Day	5,120	0.581	0.714	Diet:Sex:Day	n.a.	n.a.	n.a.

n.a.: not available due to model reduction based on the AIC.

3.5. **Multiple T-Maze – detailed error analysis**

Regarding forward errors during the learning phase, we found significant effects of diet and day (Table 4). PUFA animals made more forward errors than control animals, especially on days three and four (Fig. 11A). The number of backward errors decreased but no diet-related effects were detected (Table 4). On the last day of the learning phase, all groups except the PUFA males made no backward errors (Fig. 11B).

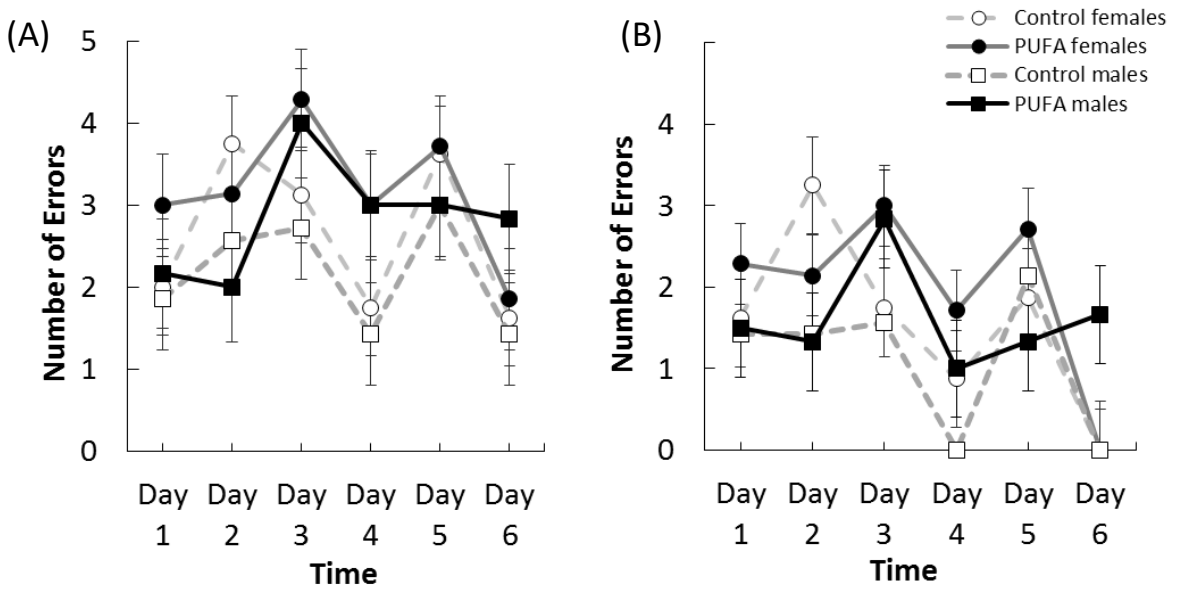

Fig. 11. Number of forward (A) and backward (B) errors during the learning phase in the multiple T-maze (for statistical details see text and Table 3).

Independent of the day, we found significant differences between the single errors (A-F) made during the learning phase (Fig. 12, Table 4). Error B was made significantly more often than all other errors. In contrast, error D was the least frequent error made (for post-hoc tests see Table S1a).

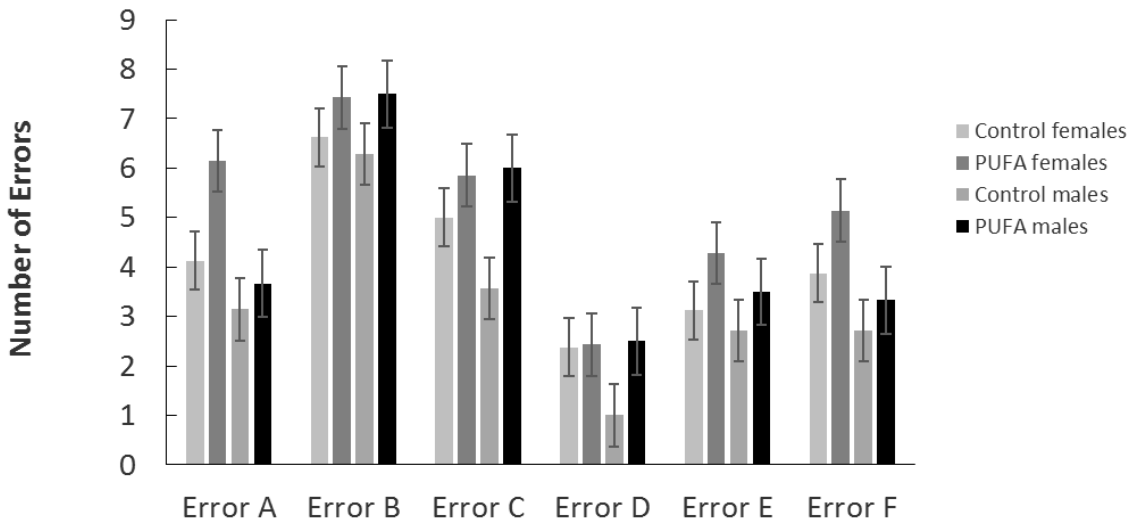

Fig. 12. Number of single errors (A-F) in the multiple T-maze during the learning phase (for statistical details see text, Table 4 and Table S1a). Note that errors A, B and C are forward errors and errors D, E and F are backward errors.

Most errors were made in the second T (errors BE), while the number of errors in the first (errors AF) and third (errors CD) T were similar (Fig. 13, Table 4, for post-hoc tests see Table S1b).

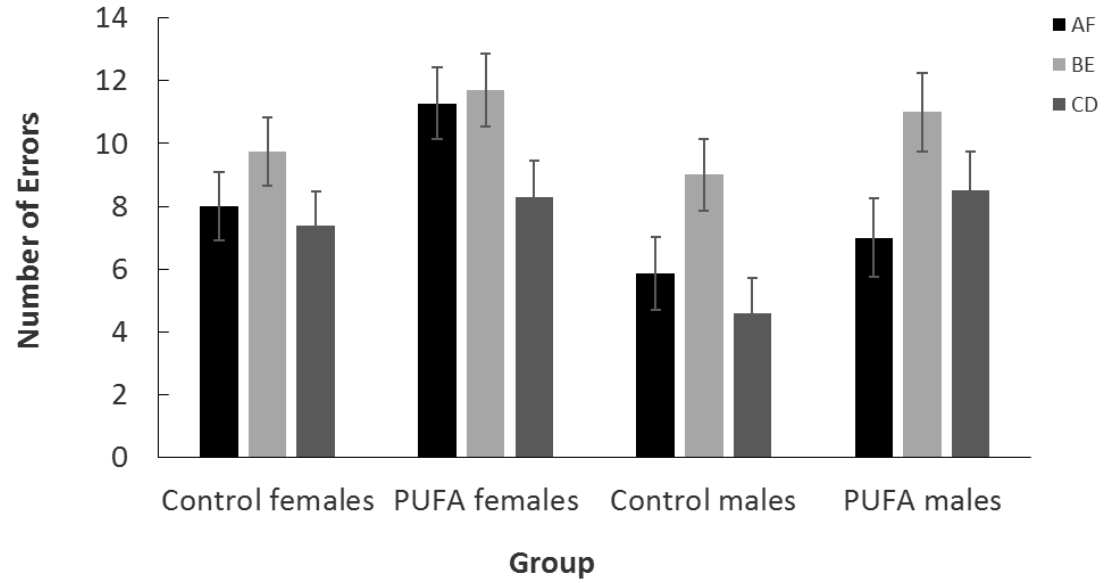

Fig. 13. Number of errors made in the first (AF), second (BE) and third (CD) T of the multiple T-maze during the learning phase (for statistical details see text and Table 4).

Table 4. ANOVA (Type 3) results of full and fitted linear mixed model (LME) analysis of number of errors forward and backward, number of single errors and number of errors per T made in the multiple T-maze during the Learning phase.

	Full model				**Fitted model**			
Response	**Predictor**	**Statistics**			**Predictor**	**Statistics**		
		df	**F-value**	**p-value**		**df**	**F-value**	**p-value**
Errors forward	Diet	1,24	1.186	0.287	**Diet**	**1,25**	**4.776**	**0.038**
	Sex	1,24	0.024	0.878	Sex	1,25	2.335	0.139
	Day	**5,120**	**2.373**	**0.043**	**Day**	**5,135**	**4.03**	**0.002**
	Diet:Sex	1,24	0.262	0.613	Diet:Sex	n.a.	n.a.	n.a.
	Diet:Day	5,120	0.643	0.667	Diet:Day	n.a.	n.a.	n.a.
	Sex:Day	5,120	0.174	0.972	Sex:Day	n.a.	n.a.	n.a.
	Diet:Sex:Day	5,120	0.204	0.960	Diet:Sex:Day	n.a.	n.a.	n.a.
Errors backward	Diet	1,24	0.553	0.464	Diet	1,25	3.028	0.094
	Sex	1,24	0.049	0.827	Sex	1,25	2.511	0.126
	Day	**5,120**	**3.225**	**0.009**	**Day**	**5,135**	**5.569**	**0.001**
	Diet:Sex	1,24	0.204	0.655	Diet:Sex	n.a.	n.a.	n.a.
	Diet:Day	5,120	0.920	0.470	Diet:Day	n.a.	n.a.	n.a.
	Sex:Day	5,120	0.744	0.592	Sex:Day	n.a.	n.a.	n.a.
	Diet:Sex:Day	5,120	0.809	0.546	Diet:Sex:Day	n.a.	n.a.	n.a.
Single errors (A-F)	**Diet**	**1,24**	**4.7398**	**0.04**	**Diet**	**1,25**	**5.053**	**0.034**
	Sex	1,24	1.123	0.3	Sex	1,25	3.243	0.084
	Error_Arm	**5,120**	**10.859**	**< 0.001**	**Error_Arm**	**5,135**	**45.119**	**< 0.001**
	Diet:Sex	1,24	1.206	0.283	Diet:Sex	n.a.	n.a.	n.a.
	Diet:Error_Arm	5,120	0.95	0.452	Diet:Day	n.a.	n.a.	n.a.
	Sex:Error_Arm	5,120	0.51	0.768	Sex:Day	n.a.	n.a.	n.a.
	Diet:Sex:Error_Arm	5,120	1.562	0.176	Diet:Sex:Day	n.a.	n.a.	n.a.
Errors per T (AF, BE, CD)	Diet	1,24	3.684	0.067	**Diet**	**1,25**	**4.863**	**0.037**
	Sex	1,24	1.567	0.223	**Sex**	**1,25**	**6.501**	**0.017**
	Error_T	2,48	2.263	0.115	**Error_T**	**2,52**	**5.713**	**0.006**
	Diet:Sex	1,24	0.727	0.402	Diet:Sex	n.a.	n.a.	n.a.
	Diet:Error_T	2,48	0.987	0.380	Diet:Error_T	n.a.	n.a.	n.a.
	Sex:Error_T	2,48	0.766	0.471	Sex:Error_T	2,52	1.96	0.151
	Diet:Sex:Error_T	2,48	2.169	0.125	Diet:Sex:Error_T	n.a.	n.a.	n.a.

Error_Arm represents single errors (A, B, C, D, E, F); Error_T represents errors per T (AF, BE, CD)

n.a.: not available due to model reduction based on the AIC.

3.6. **Multiple T-Maze - Memory**

In the memory test, a tendency of a three-way interaction between diet:sex:day was found. However, only a two-way interaction between diet:day was significant (Table 5). Although PUFA females showed a strong increase in the time needed to complete the maze in the short term memory test, the general performance of PUFA animals did not differ to the last day of the learning phase (Fig. 14, Table 6). However, PUFA females were faster again in the long term memory test and they performed similar compared to the last day of the learning phase, which resulted in a similar performance of PUFA males and females compared to the learning phase. In control animals, however, the time needed steadily increased during memory testing (Fig. 14, Table 6).

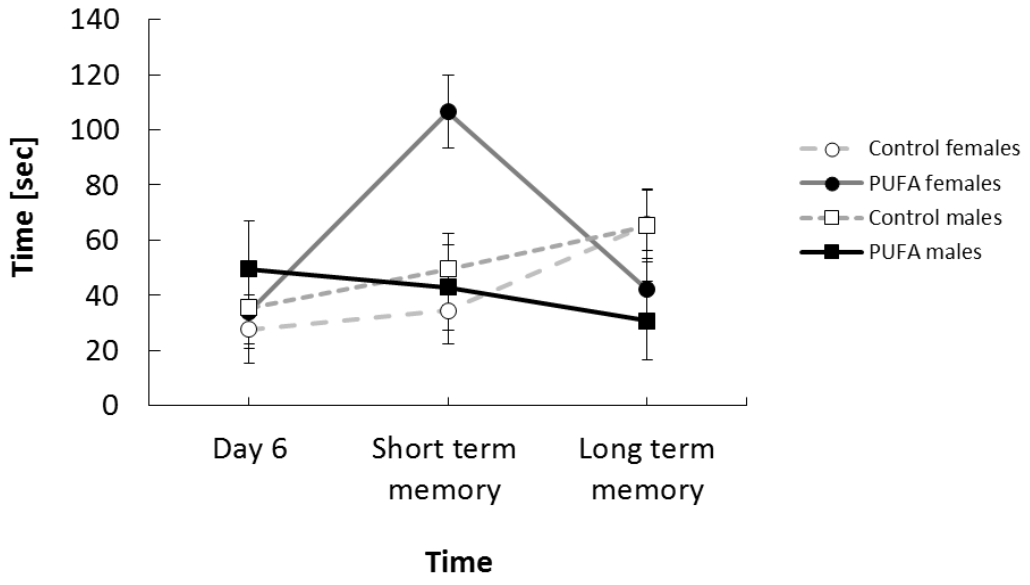

Fig. 14. Time needed to complete the multiple T-maze task during the memory tests compared to the last day of the learning phase (for statistical details see Tables 5 and 6).

A significant three-way interaction between diet:sex:day regarding errors made during the memory phase was found (Table 5). The number of errors showed a similar pattern to the time the animals needed in the memory tests. In PUFA females, an increase in the number of errors was observed in the short-term, followed by a decrease in the long-term memory test (Fig. 15). However, the number of errors did not differ to the last day of the learning phase in PUFA animals (Table 6). Control animals showed an increasing number of errors during memory testing compared to the learning phase (Fig. 15, Table 6).

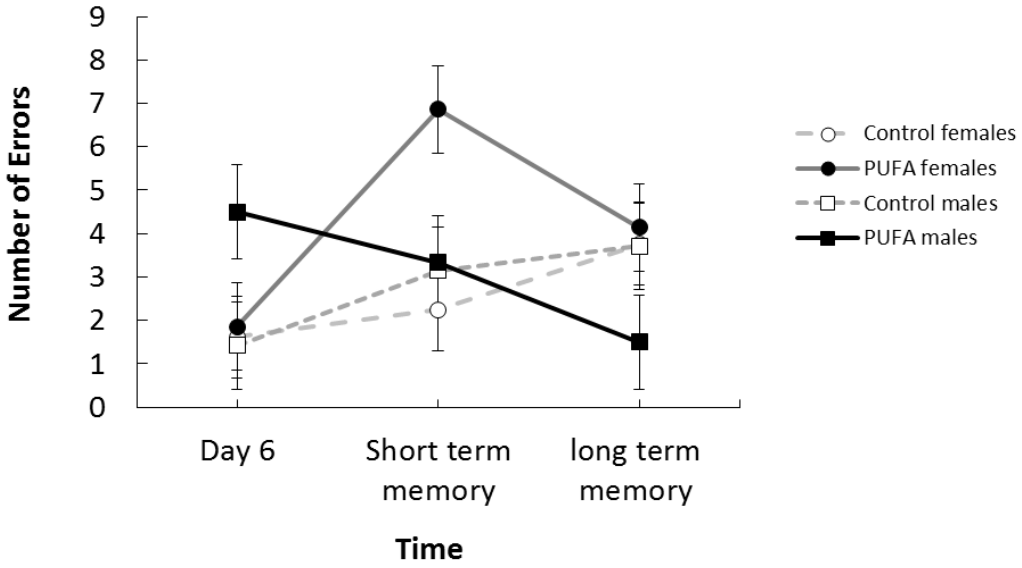

Fig. 15. Number of errors in the multiple T-maze task during the memory tests compared to the last day of the learning phase (for statistical details see Tables 5 and 6).

Table 5. ANOVA (Type 3) results of linear mixed model (LME) analysis for time needed and errors made in the multiple T-maze during the memory phase.

Response	**Memory phase Predictor**	**Statistics**		
		df	**F-value**	**p-value**
MTM Time	Diet	1,24	0.105	0.749
	Sex	1,24	0.163	0.69
	Day	2,44	2.6	0.086
	Diet:Sex	1,24	0.061	0.807
	Diet:Day	**2,44**	**6.810**	**0.003**
	Sex:Day	2,44	0.175	0.84
	Diet:Sex:Day	2,44	2.595	0.086
MTM Errors	Diet	1,24	0.0243	0.877
	Sex	1,24	0.017	0.896
	Day	2,48	1.453	0.244
	Diet:Sex	1,24	1.687	0.206
	Diet:Day	**2,48**	**3.499**	**0.038**
	Sex:Day	2,48	0.197	0.822
	Diet:Sex:Day	**2,48**	**3.761**	**0.030**

Table 6 Post-hoc analyses for time needed and number of errors in the multiple-T-maze memory tests compared to the last day of the learning phase.

Response	Diet	Learning phase vs. Short-term memory		Learning phase vs. Long-term memory	
		χ2	**p-value**	**χ2**	**p-value**
Time	Control	1.814	0.712	**6.450**	**0.044**
	PUFA	4.216	0.160	0.146	1.000
Errors	Control	0.751	1.000	**7.970**	**0.019**
	PUFA	5.455	0.078	0.139	1.000

Post hoc analysis was performed with Bonferroni corrections.

4. Discussion

The present study investigated modulatory effects of dietary PUFAs on spatial abilities in relation to FCM concentrations in male and female rats. Our findings demonstrate not only sex-specific effects of PUFAs on FCM concentrations and memory retention, but also an impact of FCM concentrations on learning abilities. Sex differences in body mass and FCM concentrations were expected. In rats, males are larger, more active, and establish a pronounced social hierarchy where individual confrontations are common (Macdonald et al., 1995). Therefore, socially housed males usually show higher FCM concentrations than females. Social defeat stress, as a result of conflicts when establishing and maintaining a hierarchy, can impact male rodent physiology and behavior and can lead to social avoidance (Beery & Kaufer, 2015). With regard to the central nervous system, social defeat stress affects hippocampal morphology and function, i.e. leading to a reduction in hippocampal volume, which was also documented as a result of cognitive impairment (McEwen, 2012). A high social status can also cause high stress load, particularly in societies in which dominance must be continuously defended (Sapolsky, 2005). Not only different CORT secretion *per se*, but especially different glucocorticoid receptor densities and the related affinity for CORT of different brain regions may sex-specifically affect specific brain structures (Turner, 1985). Hence, differences in physiological stress reactions between males and females may also contribute to differences in cognitive performance as demonstrated in this experiment.

In contrast to our expectations, PUFA males showed increasing body mass and FCM concentrations during the 5-week initial feeding phase compared to control males. It was expected that PUFAs counteract increasing FCM concentrations but show no effect on body

mass because of the isocaloric diets. Ferraz et al. (2011) demonstrated that n-3 PUFAs reduce CORT concentrations under chronic stress. In addition to decreased CORT concentrations after PUFA supplementation, Hennebelle et al. (2012) also showed a reduction in body mass loss as a result of chronic stress. However, we did not systematically expose rats to chronic stress in our experiments. Therefore, we assume that our rats did not experience chronic stress in contrast to the experiments previously mentioned. Findings by Sanz et al. (2000) suggest that a PUFA-linked increase in β-oxidation results in a higher energy turnover, which might be paralleled by increasing CORT concentrations, although this was not addressed in the mentioned study. However, a direct and fast incorporation of PUFAs in the energy balance could have also enabled a body mass gain despite increased FCM concentrations. Males appear to be more affected by a change in dietary fatty acid composition than females. Differences in physiological parameters and/or cognition due to PUFA supplementation can already be detected after a couple of weeks. The supplementation period used in this study can be assumed as sufficient because previous studies have shown that changes in spatial performance can already be detected after 4 weeks of PUFA administration in rats (Haider et al., 2011) and cortisol concentrations were affected after 3 weeks in guinea pigs (Nemeth et al., 2014). Contrary to that, Nemeth et al. (2016) did not find any effect of a two-month walnut oil administration on cortisol concentrations in guinea pigs. Nevertheless, the same animals showed the strongest increase in cortisol during social confrontations with unfamiliar conspecifics, which seemingly enabled adequate behavioral responses to cope with the situation. Since this study did not investigate social behavior, potential effects of FCM concentrations especially in PUFA males remain unknown. Effects of PUFAs on FCM concentrations have been shown to be context specific and diminishing effects on stress responses under chronic stress situations are seemingly mainly attributable to high n-3 PUFA sources (Ferraz et al., 2011; Hennebelle et al., 2012) and not to walnut oil, which also contains n-6 PUFAs.

To test for effects of PUFAs and FCM concentrations on spatial cognitive abilities, we used two different maze setups, the T-maze and the multiple T-maze. In the T-maze experiment, learning performance did not differ between experimental groups. The T-maze experiment might have been too simple for the animals, so that differences in cognitive abilities between sexes or diets could not be identified. Nevertheless, when T-maze performance was corrected for FCM concentrations, PUFA animals showed a lower number of trials to finish the task.

Positive effects of PUFAs on spatial learning abilities correspond to results from experiments using different types of maze tasks (Fedorova et al., 2009; Lim et al., 2005; Moriguchi et al., 2001). Ferraz et al. (2011) showed that n-3 fatty acid supplementation counteracts anxiogenic effects of stress on cognitive functions in rats. Moreover, n-3 supplemented animals outperformed control animals in chronic stress situations, again indicating an "anti-stress" effect of n-3 PUFAs. Although n-3 PUFAs significantly diminished FCM concentrations caused by chronic stress in the mentioned study, whereas no such effect was found here, these findings match our results: FCM concentrations correlated positively with the number of trials needed in the T-maze, hence it impaired cognitive performance, but PUFA animals (especially males) performed similar to controls despite higher FCM concentrations. Negative behavioral influences of CORT concentrations have also been shown in other studies as, for example, rats in an elevated plus maze showed more anxiety-like behavior and inferior learning performance with increasing individual CORT concentrations (Ferraz et al., 2011; Pérez et al., 2013). Therefore, interaction-effects of dietary PUFAs and CORT concentrations on learning abilities can be assumed and should be considered in future studies to better understand the effects of PUFAs on cognitive abilities.

The multiple T-maze featured a unique modification - the dead-end branches – to ensure that the rats were not able to predict the correct path from the intersection in each T. Although this definitely represented a more challenging learning paradigm as the T-maze, a significant learning performance was found independent of sex or group. Success rates increased during the learning phase, while the time to finish the task and the number of errors decreased. However, there was a tendency of PUFA animals needing more time and making more errors than control animals, which contrasts previous findings (Moriguchi & Salem Jr, 2003; Ferraz et al., 2011; Pérez et al., 2013). Especially at the end of the learning phase PUFA males made more errors than the other groups. Correcting multiple T-maze performance for FCM concentrations did not explain this effect, which is not surprising considering that four weeks passed from FCM sampling to the learning phase. With regard to the T-maze results, it would have been appropriate to include accurately timed FCM concentration measurements as a physiological parameter also in the analysis of the multiple T-maze.

The PUFA males were the only group that started the learning phase in the multiple T-maze with a success rate of 100 % but ended the learning phase as the only group with less than

100 % (67.7 %). The decreasing success rate in PUFA males could be a result of increasing explorative behavior during the learning phase. All animals of this group found the food reward on the first day of the learning phase and perhaps investigated the maze for other resources on the following days. Increasing exploration would have resulted in animals taking more time in the maze, ultimately not finishing the task within the time limit. Kabbaj et al. (2000) demonstrated that in rats high explorative behavior in an anxiogenic environment was related to elevated CORT concentrations. Therefore, high individual exploration activity in the multiple T-maze may have resulted in increased FCM concentrations, possibly indicating a better ability to activate the HPA-axis compared to rats who showed less explorative behavior. During the learning phase, PUFA males may have actively explored the maze environment more, which might have been accompanied by an enhanced stress induced secretion of CORT resulting in a decreased success rate.

Contrary to PUFA males, all other groups improved their success rate during the learning phase. Here, explorative behavior could have also contributed to animals performing worse, but this time, rather in the beginning of the learning phase than in the end. Notably, early in the learning phase, we assume that explorative behavior impacts the performance in the experiment. As the learning phase progresses, the animals get used to the testing environment and explorative behavior declines. All groups beside the PUFA males seemed to decrease exploration to a minimum until the end of the learning phase, resulting in improved performances (higher success rates). This would suggest that PUFAs allowed males to get accustomed faster to this new environment and the task, which could explain their superior success rate from the start of the learning phase.

Testosterone has been shown to be an important modulator of spatial cognition in male rats (Hawley et al., 2013). Retana-Márquez et al. (2003) did not find any correlations between testosterone and CORT concentrations in acute and chronically stressed male rats. A direct relation between CORT and testosterone concentrations with regard to our findings could therefore be excluded. Nevertheless, Tran et al. (2016) found that an n-3 rich diet increases testosterone secretion in male buffalos and Nemeth et al. (2018) found an increase in testosterone in male guinea pigs as a result of saturated fatty acid supplementation. In females, PUFAs may also lead to a testosterone increase (Mumford et al., 2016). Hence differences in individual testosterone concentrations could have been caused by the dietary

regimes and may have affected cognitive performance in our maze experiments. However, as we did not analyze testosterone concentrations in this study, effects of testosterone should be investigated in future studies.

To determine if the rats used certain learning strategies to reach the goal, we performed a detailed error analysis in the multiple T-maze experiments. The sequence of errors made by the rats, did not indicate general learning strategies. The rats performed individually different and independent of diet and sex. Schmitzer-Torbert & Redish (2002) also did not document any subsequent changes in performance strategy after the rats learned to complete a multiple T-maze task. In general, the task seemed to be a cognitive challenge for the animals. They made many errors, especially early in the learning phase. Given that the animals did not have any external cues for orientation, they had to remember the path. The second T in the multiple T-maze caused most problems for the rats. Error B was made more frequently than any other error. Since most of the time the rats started off taking a left turn at the first intersection, they continued doing so at the second intersection until they seemingly remembered (on day 4 of the learning phase) that they also have to switch to a right turn at some point to reach the goal. Each time they reached the third T they made less backward errors henceforward, resulting in error D being the least frequent error made. At the end of the learning phase rats seemed to remember the way they came from and that the goal is in an arm they have not visited before. At this point, only PUFA males still made backward errors. This was not the case early in the learning phase. First, they had to learn the right path to the goal and with that also the fact that revisiting arms did not make a difference, so they needed to visit arms they have not been in before. The lack of clear differences between the experimental groups could be due to the higher complexity of the multiple T-maze task (compared to the relatively simple T-maze). Nonetheless, rats are able to complete challenging tasks like our multiple T-maze independent of a dietary treatment.

In the short-term memory test, PUFA females performed worse compared to all other groups. We do not have a reasonable explanation for this result. Perhaps something unfamiliar happened inside the cage or inside the room the cage was placed so that the rats in this group where aroused before the experiment was carried out. Anyway, PUFA females did not show untypical behavior at the time of the experiment. Such an impaired short-term memory performance in PUFA supplemented female rodents was not yet documented before (see e.g.

in guinea pigs; Nemeth et al., 2015). However, the short-term memory performance of each experimental group did not differ significantly compared to the learning phase. All groups were still able to pass the multiple-T-maze task, but PUFA females inexplicably needed more time and made more errors than all other groups. Accordingly, rats showed pronounced short-term memory abilities within the context of our maze task, which reveals pronounced cognitive abilities in these animals. However, four weeks later in the long-term memory test, PUFA animals still performed similar to the learning phase, while control animals showed an increased number of errors and needed more time to finish the task. Hence, control animals which did not receive additional PUFAs showed impaired long-term memory performance. They were seemingly unable to fully remember the path four weeks after the short-term memory test and six weeks after the learning phase, respectively. Therefore, PUFA supplementation had positive effects on long-term memory performance in male and female rats as they were still able to remember the path inside the maze. Based on these findings it could be concluded that PUFAs positively affected solely long-term memory in both sexes. However, short-term memory performance suggests sex-specific effects of PUFAs, which correspond to findings in guinea pigs (Nemeth et al., 2015). Other studies have also shown that male rather than female physiology and behavior are affected by high PUFA intake (Davis et al., 2017; Levant et al., 2006). The findings presented here support the existence of sex-specific effects of PUFAs not only on physiology but also on cognitive functions.

Although PUFA males made more errors in the same time span compared to the other groups in the learning phase of the multiple T-maze and also showed a decreasing success rate, they showed outstanding memory performance. On the other hand, PUFA supplemented males showed a 100 % success rate already in the beginning of the learning phase. It could be questioned if these animals simply needed a shorter learning phase than females to “remember” the correct way in the short- and long-term memory tests. This would imply that PUFAs stimulate a fast memory formation perhaps in a sex-specific way. Even though sex-specific effects of PUFAs on LTP have not been documented before, PUFAs may differently affect LTP in male and female individuals. All other groups seemingly needed the complete learning phase to successfully solve the multiple T-maze task. Perhaps an even longer learning phase would have been necessary for these groups to keep up with the memory performance of PUFA males in the short- and long-term.

The present findings demonstrate that dietary PUFAs affect learning and memory performance in adult male and female rats. Furthermore, possible interaction-effects of PUFAs and FCM concentrations on learning abilities are assumed. We suggest that due to differences in physiological conditions such as glucocorticoid concentrations, neural plasticity and cognition, PUFAs differently affect memory abilities in male and female individuals (see e.g. Chow et al., 2013). Sex-specific effects of PUFAs on cognitive performance (learning & memory) have, however, not been studied in detail and should be strongly considered in the future. Furthermore, additional physiological parameters such as androgens and estrogens should be included in the analyses, as they have been shown to affect spatial memory as well (Hawley et al., 2013; Jiménez-Rubio et al., 2020; Korol et al., 2004). Considering possible sex differences and the involvement of steroid hormones would increase our understanding of how PUFAs affect physiology and cognition. This would be highly valuable with regard to possible therapeutic effects of PUFAs in Alzheimer's disease or dementia, which are characterized by cognitive impairment.

5. References

Beery, A. K., & Kaufer, D. (2015). Stress, social behavior, and resilience: insights from rodents. *Neurobiology of Stress*, *1*, 116–127.

Brenna, J. T. (2011). Animal studies of the functional consequences of suboptimal polyunsaturated fatty acid status during pregnancy, lactation and early post-natal life. *Maternal & Child Nutrition*, *7*, 59–79.

Chow, C., Epp, J. R., Lieblich, S. E., Barha, C. K., & Galea, L. A. M. (2013). Sex differences in neurogenesis and activation of new neurons in response to spatial learning and memory. *Psychoneuroendocrinology*, *38*(8), 1236–1250.

Chung, W.-L., Chen, J.-J., & Su, H.-M. (2008). Fish oil supplementation of control and (n-3) fatty acid-deficient male rats enhances reference and working memory performance and increases brain regional docosahexaenoic acid levels. *The Journal of Nutrition*, *138*(6), 1165–1171.

Das, U. N. (2003). Can memory be improved? A discussion on the role of ras, GABA, acetylcholine, NO, insulin, TNF-α, and long-chain polyunsaturated fatty acids in memory formation and consolidation. *Brain and Development*, *25*(4), 251–261.

Davis, D. J., Hecht, P. M., Jasarevic, E., Beversdorf, D. Q., Will, M. J., Fritsche, K., & Gillespie, C. H. (2017). Sex-specific effects of docosahexaenoic acid (DHA) on the microbiome and behavior of socially-isolated mice. *Brain, Behavior, and Immunity*, *59*, 38–48.

De Rosario-Martinez, H. (2015). *phia: Post-hoc interaction analysis. R package. 2015*. CRAN:

The R Foundation for Statistical Computing. http://CRAN.R-project.org/package=phia

de Souza, A. S., Rocha, M. S., & do Carmo, M. das G. T. (2012). Effects of a normolipidic diet containing trans fatty acids during perinatal period on the growth, hippocampus fatty acid profile, and memory of young rats according to sex. *Nutrition*, *28*(4), 458–464.

Fedorova, I., Hussein, N., Baumann, M. H., Di Martino, C., & Salem Jr, N. (2009). An n-3 fatty acid deficiency impairs rat spatial learning in the Barnes maze. *Behavioral Neuroscience*, *123*(1), 196.

Ferraz, A. C., Delattre, A. M., Almendra, R. G., Sonagli, M., Borges, C., Araujo, P., Andersen, M. L., Tufik, S., & Lima, M. M. S. (2011). Chronic ω-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol. *Behavioural Brain Research*, *219*(1), 116–122.

Ferreri, C., Masi, A., Sansone, A., Giacometti, G., Larocca, A. V., Menounou, G., Scanferlato, R., Tortorella, S., Rota, D., & Conti, M. (2017). Fatty acids in membranes as homeostatic, metabolic and nutritional biomarkers: recent advancements in analytics and diagnostics. *Diagnostics*, *7*(1), 1.

Haider, S., Batool, Z., Tabassum, S., Perveen, T., Saleem, S., Naqvi, F., Javed, H., & Haleem, D. J. (2011). Effects of walnuts (Juglans regia) on learning and memory functions. *Plant Foods for Human Nutrition*, *66*(4), 335–340.

Hajjar, T., Goh, Y. M., Rajion, M. A., Vidyadaran, S., Li, T. A., & Ebrahimi, M. (2013). Alterations in neuronal morphology and synaptophysin expression in the rat brain as a result of changes in dietary n-6: n-3 fatty acid ratios. *Lipids in Health and Disease*, *12*(1), 113.

Harris, A. P., D'eath, R. B., & Healy, S. D. (2008). Sex differences, or not, in spatial cognition in albino rats: acute stress is the key. *Animal Behaviour*, *76*(5), 1579–1589.

Hawley, W. R., Grissom, E. M., Martin, R. C., Halmos, M. B., Bart, C. L. S., & Dohanich, G. P. (2013). Testosterone modulates spatial recognition memory in male rats. *Hormones and Behavior*, *63*(4), 559–565.

Hennebelle, M., Balasse, L., Latour, A., Champeil-Potokar, G., Denis, S., Lavialle, M., Gisquet-Verrier, P., Denis, I., & Vancassel, S. (2012). Influence of omega-3 fatty acid status on the way rats adapt to chronic restraint stress. *PloS One*, *7*(7).

Ikemoto, A., Ohishi, M., Sato, Y., Hata, N., Misawa, Y., Fujii, Y., & Okuyama, H. (2001). Reversibility of n-3 fatty acid deficiency-induced alterations of learning behavior in the rat: level of n-6 fatty acids as another critical factor. *Journal of Lipid Research*, *42*(10), 1655–1663.

Jiménez-Rubio, G., Herrera-Pérez, J. J., Martínez-Becerril, H. A., Márquez-Baltazar, M. S., & Martínez-Mota, L. (2020). Age-dependent effects of testosterone on spatial memory in male rats. *Hormones and Behavior*, *122*, 104748.

Kabbaj, M., Devine, D. P., Savage, V. R., & Akil, H. (2000). Neurobiological Correlates of Individual Differences in Novelty-Seeking Behavior in the Rat: Differential Expression of Stress-Related Molecules. *The Journal of Neuroscience*, *20*(18), 6983 LP – 6988.

https://doi.org/10.1523/JNEUROSCI.20-18-06983.2000

Kelly, L., Grehan, B., Della Chiesa, A., O'Mara, S. M., Downer, E., Sahyoun, G., Massey, K. A., Nicolaou, A., & Lynch, M. A. (2011). The polyunsaturated fatty acids, EPA and DPA exert a protective effect in the hippocampus of the aged rat. *Neurobiology of Aging*, *32*(12), 2318-e1.

Kesner, R. P., Lee, I., & Gilbert, P. (2004). A behavioral assessment of hippocampal function based on a subregional analysis. *Reviews in the Neurosciences*, *15*(5), 333–352.

Korol, D. L., Malin, E. L., Borden, K. A., Busby, R. A., & Couper-Leo, J. (2004). Shifts in preferred learning strategy across the estrous cycle in female rats. *Hormones and Behavior*, *45*(5), 330–338.

Lepschy, M., Touma, C., Hruby, R., & Palme, R. (2007). Non-invasive measurement of adrenocortical activity in male and female rats. *Laboratory Animals*, *41*(3), 372–387.

Levant, B., Ozias, M. K., & Carlson, S. E. (2006). Sex-specific effects of brain LC-PUFA composition on locomotor activity in rats. *Physiology & Behavior*, *89*(2), 196–204.

Lim, S.-Y., Hoshiba, J., Moriguchi, T., & Salem, N. (2005). N-3 fatty acid deficiency induced by a modified artificial rearing method leads to poorer performance in spatial learning tasks. *Pediatric Research*, *58*(4), 741–748.

Macdonald, D. W., Berdoy, M., & Smith, P. (1995). Stability of social status in wild rats: age and the role of settled dominance. *Behaviour*, *132*(3–4), 193–212.

Martin, S. J., & Clark, R. E. (2007). The rodent hippocampus and spatial memory: from synapses to systems. *Cellular and Molecular Life Sciences*, *64*(4), 401.

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiological Reviews*, *87*(3), 873–904.

McEwen, B. S. (2012). Brain on stress: how the social environment gets under the skin. *Proceedings of the National Academy of Sciences*, *109*(Supplement 2), 17180–17185.

Moriguchi, T., Loewke, J., Garrison, M., Catalan, J. N., & Salem, N. (2001). Reversal of docosahexaenoic acid deficiency in the rat brain, retina, liver, and serum. *Journal of Lipid Research*, *42*(3), 419–427.

Moriguchi, T., & Salem Jr, N. (2003). Recovery of brain docosahexaenoate leads to recovery of spatial task performance. *Journal of Neurochemistry*, *87*(2), 297–309.

Mumford, S. L., Chavarro, J. E., Zhang, C., Perkins, N. J., Sjaarda, L. A., Pollack, A. Z., Schliep, K. C., Michels, K. A., Zarek, S. M., & Plowden, T. C. (2016). Dietary fat intake and reproductive hormone concentrations and ovulation in regularly menstruating women. *The American Journal of Clinical Nutrition*, *103*(3), 868–877.

Nemeth, M., Millesi, E., Puehringer-Sturmayr, V., Kaplan, A., Wagner, K.-H., Quint, R., & Wallner, B. (2016). Sex-specific effects of dietary fatty acids on saliva cortisol and social behavior in guinea pigs under different social environmental conditions. *Biology of Sex Differences*, *7*(1), 51.

Nemeth, M., Millesi, E., Schuster, D., Quint, R., Wagner, K.-H., & Wallner, B. (2018). Dietary fatty acids sex-specifically modulate guinea pig postnatal development via cortisol concentrations. *Scientific Reports*, *8*(1), 1–14.

Nemeth, M., Millesi, E., Wagner, K.-H., & Wallner, B. (2014). Effects of diets high in unsaturated fatty acids on socially induced stress responses in guinea pigs. *PLoS One*, *9*(12), e116292.

Nemeth, M., Millesi, E., Wagner, K.-H., & Wallner, B. (2015). Sex-specific effects of diets high in unsaturated fatty acids on spatial learning and memory in guinea pigs. *PLoS One*, *10*(10).

Okaichi, Y., Ishikura, Y., Akimoto, K., Kawashima, H., Toyoda-Ono, Y., Kiso, Y., & Okaichi, H. (2005). Arachidonic acid improves aged rats' spatial cognition. *Physiology & Behavior*, *84*(4), 617–623.

Pérez, M. Á., Terreros, G., & Dagnino-Subiabre, A. (2013). Long-term ω-3 fatty acid supplementation induces anti-stress effects and improves learning in rats. *Behavioral and Brain Functions*, *9*(1), 25.

Péter, A. (2011). Solomon Coder (version beta 11.01. 22): a simple solution for behavior coding. *Computer Programm Available at Http://Solomoncoder. Com*.

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2017). *R Core Team (2017) nlme: linear and nonlinear mixed effects models. R package version 3.1-131*. https://CRAN.R-project.org/package=nlme

Retana-Márquez, S., Bonilla-Jaime, H., Vázquez-Palacios, G., Martínez-García, R., & Velázquez-Moctezuma, J. (2003). Changes in masculine sexual behavior, corticosterone and testosterone in response to acute and chronic stress in male rats. *Hormones and Behavior*, *44*(4), 327–337. https://doi.org/https://doi.org/10.1016/j.yhbeh.2003.04.001

Sapolsky, R. M. (2005). The influence of social hierarchy on primate health. *Science*, *308*(5722), 648–652.

Schmitzer-Torbert, N., & Redish, A. D. (2002). Development of path stereotypy in a single day in rats on a multiple-T maze. *Archives Italiennes de Biologie*, *140*(4), 295–301.

Sinclair, A. J. (1975). Long-chain polyunsaturated fatty acids in the mammalian brain. *Proceedings of the Nutrition Society*, *34*(3), 287–291.

Sprecher, H., Luthria, D. L., Mohammed, B. S., & Baykousheva, S. P. (1995). Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. *Journal of Lipid Research*, *36*(12), 2471–2477.

Svennerholm, L. (1968). Distribution and fatty acid composition of phosphoglycerides in normal human brain. *Journal of Lipid Research*, *9*(5), 570–579.

Team, R. C. (2019). R: a language and environment for statistical computing computer program, version 3.6. 1. *R Core Team, Vienna, Austria*.

Tran, L. V, Malla, B. A., Sharma, A. N., Kumar, S., Tyagi, N., & Tyagi, A. K. (2016). Effect of

omega-3 and omega-6 polyunsaturated fatty acid enriched diet on plasma IGF-1 and testosterone concentration, puberty and semen quality in male buffalo. *Animal Reproduction Science*, *173*, 63–72.

Turner, B. B. (1985). Sexual dimorphism of glucocorticoid binding in rat brain. *Brain Research*, *343*(1), 16–23.

Yehuda, S. (2003). Omega-6/omega-3 ratio and brain-related functions. *World Review of Nutrition and Dietetics*, *92*, 37–56.

6. Supplementary tables

Table S1 Post hoc analyses for numbers of errors made per arm (a) and numbers of errors made per T (b).

(a)

Error_Arm_Sum	χ2	p-value
A-B	61.066	< 0.001
A-C	5.397	0.303
A-D	42.867	< 0.001
A-E	6.970	0.124
A-F	2.186	1
B-C	30.154	< 0.001
B-D	206.259	< 0.001
B-E	109.296	< 0.001
B-F	86.357	< 0.001
C-D	78.685	< 0.001
C-E	24.634	< 0.001
C-F	14.452	0.002
D-E	15.266	0.001
D-F	25.693	< 0.001
E-F	1.349	1

(b)

Error_T_Sum	χ2	p-value
AF-BE	13.125	0.001
AF-CD	2.235	0.405
BE-CD	26.193	< 0.001

Post hoc analysis was performed with Bonferroni corrections.

7. Zusammenfassung

Mehrfach ungesättigte Fettsäuren (polyunsaturated fatty acids, PUFAs) sind nicht nur essentielle Nahrungsbestandteile von Säugetieren, sondern beeinflussen auch die Aktivität von Neuronen, die räumliche Orientierung und die Gedächtnisleistung. Das Ziel dieser Studie war es herauszufinden welchen Einfluss PUFAs auf die räumliche Orientierung haben und inwiefern Corticosteron (CORT) dies beeinflusst, da sich CORT nachweislich negativ auf die räumliche Orientierungsfähigkeit auswirkt. Aufgrund von geschlechtsspezifischen Unterschieden bei neurophysiologischen Funktionen wird angenommen, dass PUFAs Männchen und Weibchen unterschiedlich stark beeinflussen. Männliche und weibliche Ratten wurden mit einer PUFA-angereicherten (10% Walnussöl) bzw. mit einer Kontrolldiät gefüttert. Zu Beginn wurden während einer fünfwöchigen Fütterungsphase wöchentlich das Körpergewicht gemessen und Kotproben gesammelt um anschließend Corticosteronmetabolitkonzentrationen (fecal corticosterone metabolites, FCM) aus den Proben analysieren zu können. In den folgenden Experimenten, dem T-Maze und Multiple T-Maze, wurden die Ratten auf ihre räumliche Lernfähigkeit sowie Kurz- und Langzeitgedächtnis getestet. Die Leistungen der Ratten in diesen Experimenten wurden statistisch auf individuelle FCM Konzentrationen korrigiert. PUFA-Männchen nahmen im Laufe der fünfwöchigen Fütterungsphase am stärksten zu und hatten die höchsten FCM Konzentrationen. Im T-Maze wirkten sich PUFAs unter Berücksichtigung von individuellen FCM Konzentrationen negativ auf die Anzahl an Versuchen aus, um den Test zu bestehen. PUFA-Männchen schnitten in der Lernphase des Multiple T-Maze schlechter ab als alle anderen Gruppen. Im Multiple T-Maze-Langzeitgedächtnistest zeigten die PUFA-Tiere signifikant bessere Leistungen als die Kontrolltiere, obwohl PUFA-Weibchen im Kurzzeitgedächtnistest deutlich schlechter waren als die anderen Gruppen. Diese Ergebnisse verdeutlichen den Einfluss von PUFAs auf die Lern- und Gedächtnisleistung und zeigen, dass physiologische Konditionen wie CORT und das Geschlecht hier ebenfalls eine wichtige Rolle spielen.