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Chronic consumption of a high linoleic acid diet during pregnancy, lactation and post-weaning period increases depression-like behavior in male, but not female offspring

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ABSTRACT

Polyunsaturated fatty acids (PUFAs) play an essential role in brain development. Emerging data have suggested a possible link between an imbalance in PUFAs and cognitive behavioral deficits in offspring. A diet rich in high linoleic acid (HLA), typically from preconception to lactation, leads to an increase in the ratio of omega-6 (n-6) to omega-3 (n-3) fatty acids in the fetus. Arising research has suggested that a deficiency in omega-3 fatty acids is a potential risk factor for inducing autism spectrum disorder (ASD)-like behavioral deficits. However, the impact of a high n- diet during preconception, pregnancy, lactation, and post-weaning on the brain development of adolescent offspring are yet to be determined. This study examined whether consumption of an HLA diet during pregnancy, lactation, and post-weaning induced social and cognitive impairments in female and male offspring rats that resemble autistic phenotypes in humans. Female Wistar Kyoto rats were fed with either HLA or low linoleic acid (LLA) control diet for 10 weeks before mating, then continued with the same diet throughout the pregnancy and lactation period. Female and male offspring at 5 weeks old were subjected to behavioral tests to assess social interaction behavior and depression-/anxiety-like behavior. Our result showed that chronic consumption of an HLA diet did not affect sociability and social recognition memory, but induced depression-like behavior in male but not in female offspring.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social interaction disability, repetitive behavioral patterns, and cognitive rigidity [1]. ASD patients can also present learning and memory impairments [2,3] and emotional dysregulation such as depression [4] and anxiety [5]. The prevalence of ASD is growing, with 1 out of 54 children in the USA [6–8] and a higher ASD susceptibility in males [9]. Although the underlying causes of ASD are not fully understood, accumulating evidence has suggested that in addition to genetic factors, maternal perturbations in nutrition could lead to ASD [10].

Polyunsaturated fatty acids (PUFAs) are essential for normal brain growth and development by regulating neurogenesis and synapse formation [11]. α-linoleic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:3n-6) are regarded as essential PUFAs because they cannot be synthesized in mammals [12,13]. Therefore, maternal diet is the sole supply of fetal n-3 and n-6 fatty acids, which crosses the placenta and reach the developing fetus [14,15].

The Western diet has shifted to a higher n-6:n-3 PUFA ratio, from 1:1 to 15:1 [16]. During pregnancy, an imbalanced n-6:n-3 PUFA ratio could be detrimental to fetal growth [17,18] and brain development [16]. ALA and LA are regarded as the precursors of n-3 and n-6 PUFAs, respectively. ALA usually converts to eicosapentaenoic acid (EPA, 20:5n-3)

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and docosahexaenoic acid (DHA, 22:6n-3), whereas LA converts to arachidonic acid (AA, 20:4n-6). Both ALA and LA rely on the same set of enzymes for conversion, including Δ-6 desaturase and Δ-5 desaturase, and thus they compete for the same enzymes [19]. High dietary LA would compete with ALA for the Δ-6 desaturase, resulting in a lower amount of ALA being converted to n-3 PUFAs [6,20]. DHA and AA are required for brain development because of their important roles in maintaining cellular fluidity, flexibility, and thickness, which are critical for regulating neuronal growth and synaptogenesis. Therefore, a balanced maternal dietary intake of n-6 and n-3 PUFAs is vital for proper fetal neurodevelopment[21,22].

An imbalanced PUFAs intake could be detrimental to normal brain development. Long-term consumption of different types of FA, such as interesterified fat, can result in high levels of saturated FA and LA, low levels of DHA in the hippocampus, and impaired memory performance in adult rats [23]. Also, interesterified fat consumption since gestation impairs motor skill learning and sensorimotor behavior in the adult offspring [24]. Another study showed that an increase in maternal dietary n-6:n-3 ratio could also harm brain neurodevelopment in children [25]. In a cohort study, increased n-6:n-3 PUFA ratio in cord plasma of children was associated with a higher incidence of attention-deficit/hyperactivity disorder (ADHD) [26]. Similarly, a murine study found that maternal consumption of an n-6-rich diet during gestation and lactation increased anxiety levels and impaired sociability in the offspring [27]. We have previously reported that increased consumption of an HLA (6.21 % of energy) diet for 10 weeks before mating and during pregnancy increases maternal and fetal n-6:n-3 ratio in the plasma [28]. Furthermore, perturbations *in utero* are often more deleterious to the early development of male offspring [29] than females. Similar to interesterified fat, maternal HLA diet increases total placental n-6 and LA concentrations and decreases total n-3 PUFA, ALA, and DHA [30]. However, it is unclear whether increased HLA diet consumption during the pregnancy, lactation and post-weaning period can induce behavioral deficits that resemble autistic-like behaviors in adolescent male and female offspring. Using the same diet treatment and rat model as previously reported [28], we here examined whether chronic consumption of an HLA diet during the pregnancy, lactation and post-weaning period could affect social interaction and depression-/anxiety-like behavior in the offspring, and whether these putative alterations were sex-specific.

2. Materials and methods

2.1. Animals and experimental design

All experimental procedures were approved by the Animal Subjects Ethics Sub-Committee, Hong Kong Polytechnic University. Wistar Kyoto (WKY) rats received either an LLA diet as a control diet or an HLA diet (Specialty Feeds, Australia) and water *ad libitum* in the animal holding room in a 12:12-hr light-dark cycle. The diets were the same as previously described [28], and the compositional differences between the HLA and LLA diets are listed in Table 1. Twenty 10-week-old female rats were randomly assigned to the HLA diet group or the LLA diet group in five batches. The females were pair-housed and fed with the assigned diet for 10 weeks before mating [28,31]. A male rat was then introduced into each cage for mating. The HLA and LLA diets were continued throughout the prenatal and postnatal period for the dams and post-weaning period for the pups until they were sacrificed.

Starting from postnatal day (PND) 20, pups can consume solid food and water, maintain their body temperature, and show self-grooming behavior, indicating the time ready for weaning [32,33]. Thus, pups were weaned at PND21 and were group-housed to avoid stress induced by social isolation [34,35].

2.2. Behavioral tests

At PND35, three male and three female offspring from each litter were randomly assigned for behavioral tests [36]. Five litters of offspring were subjected to the sucrose preference test (SPT), forced swim test (FST), open field test (OFT), and three-chamber social interaction test. Total animal numbers for each behavioral test were shown in Table 2.

Table 1
Compositional differences in fatty acid levels between the HLA and LLA diets (% energy).

Calculated Fatty Acid Composition as Fed	SF7−110 HLA diet	SF7−109 LLA diet
Saturated Fat C12:0 and less	0.09 %	0.05 %
Myristic Acid 14:0	0.04%	0.02 %
Palmitic Acid 16:0	0.54%	0.99 %
Stearic Acid 18:0	0.21%	0.25 %
Arachidic Acid 20:0	0.03 %	Trace
Palmitoleic acid 16:1	0.04%	0.07 %
Oleic Acid 18:1	1.40%	5.69 %
Gadoleic Acid 20:1	0.03 %	0.03 %
Linoleic Acid 18:2 (n6)	5.86 %	1.44 %
α-Linolenic Acid 18:3 (n3)	0.24 %	0.36 %
Total n3	0.24 %	0.36 %
Total n6	5.86 %	1.44 %
Total Mono Unsaturated Fats	1.51 %	5.79 %
Total Polyunsaturated Fats	6.10 %	1.80 %
Total Saturated Fats	0.98 %	1.34 %
n-6/n-3 ratio	24.42	4

On the test days, rats were habituated in the behavioral test room two hours before assessments [37]. Behavioral tests were carried out in different subsequent days in the following order: SPT, OFT, three-chamber social interaction test, and FST (Fig. 1).

2.2.1. Forced swim test

The forced swim test (FST) was performed to assess behavioral despair, an indicator of depression-like behavior, as previously performed [37,38]. In brief, rats were placed in a cylinder (30 cm × 40 cm) for 15 min on Day 1 and 5 min on Day 2. The session on Day 2 was videotaped for scoring in an observer-blinded manner. Immobility was interpreted when the rats were not moving or moving solely to keep the head above the water, as we previously performed [37,38].

2.2.2. Sucrose preference test

The sucrose preference test (SPT) was performed to assess anhedonia-like behavior as we previously performed [39]. Rats were single housed and provided with a bottle of tap water and a bottle of 1% sucrose solution for 24 h. Bottles were weighed before and after the test to measure the amount of liquid consumption. Sucrose preference over tap water (%) was calculated as sucrose consumption/total liquid consumption of water and sucrose × 100 %.

2.2.3. Open field test

The exploratory behavior and locomotor activity were assessed through the open field test (OFT) as previously performed [40]. Rats were allowed to freely explore an open field (100 cm × 100 cm × 40 cm) for 5 min while being video-recorded. The video was analyzed using ANYMAZE software (Stoelting Co., IL, USA). Locomotor activity was defined by the total distance traveled for 5 min in the arena. The center zone was defined as the center (50 cm × 50 cm). Anxiety-like behavior was calculated as time spent in the center zone/time spent in the center zone and the periphery zone × 100 %.

2.2.4. Three-chamber social interaction test

The social interaction test measures the sociability and social novelty preference of the rat [41]. The test consisted of three phases: habituation, sociability test, and social novelty test, and was conducted as previously described [42]. An apparatus box that was separated into three chambers was used. During a 5-min habituation period, a test rat was placed in the middle chamber of the box, and an empty wire

Table 2
The number of batch and litter size of the HLA and LLA groups.

Batch	Litter Size (n=)				Behavioral Tests			
	HLA group		LLA group		SPT	OFT	Three-chamber Social Interaction test	FST
	Males	Females	Males	Females				
1	3	3	3	3	✓	✓	✓	✓
2	3	3	3	3	✓	✓	✓	✓
3	3	3	3	3	✓	✓	✓	✓
4	3	3	3	3	✓	✓	✓	✓
5	3	3	3	3	✓	✓	✓	✓

HLA: High linoleic acid; LLA: Low linoleic acid; SPT: Sucrose preference test; OFT: Open field test; FST: Forced swim test.

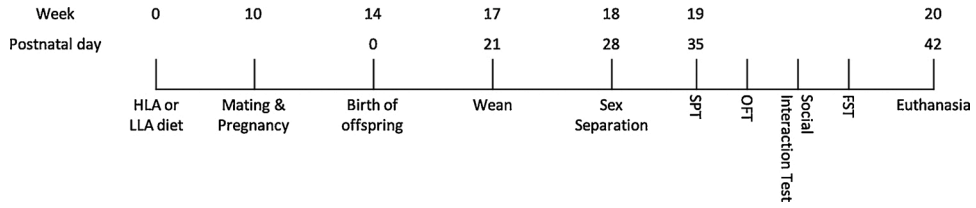

Fig. 1. Experimental timeline. Female rats received either HLA or LLA diets for 10 weeks prior to mating. The HLA and LLA diets continued until the offspring were sacrificed. 5-weeks-old offspring were subjected to a battery of subsequent, daily behavioral tests (SPT, OFT, three-chamber social interaction test, and FST). They were sacrificed on the day following the last behavioral test.

enclosure was placed in both the first and third chambers. The test rats were allowed to freely access and explore the three chambers through the doorways. There was a 5-min interval before the next phase started. In the 10-min sociability test, a stimulus rat was placed in the wire cage in one chamber, while the wire enclosure in the other chamber remained empty. The test rat was then returned to the middle chamber and allowed to roam around the three chambers freely. Sociability was determined by the preference of the testing rat for the stimulus rat or the empty enclosure. Any direct contact or sniffing of the cage was assessed as evidence of direct exploration or touch of an enclosure or social interest in the stimulus rat. The exploration ratio was calculated as $T_A/(T_A + T_B)$ for the rat and $T_B/(T_A + T_B)$ for the empty enclosure. The exploration index was calculated as $(T_A - T_B)/(T_A + T_B)$, where T_A = time spent exploring the rat and T_B = time spent exploring the empty enclosure. A 5-min interval was given before the final phase.

In the 10-min social novelty test, a new stimulus rat (novel rat) was placed in the empty wire enclosure, and the test rat was allowed to explore the chambers for 10 min. Social novelty recognition memory was assessed by the preference for approaching the novel or the familiar rat. The exploration ratios for the familiar and novel rats were calculated as $T_F/(T_F + T_N)$ and $T_N/(T_F + T_N)$, respectively. The exploration index was calculated as $(T_N - T_F)/(T_N + T_F)$, where T_F = time spent exploring the familiar rat and T_N = time spent exploring the novel rat. The test was videotaped and analyzed by a well-trained researcher manually in a sample-blinded manner.

All animals that went through behavioral tests included in Fig. 4A & D. When the rats did not approach both cages in the sociability test and the novelty test, their ratio and index cannot be computed. Thus, they were excluded from the exploration ratio and index data set. In the sociability phase, one male offspring from the HLA diet group did not explore the empty or the stimulus rat enclosure, so it was excluded from the sociability test, only fourteen offspring were included in Fig. 4B & C. Similarly, two male offspring from HLA-fed litters group did not explore both the familiar and novel rat enclosures, their exploration ratio and index could not be calculated, therefore only thirteen rats for offspring with HLA-fed litters were presented in Fig. 4E & F.

2.3. Statistical analyses

Two-way ANOVA with sex and diet as factors was performed with Tukey post-hoc test to compare differences among treatment groups using Prism 8.0 software (GraphPad Software, USA). A paired *t*-test was used to compare the exploration time and ratio of the novel to familiar rats for each individual rat in the three-chamber social interaction test. Grubbs' test was applied to detect and exclude significant outliers. $P < 0.05$ was considered statistically significant. Data are shown as mean ± SEM. n values represent each individual offspring.

3. Results

3.1. Chronic consumption of HLA diet induced depression-like behavior in male offspring

Male offspring from dams with the HLA diet showed a significant increase in immobility time in the FST, indicating an increase in depression-like behavior (Fig. 2A; $P < 0.05$ vs Males-LLA). The two-way ANOVA indicated a significant main effect of diet on behavioral despair in the FST (effect of interaction: $F_{1,56} = 3.250$, $P = 0.0768$; effect of sex: $F_{1,56} = 2.018$, $P = 0.1610$; effect of diet: $F_{1,56} = 4.063$, $P = 0.0486$). However, the female offspring from dams with the HLA diet was not affected (Fig. 2A; $P = 0.9988$ vs Females-LLA).

The HLA diet did not affect anhedonia-like behavior expression, neither in male nor in female offspring (Fig. 2B; $P = 0.4105$ vs Males-LLA & $P = 0.1278$ vs Females-LLA). The two-way ANOVA showed a significant effect of diet on the sucrose preference; however, there was no significant effect of sex or interaction (Fig. 2B; effect of interaction: $F_{1,56} = 0.2253$, $P = 0.6368$; effect of sex: $F_{1,56} = 3.906$, $P = 0.0531$; effect of diet: $F_{1,56} = 7.173$, $P = 0.0097$).

3.2. Chronic consumption of the HLA diet decreased locomotor activity in the male offspring

Chronic HLA diet significantly reduced the distance travelled of the male offspring in the OFT, suggesting reduced locomotor activity (Fig. 3A; $P = 0.0284$ vs Males-LLA; effect of interaction: $F_{1,56} = 0.8851$, $P = 0.3508$; effect of sex: $F_{1,56} = 3.643$, $P = 0.0614$; effect of diet: $F_{1,56} = 9.758$, $P = 0.0028$). Post-hoc test revealed no significant difference on the time spent in center among groups (Fig. 3B; Males-HLA: $P = 0.3655$ vs Males-LLA; Females-HLA: $P = 0.1284$ vs Females-LLA), while two-way ANOVA analysis indicated a significant main effect of diet on

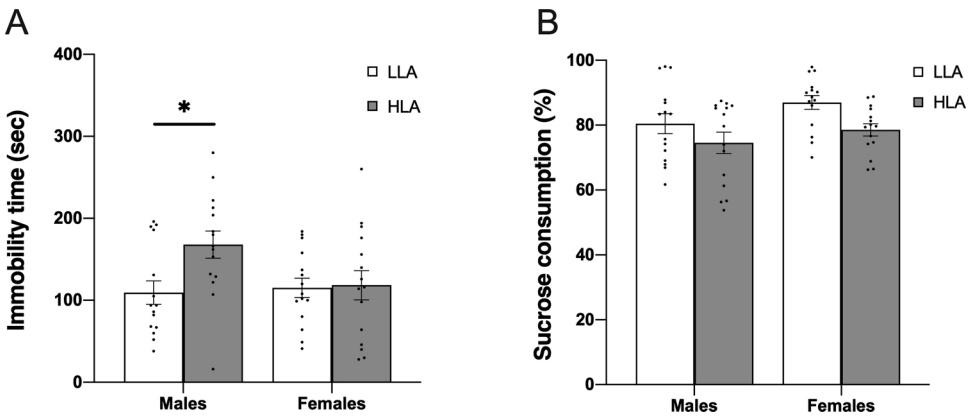

Fig. 2. HLA diet increased depression-like behavior in male offspring, but not in female offspring. A: Male offspring with maternal HLA diet showed increased immobility time in the FST compared with those receiving maternal LLA diet (Tukey post hoc test: $^*P < 0.05$ vs Males-LLA). **B:** The HLA diet did not affect anhedonia-like behavior in both male (Tukey's post hoc test: $P > 0.05$ vs Males-LLA) and female (Tukey post hoc test: $P > 0.05$ vs Females-LLA) offspring in the SPT. n=15 animals per group.

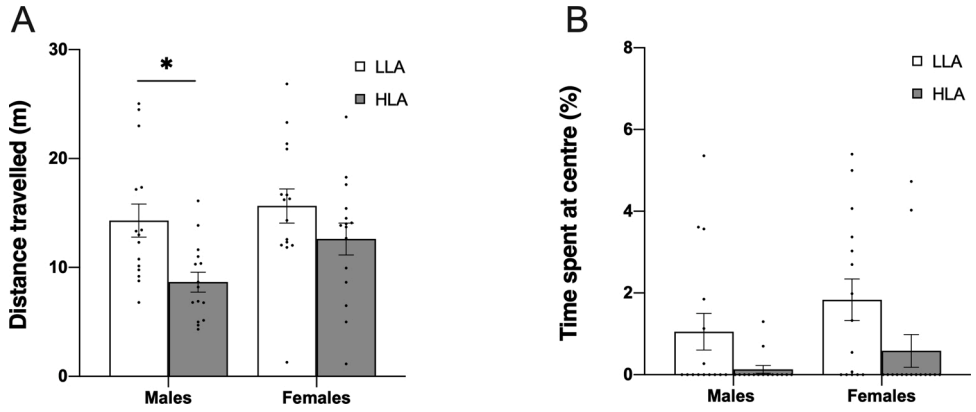

Fig. 3. Maternal HLA diet affected locomotor activity and anxiety-like behavior in the offspring. A: Male offspring with a maternal HLA diet had reduced locomotor activity (Tukey post hoc test: $^*P < 0.05$ vs Males-LLA). However, maternal HLA diet did not show significant effect in the female offspring (Tukey post hoc test: $P > 0.05$ vs Females-LLA). **B:** Both offspring with HLA diet showed no differences in the time spent at the center. n = 15 animals per group.

increasing anxiety-like behavior (effect of interaction: $F_{1,56} = 0.1730$, $P = 0.6791$; effect of sex: $F_{1,56} = 2.408$, $P = 0.1264$; effect of diet: $F_{1,56} = 7.474$, $P = 0.0084$).

3.3. Chronic consumption of the HLA diet did not affect social interaction performance in offspring

In the sociability test, both male and female offspring with chronic LLA diet and female offspring with chronic HLA diet showed a significantly greater preference towards a stimulus rats than an empty enclosure (Fig. 4A, Male-LLA: $t_{14} = 4.2704$, $P < 0.005$; Female-LLA: $t_{14} = 6.6817$, $P < 0.005$; Female-HLA: $t_{14} = 8.2612$, $P < 0.005$). However, male offspring from dams with the HLA diet showed no significant preference to towards stimulus rats (Fig. 4A; Male-HLA: $t_{14} = 2.063$, $P = 0.0582$). For the exploration ratio, both male and female offspring with the maternal LLA diet showed significant preference towards stimulus rat (Fig. 4B; Male-LLA: $t_{14} = 2.3742$, $P < 0.05$; Female-LLA: $t_{14} = 2.3742$, $P < 0.005$). Female offspring but not male offspring from dams with maternal HLA diet showed significantly higher exploration ratio to the stimulus rats (Fig. 4B; Female-HLA: $t_{14} = 10.1763$, $P < 0.005$; Male-HLA: $t_{13} = 0.9317$, $P = 0.3685$). However, two-way ANOVA analysis on the exploration index revealed no significant effect of interaction (Fig. 4C; $F_{1,55} = 0.6259$, $P = 0.4323$) and effect of diet ($F_{1,55} = 0.2007$, $P = 0.6559$), but significant effect of sex ($F_{1,55} = 6.184$, $P = 0.0160$). Post-hoc analysis showed no significant difference in exploration index among groups, suggesting chronic HLA consumption did not affect sociability in both male and female offspring.

In the social novelty test, male offspring from the maternal LLA diet showed preference to novel stimulus rats as shown in exploration time and exploration ratio (Fig. 4D; Male-LLA: $t_{14} = 2.289$, $P < 0.05$; Fig. 4E; Male-LLA: $t_{14} = 3.579$, $P < 0.005$). Both male and female offspring from dams with the HLA diet showed no significant difference in exploration time, ratio, or index to novel stimulus rats compared to the LLA ones (Fig. 4D–F). Two-way ANOVA revealed a significant main effect of diet (Fig. 4F; $F_{1,54} = 5.709$, $P = 0.0204$), but no significant effect of interaction ($F_{1,54} = 0.$ 0.00002041, $P = 0.9964$) and effect of sex ($F_{1,54} = 1.518$, $P = 0.2232$), suggesting that maternal HLA diet did not affect social novelty recognition memory, though there was a trend with lower exploration indexes in offspring from dams with HLA diet.

4. Discussion

An imbalanced PUFA level has been suggested as a risk factor for developing ASD in the offspring [10]. Maternal consumption of HLA increases the n-6:n-3 ratio in the brain and bloodstream [43], which is detrimental to neural development and can induce behavioral deficits in the offspring [44,45]. This study demonstrated that chronic consumption of a diet with HLA during the pregnancy, lactation, and post-weaning period induced behavioral changes in the offspring, with a sex-specific effect on males . They showed increased depression-like behavior, reduced locomotor activity, but were not affected in their social interaction performance. The data revealed that a lifelong intake of the HLA diet can be a risk factor for depression onset in the offspring, with a greater susceptibility for males.

ASD patients have specific behavioral dysfunctions, including impaired sociability, restricted interests, repetitive behaviors, anxiety, and depression [46]. Animal models of ASD exhibit behavioral deficits that resemble ASD, including impaired sociability, restricted interests in

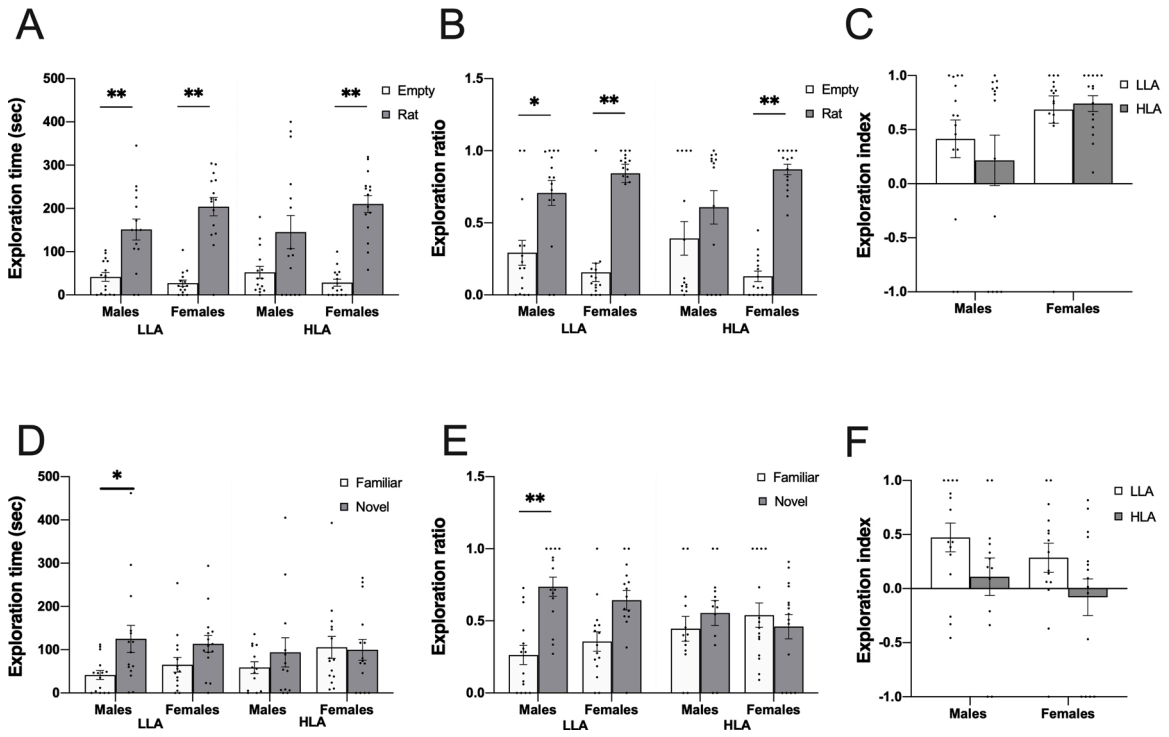

Fig. 4. Effect of HLA diet on sociability and social novelty.
A: Offspring with maternal LLA diet spent more time exploring the cage with a rat (paired *t*-test: $^{**}P < 0.005$ vs empty enclosure); female offspring with maternal HLA diet also explored the rat cage for longer (paired *t*-test: $^{**}P < 0.005$ vs empty enclosure). However, maternal diet with HLA abolished the preferences of the male offspring towards the rat cage (paired *t*-test: $P = 0.0579$ vs empty enclosure). B: In terms of exploration ratio, both male and female offspring with the LLA diet showed a greater preference to interact with a stimulus rat (paired *t*-test: Males-LLA: $^{*}P < 0.05$, Females-LLA: $^{**}P < 0.005$ vs empty enclosure). On the contrary, the HLA diet vanished the preference of male offspring towards the stimulus rat (paired *t*-test: $P = 0.3684$ vs empty enclosure), but not in female offspring (paired *t*-test: $^{**}P < 0.005$ vs empty enclosure). C: Maternal HLA diet did not alter the sociability preference of the female offspring (Tukey post hoc test: $P > 0.05$ vs Females-LLA). A trend towards reduced sociability was shown in the male offspring with HLA diet (Tukey post hoc test: $P = 0.8210$ vs Male-LLA). D: Male offspring from the LLA diet showed a greater preference to interact with a novel stimulus rat (paired *t*-test: $^{*}P < 0.05$ vs familiar rat). Male and female offspring with maternal HLA diet spent an equal amount of time exploring the familiar and novel stimulus rat. Female offspring with maternal LLA diet also showed no preference in exploring either the familiar or novel stimulus rat. E: Male offspring receiving maternal LLA diet showed greater preference to interact with a novel stimulus rat (paired *t*-test: $^{**}P < 0.005$ vs familiar rat), while male offspring receiving maternal HLA diet presented no social novelty preference towards the stimulus rats. In a similar case, female offspring with maternal LLA diet had a slightly higher exploration ratio towards the novel stimulus rat (paired *t*-test: $P = 0.0527$ vs familiar rat), while the one with HLA diet showed no time differences in exploring the familiar and novel stimulus rat (paired *t*-test: $P > 0.05$ vs familiar rat). F: Offspring with maternal HLA diet did not have social novelty preference towards the novel stimulus rat or the familiar stimulus rat. n = 13–15 animals per group.

the surrounding environment, decreased locomotor activity, and increased anxiety and depression [47–51]. Social interaction impairment with less eye contact and response to the surrounding environment is one of the core symptoms of ASD [52]. The three-chamber social interaction test has been commonly used to measure decreased social interaction in rodent models of ASD [41]. The OFT and FST have also been commonly used to measure affective deficits in ASD animal models [53]. LA can pass across the placenta from mother to fetus and affect fetal development directly [54]. Notably, our previous studies have reported that maternal HLA diet modifies placental fatty acid composition, increasing total n-6 and LA concentrations, and decreaseing total n-3 PUFA, ALA, and DHA [30]. Similar changes in PUFA level are also observed in male and female offspring, showing decreased n-3 PUFA and ALA levels in the blood, but increased n-6 PUFA, LA and AA [55]. A chronic HLA diet affects physiology in both mothers and their offspring [28,30,56]. It also significantly changes the placental fatty acid composition, gene expressions of fatty acid, glucose transporters, and placental inflammatory response [30].

Furthermore, a chronic HLA diet alters leptin synthesis and lipogenesis in the maternal adipose tissue, promotes circulating levels of pro-inflammatory cytokines, and alters maternal and fetal plasma fatty acid compositions [28]. A higher ratio of AA:DHA was reported in the plasma and red blood cells of autistic patients when compared to healthy controls [57]. The dietary PUFA composition affects the levels of blood PUFA in that the higher the level of dietary LA, the lower the level of DHA. PUFA serves as the precursor of second messengers responsible for regulating inflammation, immunity, and synaptic plasticity. Notably, the n-3 fatty acid is known to inhibit inflammation, whereas n-6 fatty acid promotes inflammation [18]. Consumption of a high n-6:n-3 diet could increase the risk of depression [58]. Rodent studies have shown that high n-6 concentrations in the brain are associated with depressive phenotypes [59,60]. A further study has demonstrated that a high n-6 diet promotes behavioral deficits. Upon weaning, 15 weeks of excessive n-6 (26 %) and deficient n-3 (< 0.1 %) diet induced depression-like behavior as observed in the FST in male rats [61]. Additionally, exposure to a high n-6 and low n-3 diet in utero resulted in depressive behavior in the FST in offspring with a C57BL6/J background [62]. The current results demonstrated that prolonged exposure to an HLA diet induced depression-like behavior specific to male offspring, but not female offspring. However, conflicting results have been found, including that exposure to a high n-6 diet from fertilization to postnatal day 10 did not induce depression phenotypes in the offspring [63]. It is possible that exposure to an elevated n-6 diet before pregnancy could be critical in programming the susceptibility of offspring to affective behavior. Our results echo the longitudinal clinical findings reporting that LA levels are correlated with an increased risk of depression, especially in males [64]. However, the underlying mechanisms for the sex-specific effect of HLA require further investigation.

A significant decrease in locomotor activity was observed in the male offspring from dams with HLA diet. Animal studies have demonstrated that chronic interesterified fat consumption or a western-based diet resulting in a high n-6:n-3 PUFA ratio can alter brain functions [23,24,65]. Intersterified fat consumption since gestation could modify expression levels of dopamine receptors and dopamine transporter in the striatum and impair locomotion in adult offspring. It is reasonable to speculate that reduction in locomotor activity in our male offspring with chronic HLA consumption could be due to altered dopaminergic function in the striatum. However, this hypothesis warrants further investigation. Furthermore, we observed that female offspring displayed a longer immobility time than male offspring with mothers consuming the LLA diet (control diet). This result is consistent with the literature reporting higher immobility in females than male rodents [66–69]. Sex-specific responses in serotonergic and dopaminergic activation in the hypothalamus and hippocampus could contribute to the higher immobility level observed in females. Forced swimming reduces the conversion of serotonin (5-HT) to metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the hypothalamus and hippocampus of female rats, implicating reduced serotonergic activities. Conversely, forced swimming promotes serotonergic activity in the hypothalamus and increases the hippocampal 5-HT1A mRNA levels in males [67]. Moreover, forced swimming enhances dopaminergic activity in the hippocampus and prefrontal cortex of male, but not female, rats [66], suggesting the differential response between male and female rats to forced swimming. Anxiety-like behavior is often associated with ASD subtypes [70]. Locomotor activity indicates novelty-seeking behavior which is negatively associated with depression and anxiety symptoms in humans and animals [71]. Rodent studies have reported that a maternal diet composed of a high n-6:n-3 ratio increases anxiety-like behavior in the offspring, evidenced by a reduced number of entries to the center of the open field and reduced time spent in the open arm in the elevated plus maze [27,63,72]. Our findings further showed a reduced trend in time spent at the center of the open field of offspring from dams fed with the HLA diet. Reduced locomotor activity in the offspring from mothers consuming an HLA diet was observed in the OFT. Likewise, murine male offspring fed with a high n-6:n-3 ratio diet are more susceptible to anxiety induced by prolonged isolation stress [73]. A chronic HLA diet may impact the sensitivity to anxiety of the offspring.

Social impairment is another prominent feature of autism [74,75]. It has been reported that an n-6-rich maternal diet induces autistic-like sociability deficits in adult offspring [27], and a high n-6 prenatal diet can impair neuronal growth [76]. However, our current results report no significant effect of maternal HLA on social interaction in the three chamber social interaction test, but a significant male specific effect on depression-like behavior. LA is a precursor of eicosanoids, which promote and regulate inflammation [18]. An elevated amount of n-6 fatty acids produces a higher amount of prostaglandins (PGs) [77], which activate the microglia and increase the cell number in response to injuries and brain degradation [78]. Prostaglandin E2 (PGE2), derived from the phospholipid membrane, is responsible for inflammation in the brain [79]; it can also induce the expression of cytokines [80,81]. Increased levels of cytokines in the brain can alter neuronal development and function of the nervous system [82,83], decreasing the number of neurons or dendrites [84], and consequently result in autistic-like traits such as cognitive deficits and learning impairment. One study reported that exposure to HLA decreases cell viability and increases pro-inflammatory cytokines [85], suggesting that HLA levels induce inflammation in the brain [86]. We observed increased neuroinflammation in the hippocampus of male offspring, showing increased microglial activation in the hippocampal dentate gyrus with the HLA diet (data not shown). Changes in neuroinflammation in offspring should be studied further. It is also known that lifelong consumption with interesterified fat which reduces DHA and increases saturated FA and LA incorporation in the rat hippocampus, impairs short-term and long-term memory [23]. The behavioral deficits could be due to increased oxidative stress and decreased hippocampal brain-derived neurotrophic factor (BDNF) and its TrkB receptor expression. Furthermore, lifelong consumption of interesterified fat diet impairs sensorimotor development, decreases dopamine receptor type 2 receptor expression and glial cell line-derived neurotrophic factor (GDNF) levels in the striatum of the adult offspring, suggesting diets that change PUFA composition in the brain could impair brain function. Behavioral deficits observed in the offspring with a lifelong HLA diet could be due to increase inflammation, decreased neurotrophic factors in the presence of a low n-3:n-6 ratio in the hippocampus. Further studies will be expanded to examining structural and molecular changes in the hippocampus, since an impaired hippocampal function could be contributed by impaired adult neurogenesis, decreased dendritic complexity and decreased neurotrophic factors as we observed previously in animal models of depression [36–38].

An emerging new theory suggests that ASD symptoms could be more severe and distinct in males than females [87,88]. The current data indicate that male offspring are more vulnerable to the effect of an HLA diet. This could be because females have a higher conversion rate of DHA, which has a more protective effect in the central nervous system, compared with males [89]. For a healthy young woman, around 9% of dietary ALA could convert to DHA [90], whereas a man could only convert 0–4 % of dietary ALA to DHA [91]. The higher ALA to DHA conversion ability in females has been suggested to be caused by the sex hormone estrogen [92]. Some clinical studies have reported a positive correlation between ASD and a high n-6:n-3 ratio in children [93], whereas others have shown that maternal intake of LA promotes better brain development and decreases the risk of ASD in offspring [94]. Discrepancies have also been noted in animal studies [95]. One study showed that both 23.9:1 and 4.5:1 (n-6:n-3 ratio) diets led to autistic symptoms with anxiety and depressive behavior, as well as learning deficits, but not in rats receiving the 8.6:1 control diet [96], suggesting that an n-6:n-3 ratio that is too high or too low could be detrimental. An optimized balance of n-6:n-3 could be necessary to maintain normal cognitive functions.

5. Conclusion

Emerging data have suggested that a balanced ratio of PUFAs in diet could be essential for maintaining brain health. Our results demonstrated that chronic consumption of an HLA diet throughout the pre-pregnancy, lactation, and post-weaning period, reduced locomotor activity and increased depression-like behavior, specifically in male offspring. There was no significant effect on sociability and social recognition memory, suggesting that lifelong consumption with HLA diet could induce depression-like behavior in a sex-specific manner, but not social behavior. In order to offer a comprehensive understanding of the transgenerational effect of maternal HLA diet on offspring behaviors, future studies should highlight histological, molecular, and cellular changes in the brain, as well as adopting more behavioral assessments for autism-like behavior. Based on the current data and the previous work, these findings suggest that long-term consumption of HLA could potentially increase depression-like behavior and alter locomotor activity in adolescent offspring in a sex-specific manner. Whether these behavioral deficits associated with chronic consumption of the HLA diet are attributed to impaired brain function, e.g., hippocampal or striatum function in offspring, warrants further investigation.

Author statement

We have addressed all reviewers' comments and had substantial revision to improve the manuscript.

The material in this manuscript has not been published, or is it being considered for publication elsewhere, either in whole or in part.

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