Chronically Maternal Exposure to Fenvalerate during Medium-Late Pregnancy Accelerated Cognitive Decline in Middle-Aged Offspring Mice

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Abstract: Epidemiological evidence suggests that pesticide exposure may be involved in the pathogenesis of Alzheimer's disease (AD). However, whether maternal exposure to fenvalerate (FV) can affect AD-type behaviors in middle-aged offspring has not been determined yet. In this study, CD-1 mothers received 7.5 mg/kg FV or corn oil by gavage daily during gestational days 8–18. Body weight of the offspring was recorded at ages 4–34 weeks. A battery of behavioral tasks was conducted at 13-month, and 5-month-old mice were set as a young control. The results showed that there was insignificant difference in body weight between the FV-treated and control mice. Compared to the young mice, the middle-aged control mice exhibited decreased burrowing activity, decreased spontaneous exploration and sensorimotor ability, increased anxiety, and impaired abilities of spatial and non-spatial learning and memory. Compared to the controls, the FV-treated mice exhibited similar species-typical behaviors, locomotor activity, sensorimotor abilities, but increased anxiety, and decreased abilities of learning and memory. Our results suggested that chronically maternal exposure to FV at a low dose in medium-late gestation could accelerate the impairment in the behaviors of learning and memory and anxiety in the middle-aged offspring, which experience a normal duration of development.

Keywords: Aging, Alzheimer's disease, Fenvalerate, Mouse, Pregnancy.

1. INTRODUCTION

With rapid expansion of old population, the incidence of dementia sharply increases, growingly becoming a serious burden to society. Alzheimer's disease (AD), the most common age-related dementia, has affected millions of people around the world [1]. Thus far, the etiology of AD remains unknown, especially for sporadic AD, which accounts for over 95% of the total cases of AD. Increasing evidence indicates that AD could be the consequence of a multifactorial process, involving the interactions of gene-environment [2].

Epidemiological evidence suggests that environmental exposure, such as pesticide exposure, can impact the progression of AD-related symptoms and pathologies [3-6]. However, investigation of these effects in an epidemiological background is particularly difficult because of the large time span between exposure and clinical manifestation of AD, as well as the uncontrolled nature of the exposures (such as number, type, and

frequency). By means of animal models, these difficulties could be resolved, and the changes of AD-related behavior and neuropathology could be detected throughout the disease [7].

Pesticides are world-widely used extensively in agriculture, farming, and other applications. Their acute toxic effects are well known, but the chronic and long-term effects still remain uncertain [8]. A possible association between chronic pesticide exposure and the increased prevalence of AD has lead to a considerable concern of the public health [8]. Pesticide exposure, even at very low doses, may cause neuronal dysfunction, resulting in cognitive decline, including impaired memory and attention [9]. These neurobehavioral disturbances could eventually lead to AD in later life. However, little is known about how pesticide exposure might affect the pathogenesis of AD. Oxidative stress and mitochondrial dysfunction have been proved to be key mechanisms underlying AD induced by pesticide exposure [10,11]. A recent study indicated that pesticide exposure appears be associated with decreased the number of neurons in the hippocampus and cerebral cortex, as well as memory-related synaptic proteins (including synaptophysin and synapsin I), and

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induced tau hyperphosphorylation at multiple AD-related phosphorylation sites, which could result in AD [12]. In recent years, it has been hypothesized that pesticide exposure can perturb AD-associated gene regulation (such as apolipoprotein E, amyloid precursor protein, or clusterin/apolipoprotein J genes) by epigenetic modification (*i.e.*, DNA methylation and chromatin remodeling), leading ultimately to late-onset AD [13].

Pyrethroid pesticides, the major class of insecticides, are commonly used in agriculture and in urban settings due to their high potency and selectivity as nerve poisons and their low amounts of persistent residues. Fenvalerate (FV), a widely used pyrethroid insecticide, has been demonstrated to have behavioral disruptive effects in small rodents [14]. For example, chronic FV exposure in puberty could impair spatial cognition, inhibit aggressive behavior, and increase anxiety activity in mice [15]. Another study of acute exposure at very low-dose found that FV increased anxiety and immobility and decreased ambulation in adult mice, with sustainment of the effects for several hours [16]. These studies have mainly focused on the short-term effects of FV exposure on behaviors, with only few studies indicating that FV exposure may have long-term effects on behaviors [18, 19]. One study indicated that maternal exposure to FV in rats during lactation could decrease exploration ability and motor condition and increase anxiety, but did not affect life habits in pubertal offspring [17]. Another study indicated that perinatal (from gestation day 18 to postnatal days 1–5) exposure to FV reduced sexual behaviors, but did not affect stereotypical and open-field behaviors in adult rats [18]. Nonetheless, the potential effects of chronic long-term FV exposure during early development on AD-type behaviors, especially cognitive function, has not been investigated yet in the murine model.

- The fetal period is one of critical period in early life, and it is characterized by high levels of epigenetic programming and imprinting, enabling disturbances to produce long-lasting effects [19]. Accumulating evidence indicates that most chronic diseases that are expressed in later life, such as AD, often find their origins in the fetal period [20]. Because the epigenome is much more susceptible to environmental factors during the fetal period than during later in life, it is important to avoid adverse environmental factors during this period to prevent adult disease [21].

- Accordingly, the present study aimed to explore whether chronic mother exposure to FV during medium-late pregnancy could alter age-related behavioral phenotypes in the middle-aged offspring mice. The dose of FV used, 7.5 mg/kg, was selected in previous experiments as a dose that did not induce overt signs of maternal toxicity or pup death. A battery of behavioral tests was used to comprehensively assess behavioral changes in the present study, including the tasks of species-typical behavior, anxiety activity, sensorimotor, learning and memory.

2. MATERIALS AND METHODS

2.1. Animals and Treatments

CD-1 mice (7–8 weeks old, 7 male and 7 female mice) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The animals were maintained on a 12-h light-dark cycle in a control temperature (20–25°C) and humidity (50 ± 5 %) environment, with free access to food and water. After they acclimated to the room for one week, the males and females were paired into breeders. The presence of a vaginal plug was set as gestational day (GD) 0. The pregnant mice were randomly assigned to two groups. In the FV treatment group, the pregnant mice were administered FV (7.5 mg/kg, 3 mg/ml dissolved in corn oil, about 1/32 LD_{50} of FV) by gavage daily from GD 8–18. Pregnant mice treated with corn oil only were designated as controls. Natural birth occurred at GD 18–19. On the postnatal day 21, the pups were separated from their mothers, and were housed 3–5 of the same sex in a plastic cage. The environment was the same as in the mother's cage, and the offspring mice received a standard rodent diet and tap water ad libitum throughout all tasks and their lifetime.

2.2. General Procedures

One male and one female offspring mice per litter were measured for body weight with 7 males and 7 females in each group. The body weight of each mouse was recorded at ages of 4, 6, 10, 14, 18, 22, 26, 30 and 34 weeks. At age of 13 months, one male and one female offspring mice per litter were randomly selected to complete the behavioral tests. Meanwhile, 14 CD-1 mice (7 males, 7 females) at age of 5 months old were set as a young control. The battery of tasks used in this study consisted of species-typical behaviors (hording, nesting and burrowing), anxiety behaviors (open field, elevated plus-maze and black-white alley), sensorimotor abilities (beam walking and tightrope), and learning and memory abilities (novel object recognition [NOR]) and radial six-arm water maze [RAWM]). The arranged sequence of these tests was: hording, nes-

ting, burrowing, open field, elevated plus maze, black-white alley, beam walking, tightrope, NOR and RAWM. All tasks were performed in the feeding room. All animal experiments complied with the Guidelines for Animal Treatment set by the Committee of Laboratory Animal Sciences at the Anhui Medical University.

2.3. Apparatuses and Behavioral Tests

The apparatuses and procedures can be found in our previous description [22].

2.3.1. Species-Typical-Behavior Tasks

2.3.1.1. Hording

A wire mesh tube with 60 cm long was attached to a testing box (30 cm × 20 cm × 15 cm), and 50 g of diet food pellets was placed at the far end of tube. Mice were singly housed in the box with wood shaving bedding before the start of the dark cycle, and they were free access to water. After an overnight, the food pellets found in the box were weighed and considered as the weight hoarded.

2.3.1.2. Nesting

Each mouse was housed in the cage with food, water and new sawdust bedding. Each cage was evenly placed with six pieces of white papery cloth (5 cm × 5 cm) for mice to build nests. In the next morning, the naturalistic scoring method was used to score each nest site from 0 to 4. No visible crater of sawdust and papery cloth received a score of 0; sawdust crater alone with no shredded cloth received a score of 1; 2 scores were referred to sawdust crater with shredded or whole papery cloth gathered around and mixed in the crater; 3 was a cup-shaped nest; and 4 was a ball-shaped nest covering the mouse.

2.3.1.3. Burrowing

A plastic cage, similar to the home cage, contained a plastic tube (4 cm in diameter, 10 cm long) and a bright iron tube (5 cm in radius, 12 cm long). Two bars (1 cm height) were transversely placed at the each end of floor in the iron tube, and the space between the bars was filled with exactly 40 g of maize. The mouse was released individually into the plastic cage, and after two hours the displaced contents from the tube was weighed.

2.3.2. Anxiety-Based Tasks

2.3.2.1. Open Field

The open field was an open black wooden box (81 cm × 81 cm floor, and 28-cm wall height), and its floor was divided into 16 equal squares with white lines. A 40-W white bulb was hung at the center of the field 2.80 m above ground. Each mouse was introduced into a corner square, and permitted to explore the environment for 5 min. The parameters recorded included time taken by the animal to cross the first square, number of squares crossed, and peripheral time (the time spent in the 12 peripheral squares). At the end of each test, the area was cleaned with water.

2.3.2.2. Elevated Plus Maze

Two opposite closed arms (30 cm × 5 cm) enclosed with walls (height 15 cm) and two opposite open arms with the same size formed the black maze of cross shape, with a central arena (5 cm × 5 cm). The cross maze was elevated to 80 cm above the ground. An animal was placed in the central arena facing an open arm. The number of entries into the open arms and the time spent on the open arms were recorded for 5 min during a single trial. The maze was thoroughly cleaned with water after each mouse was tested.

2.3.2.3. Black-White Alley

A box (120 cm × 9 cm × 30 cm) was made of narrow galvanized iron, and painted with one half black and the other half white, forming a long black-white alley. An animal was guided into the black half facing the wall. The latency to enter the white alley and the time spent in the black alley were recorded for 90 s.

2.3.3. Sensorimotor Behaviors

2.3.3.1. Beam Walking

A steel rod (long 110 cm, diameter 1.0 cm) was attached to a platform (diameter 20 cm) each end, and was elevated 50 cm above the water surface in a black circular tank (diameter 150 cm). Mouse was placed perpendicularly on the center of the beam and given three successive trials. The maximum duration of the trial was set to 60 s. The balance time was recorded and averaged for the statistical analysis.

2.3.3.2. Tightrope

A cotton rope (2 mm in diameter) was stretched across a tank (100 cm in diameter) half-filled with water. Firstly, the animal was placed in the water for 5 s. During a 60-s trial, each mouse was raised to grasp the center of the rope with forepaws, and then released slowly. Three successive trials on a single day were performed at 30 s intervals. The suspension time was averaged for the statistical analysis.

2.3.4. Cognitive Tasks

2.3.4.1. NOR Test

The Y-shaped apparatus had three equidistant arms, including a start arm (30 cm in length and 10 cm in width) and two object arms (23 cm long, 10 cm wide and 40 cm high), with painted black inside. There was a guillotine door in the start arm closed when mouse was exploring. The apparatus was placed in a sound-proof room and rounded by a black cloth curtain. A video camera was positioned above the apparatus to record the activities of mice. A bulb provided a constant illumination of about 150 lx at the center of the apparatus.

In the acclimation phase, the mice were habituated to the apparatus for 5 min per day for 3 days without objects. In the sample phase, the animal was placed into the Y-shape maze from the start arm with two identical objects in the object arms and allowed to explore for 5 min. After a 10-min or 24-h interval, the animal was allowed to explore the objects for 3 min (2 choice phases), during which one of familiar objects used in the sample phase was replaced with a novel object. The apparatus and objects were cleaned with water at the end of each trial. The mice were regarded to be exploring when they directed their noses to the objects at less than 1 cm and/or touched it with their noses. The exploring-time for the familiar (T_F) and the novel object (T_N) during choice phase were recorded. The preferential index for novel object (PI_N) in the choice phase was calculated as $T_N / (T_F + T_N) \times 100\%$.

2.3.4.2. RAWM

The apparatus was a black circular tank, with 100 cm in diameter and 21 cm in height, and contained six swimming alleys radiating out from a 40-cm-diameter center area. One of the swimming alleys had a submerged escape platform (diameter 10 cm). The pool was filled with 20–21 °C water, and rounded by a white cloth curtain with three black cardboards of different shapes to guide the spatial navigation. The mice underwent five trials a day for 10 days. The location of the platform and experimenter were kept the same on each trial, but the sequences of starting points varied for each trial. On each trial, the mouse was allowed to swim for a maximum of 60 s to find the escape platform. Once locating the platform, the mouse was left there for 30 s prior to the next trial. The number of errors (entry into an incorrect arm) and the escape latencies (time before location on the platform) were recorded and averaged daily for data analysis.

2.4. Statistical Analysis

The data are presented as mean ± standard error of the mean (S.E.M.) for the parametric data or median (25th/75th quartile) for the nonparametric data. A one-way analysis of variance (ANOVA) with treatment or age as an independent variable was used to analyze the normally distributed data, and the Mann-whitney U test was used to analyze non-normal distributed data. For the RAWM task and body weight, the repeated measures analysis of variance (rm-ANOVA) was used, with Fisher's least significant difference test for post hoc analysis. All analyses were conducted with SPSS (13.0) software. Statistically significance was assumed when $P < 0.05$.

3. RESULTS

3.1. Body Weight

The rm-ANOVA results indicated that body weight was similar in the FV-treated and control mice for the combined sexes [$F_{(1,26)} = 0.516$, $P = 0.481$], the males [$F_{(1,12)} = 1.628$, $P = 0.232$], and the females [$F_{(1,12)} = 1.712$, $P = 0.314$], as summarized in Table **1.**

3.2. Species-Typical Behavior Tasks

Only in burrowing was there a significant difference between the middle-aged control mice and the young mice, in terms of the weight burrowed [$F_{(1,26)} = 2.954$, $P = 0.007$], with the middle-aged control mice burrowing less weight than the young ones. The "single-sex" ANOVAs indicated that this age difference in the weight burrowed mainly occurred in the males [$F_{(1,12)} = 2.567$, $P = 0.025$]. There were no significant differences in the weight hoarded [$X^2_{(1,26)} = 0.161$, $P = 0.872$], weight burrowed [$F_{(1,26)} = 1.406$, $P = 0.172$], and nesting quality [$X^2_{(1,26)} = 0.380$, $P = 0.704$] between the FV-treated mice and the same-age controls. Further analysis indicated that FV exposure did not significantly affect the weight hoarded or burrowed or the nesting ability in either the males or the females ($Ps > 0.05$) (see Table **2**).

3.3. Anxiety-Based Tasks

3.3.1. Open Field

The middle-aged control mice spent significantly longer time in the periphery than the young mice when considering the combined sexes [$X^2_{(1,26)} = 4.503$, $P < 0.001$], which was attributed to both the males and the females ($Ps < 0.01$). In addition, the middle-aged control females crossed less squares than the young

Table 1: The Body Weights (g) of CD-1 Mice in the Different Ages with Maternal Fenvalerate Exposure

Age (weeks)	Fenvalerate		Control	
	Male (n=7)	Female (n=7)	Male (n=7)	Female (n=7)
4	20.1 ± 0.92	18.9 ± 0.59	18.9 ± 0.99	18.4 ± 0.73
6	30.1 ± 0.74	24.9 ± 0.74	30.1 ± 0.78	24.8 ± 0.77
10	34.8 ± 0.91	30.1 ± 0.79	36.1 ± 0.98	29.8 ± 0.76
14	36.2 ± 1.05	33.8 ± 1.27	38.1 ± 1.04	33.3 ± 1.17
18	38.2 ± 1.18	34.9 ± 1.72	41.7 ± 1.15	35.7 ± 1.23
22	40.1 ± 1.45	35.9 ± 1.44	43.7 ± 1.45	36.7 ± 1.42
26	41.8 ± 1.42	36.7 ± 1.88	44.2 ± 1.64	37.1 ± 1.87
30	42.1 ± 1.62	36.9 ± 1.97	44.8 ± 1.78	37.9 ± 1.89
34	42.9 ± 1.82	37.5 ± 1.78	45.2 ± 1.82	38.4 ± 1.82

females [$F_{(1,12)}$ = 2.405, P = 0.033]. There were no significant differences in the peripheral time and the squares crossed between the FV group and the control group for the combined sexes [$X^2_{(1,26)}$ = 0.184, P = 0.854; $F_{(1,26)}$ = 0.608, P = 0.443], the males, and the females (Ps > 0.05). However, there was a significantly longer latency in the FV-treated mice than in the control ones [$X^2_{(1,26)}$ = 2.344, P = 0.019], which was mainly attributed to the males [$X^2_{(1,12)}$ = 2.108, P = 0.035] (see Table **2**).

3.3.2. Elevated Plus Maze

The time spent on the open arm and the number of entries to the open arm were not significantly affected by age [$F_{(1,26)}$ = 0.737, P = 0.468; $X^2_{(1,26)}$ = 0.542, P = 0.588] or FV exposure [$F_{(1,26)}$ = 0.940, P = 0.356; $X^2_{(1,26)}$ = 1.139, P = 0.255] for the combined sexes, the males or the females (Ps > 0.05) (see Table **2**).

3.3.3. Black-White Alley

No significant effects of age or FV treatment were found on the latency to enter the white alley [$X^2_{(1,26)}$ = 0.276, 1.138; P = 0.783, 0.890] and the time spent in the black alley [$X^2_{(1,26)}$ = 0.001, 1.287; P = 0.990, 0.198] for the combined sexes, the males or the females (Ps > 0.05) (see Table **2**).

3.4. Sensorimotor Tasks

In the beam-walking task, the middle-aged control mice had a significantly shorter balance time than the young ones in terms of the combined sexes [$X^2_{(1,26)}$ = 3.260, P = 0.001], males [$X^2_{(1,12)}$ = 2.115, P = 0.034], and females [$X^2_{(1,12)}$ = 2.606, P = 0.009]. However, FV treatment did not affect the balance time for the combined sexes [$F_{(1,26)}$ =1.333, P = 0.194], the males

[$F_{(1,12)}$ = 1.197, P = 0.254], or the females [$F_{(1,12)}$ = 0.457, P = 0.647]. In the tightrope task, there was a marginally significant age effect on the suspension time [$F_{(1,26)}$ = 2.032, P = 0.052], which was mainly attributed to the males [$F_{(1,12)}$ = 2.675, P = 0.020]. No significant FV treatment effect on suspension time was seen for the combined sexes [$F_{(1,26)}$ = 0.258, 2.032; P = 0.798, 0.052], the males [$F_{(1,12)}$ = 1.247, P = 0.236], or the females [$F_{(1,12)}$ = 1.727, P = 0.110] (see Table **2**).

3.5. Cognitive Tasks

3.5.1. NOR Test

In the sample phase, the mice in the young control, middle-aged control and FV-treated group spent equal amounts of time exploring either of the two identical objects (data not shown), and thus there was no biased exploratory preference in the three groups of animals.

In the 10-min- and 24-h-delay choice phase, the three group mice spent similar T_N + T_F exploring the two different objects (Ps > 0.05). The middle-aged control mice showed significantly lower PI_N than the young mice, but significantly higher than the FV-treated mice for the combined sexes, the males and the females (Ps < 0.05) (see Table **3**).

3.5.2. RAWM

3.5.2.1. Learning Phase

Latency

Latency progressively decreased daily for all mice combined [$F_{(9,351)}$ = 52.323, Ps < 0.001; see Figure **1A**], suggesting that the mice were able to learn this task. The rm-ANOVA results showed that the middle-aged control mice had significantly longer latencies than the

Table 2: The Performance of CD-1 Mice in Non-Cognitive Tasks

Tasks	Measure	Fevalerate			Control			Young Control		
		Total	Males	Females	Total	Males	Females	Total	Males	Females
Hoarding	Weight hoarded (g)	3.6(0.5/ 15.1)	0.9(0.0/ 11.9)	3.8(1.5/ 26.2)	4.2(0.9/ 6.2)	2.4(0.0/ 12.4)	4.6(1.2/ 5.3)	4.3(2.3/ 9.6)	4.8(2.5/ 9.6)	3.4(1.1/ 9.7)
Burrowing	Weight displaced (g)	2.0 ± 0.2	1.6±0.3	2.3±0.3	1.5 ± 0.2*	1.3±0.3*	1.7±0.3	2.6±0.3	2.4±0.3	2.8±0.5
Nesting	Nesting scores	3.0 (1.7/ 3.0)	3.0(2.0/ 3.0)	2.0(1.0 / 3.0)	2.0(2.0/ 3.0)	2.0(2.0/ 3.0)	2.0(2.0/ 3.0)	2.5(0.7/ 4.0)	4.0(1.0/ 4.0)	1.0(0.0/ 3.0)
Open-field	Latency (s)	7.3(5.8/ 11.3) ▲	8.7(6.1/ 11.9) △	7.3(3.8/ 8.9)	5.4(4.1/ 6.3)	5.5(4.7/ 6.4)	4.4(3.1/ 6.0)	6.0(2.6/ 8.0)	6.0(4.6/ 8.0)	3.0(2.0/ 6.0)
	Peripheral time (s)	268.0 (254.0/ 293.8)	267.1 (247.4/ 297.5)	269 (256.2/ 286.0)	269.2* (253.8/ 297.2)	266.9* (254.8/ 282.5)	294.6* (250.8/ 297.9)	134.0 (82.8/ 160.5)	92 (82/ 145.0)	151.0 (114.0/ 165.0)
	Squares crossed	84.7± 11.1	78.6±16.7	90.8±15.4	94.8 ± 6.6	91.1±10.8	98.4±8.4*	118.7 ± 11.8	101.6±18.2	135.8±13.1
Elevated plus maze	Time on the open arm (s)	21.2 ± 6.5	24.8±9.2	17.6±9.8	30.1 ± 6.9	26.7±7.9	33.5±11.7	40.0 ± 11.4	40.0±14.4	40.0±19.0
	Number of entries to the open arms	1.0(0.0/ 2.0)	1.0(0.0/ 2.0)	1.0(0.0/ 2.0)	2.0(0.7/ 2.2)	2.0(0.0/ 2.0)	1.0(1.0/ 5.0)	1.0(0.0/ 4.0)	2.0(0.0/ 4.0)	1.0(0.0/ 2.0)
Black–white alley	Latency to enter the white alley	14.3 (11.8/24.9)	23.5 (12.2/41.5)	13.4 (8.4/15.1)	14.6 (12.2/22.9)	14.1 (12.8/22.9)	14.8 (10.6/23.2)	15.0 (8.5/21.5)	19.0 (15.0/24.0)	9.0 (6.0/14.0)
	Time spent in the black alley	47.5 (46.6/64.2)	51.8 (46.8/74.4)	46.8 (44.9/63.3)	52.4 (38.1/56.1)	54.7 (38.0/59.2)	48.5 (38.1/52.9)	41.0 (38.7/49.3)	39.0 (38.0/55.0)	41.0 (40.0/46.0)
Beam walking	Balance time (s)	50.91 (35.6/60.0)	45.7 (40.2/53.4)	53.2 (21.9/60.0)	29.7* (11.4/60.0)	28.8* (10.2/60.0)	30.9* (16.8/60.0)	60.0 (60.0/60.0)	60.0 (51.0/60.0)	60.0 (60.0/60.0)
Tightrope	Suspension time (s)	32.0 ± 5.3	37.8±6.7	26.2±8.2	33.7± 4.2	26.0±6.6	41.5±3.4	44.8 ± 3.4	46.2±3.6	43.4±6.2

*Compared to the same aged control mice ▲$P<0.05$ for the combined mice, and $^\triangle P<0.05$ for the same sex; Compared to the young control mice $^\star P<0.05$ for the combined mice, and $^\star P<0.05$ for the same sex.

Table 3: The Performance of CD-1 Mice in NOR Task

Groups		10 min Delay		24 h Delay	
		T_N+T_F (s)	PI_N	T_N+T_F (s)	PI_N
FV	Total	12.43±0.81	0.56±0.051▲	11.86±0.91	0.51±0.054▲
	Male	13.16±0.79	0.58±0.054$^\triangle$	12.05±0.87	0.49±0.064$^\triangle$
	Female	11.70±0.83	0.54±0.064$^\triangle$	11.67±0.93	0.53±0.058$^\triangle$
Control	Total	13.02±0.94	0.67±0.058*	12.41±0.82	0.63±0.051*
	Male	13.65±0.78	0.65±0.061*	13.12±0.96	0.65±0.054*
	Female	12.39±0.92	0.69±0.055*	11.70±0.79	0.61±0.057*
Young control	Total	13.98±1.09	0.79±0.065	13.04±0.67	0.73±0.081
	Male	14.10±0.96	0.77±0.063	13.67±0.82	0.75±0.065
	Female	13.86±0.93	0.81±0.058	12.41±0.92	0.71±0.055

*Compared to the same aged control mice ▲$P<0.05$ for the combined mice, and $^\triangle P<0.05$ for the same sex; Compared to the young control mice $^\star P<0.05$ for the combined mice, and $^\star P<0.05$ for the same sex.

young mice [$F_{(1,26)}$ = 9.709, *P* = 0.004; see Figure **1A**], which was attributable to the females [$F_{(1,12)}$ = 8.158, *P* = 0.014; see Figure **1B**]. In addition, the FV-treated mice showed significantly longer latencies than the same-age controls [$F_{(1,26)}$ = 13.54, *P* = 0.001; see Figure **1A**], for both the males [$F_{(1,12)}$ = 5.548, *P* = 0.036; see Figure **1C**], and the females [$F_{(1,12)}$ = 10.263, *P* = 0.008; see Figure **1B**].
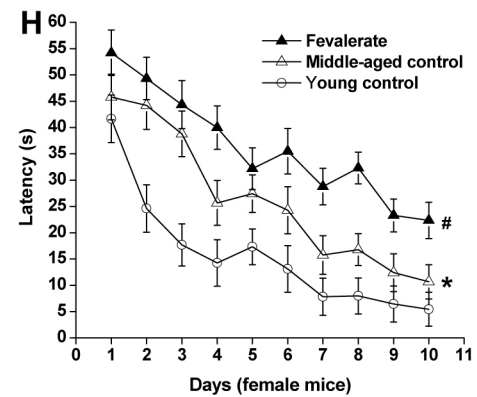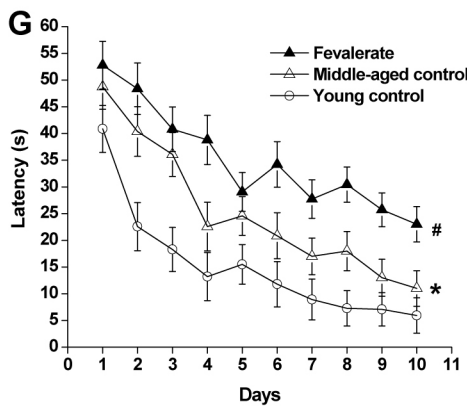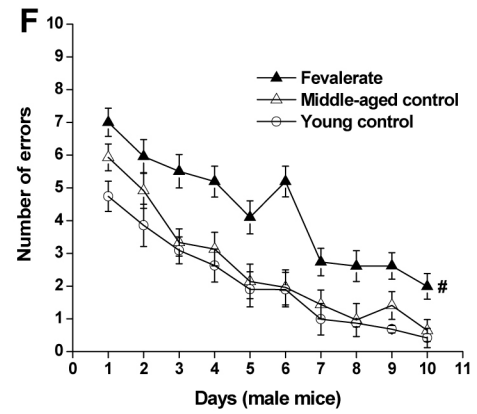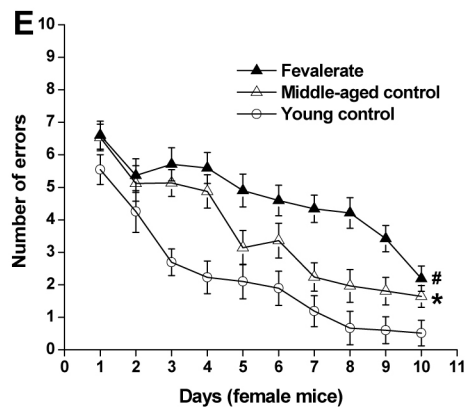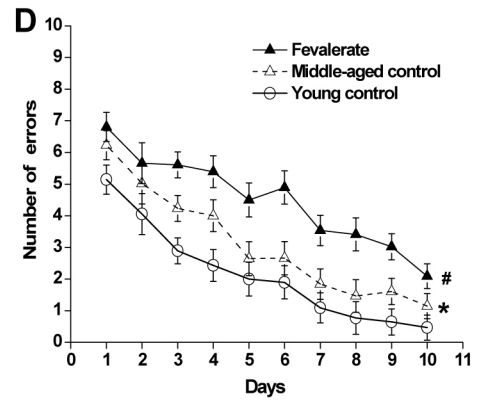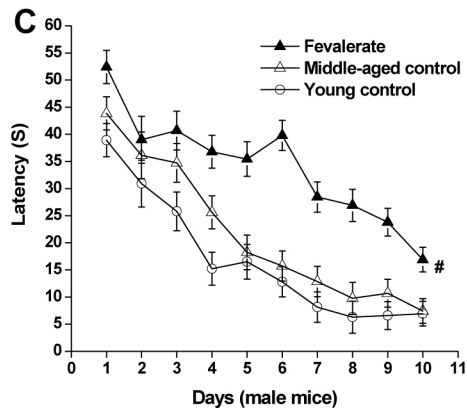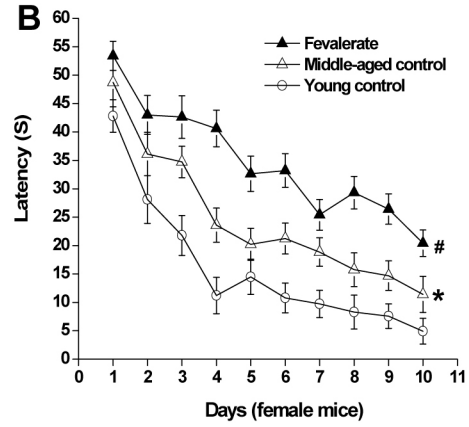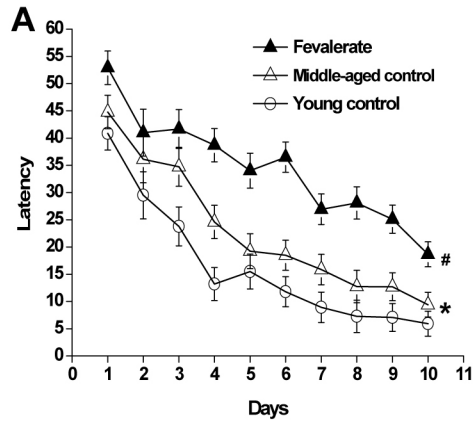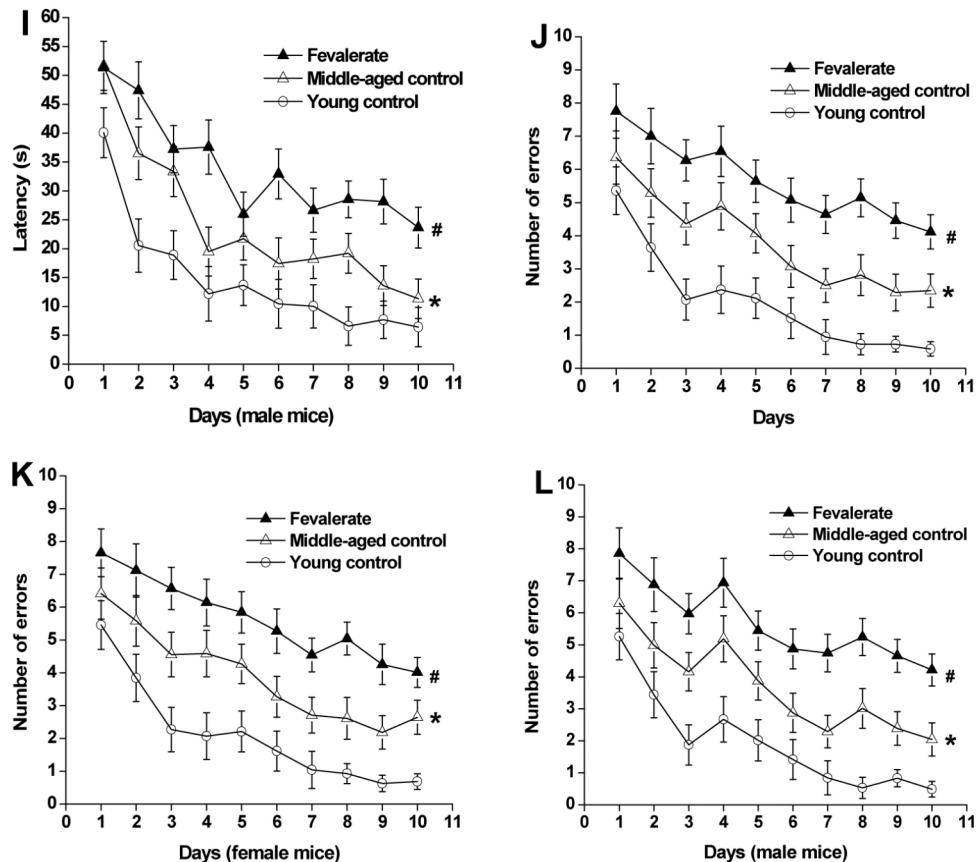
Figure 1: Performances of fevalerate-treated, middle-aged control and the young mice in the RAWM. The latency (**A, B** and **C**) and number of errors (**D, E** and **F**) during the learning phase (trials 1-4), and the latency (**G, H** and **I**) and number of errors (**J, K** and **L**) during the memory phase (trial 5). There were significant age and treatment effects on all measures in the combined sexes, males and females during both the learning and memory phases, except for age effect on the latency and number of errors for the males during the learning phase. The sample number was 42 for the three groups with 14 in each per group. The bars standing represented for S.E.M. Compared to the same aged control mice $^{#}P<0.05$, and compared to the young control mice $^{*}P<0.05$.

Errors

The number of errors progressively decreased with days for all mice combined [$F_{(9,351)}$ = 42.319, $P < 0.001$; see Figure **1D**]. Compared to the young mice, the middle-aged control mice displayed much number of errors [$F_{(1,26)}$ = 6.742, P = 0.015; see Figure **1D**], for the females [$F_{(1,12)}$ = 4.941, P = 0.046; see Figure **1E**], but not for the males [$F_{(1,12)}$ = 2.254, P = 0.159; see Figure **1F**]. The FV-treated mice had more number of errors than the middle-aged controls [$F_{(1,26)}$ = 8.059, P = 0.009; see Figure **1D**], for both the males [$F_{(1,12)}$ = 4.933, P = 0.046; see Figure **1F**] and the females [$F_{(1,12)}$ = 4.420, P = 0.039; see Figure **1E**].

3.5.2.2. Memory phase

Latency

Latency progressively also decreased over time [$F_{(9,351)}$ = 27.817, $P < 0.001$; see Figure **1G**]. The rm-ANOVA results showed that compared to the young mice, the middle-aged control mice showed signifi-

cantly long latencies [$F_{(1,26)}$ = 18.171, $P < 0.001$; see Figure **1G**], for both the males [$F_{(1,12)}$ = 6.755, P = 0.023; see Figure **1I**] and the females [$F_{(1,12)}$ = 13.253, P = 0.003; see Figure **1H**]. Moreover, compared to the middle-aged controls, the FV-treated mice had significantly long latencies [$F_{(1,26)}$ = 12.142, P = 0.002; see Figure **1G**], for both the males [$F_{(1,12)}$ = 6.823, P = 0.023; see Figure **1I**] and the females [$F_{(1,12)}$ = 4.791, P = 0.049; see Figure **1H**].

Errors

The number of errors progressively decreased with days [$F_{(9,351)}$ = 25.729, $P < 0.001$; see Figure **1J**]. The middle-aged control mice had more number of errors than the young mice [$F_{(1,26)}$ = 14.170, P = 0.001; see Figure **1J**], for both the males [$F_{(1,12)}$ = 6.492, P = 0.026; see Figure **1L**] and the females [$F_{(1,12)}$ = 6.951, P = 0.022; see Figure **1K**]. Moreover, the FV-treated mice had significantly more errors than the control mice [$F_{(1,26)}$ = 7.874, P = 0.009; see Figure **1J**], for the males

[$F_{(1,12)}$ = 4.801, *P* = 0.049; see Figure **1L**] and the females [$F_{(1,12)}$ = 3.939, *P* = 0.032; see Figure **1K**].

4. DISCUSSION

In the present study, our results indicated that there were similarities in body weight between the maternal FV-exposure and control CD-1 mice at different time points during the 34 weeks. Compared to the young mice, the middle-aged control mice exhibited decreased burrowing activity in the species-typical behavioral task, spontaneous exploration in the open field and sensorimotor ability in the beam-walking test, increased anxiety in the open field, and impaired abilities of spatial and non-spatial learning and memory in the RAWM and NOR tasks. Compared to the control mice, the FV-treated mice exhibited similar species-typical behaviors in the hoarding, burrowing and nesting tasks, similar locomotors activity in the open field, similar sensor motor abilities in the beam-walking and tightrope tasks, but increased anxiety in the open field, and decreased learning and memory in the NOR (both the 10-min and 24-h delays) and RAWM tasks. These results suggested that the normal middle-aged CD-1 mice exhibited some behavioral changes, consisting of decreased species-typical behavior, spontaneous exploratory activity, and abilities of sensor motor, learning and memory, as well as increased anxiety. The middle-aged CD-1 mice, whose mothers were repeatedly exposed to FV at low doses during the embryo stage, exhibited normal growth development, but accelerated deterioration of learning and memory, and perhaps anxiety.

Generally, there is a gradual decline in cognitive ability during normal aging, most markedly in certain types of memory, such as spatial, working, episodic, and long-term memory [23]. Spatial memory is often assessed in various mouse models of AD, because deficits in this type of memory are highly specific for hippocampal functions (one of the earliest/most severely affected brain regions in AD) [24]. One common method for assessing deficits in hippocampal-based spatial memory is through the use of exploration-based memory tasks such as the Morris water maze and RAWM [25]. Our previous study demonstrated that RAWM was more sensitive for displaying mildly impaired abilities of spatial learning and memory than the Morris water maze task [22]. Another commonly tested type of AD-associated memory is recognition memory, and the most common way to assess this memory in mice is through the use of NOR task [25]. In many AD mouse models, it appears that deficits in spatial working memory (Morris water maze and RAWM) become apparent earlier than do deficits in NOR [26]. However, the onset age of memory impairment remains to be identified. Numerous studies have suggested that some aspects of age-related memory decline begin relatively early in adulthood [27].

CD-1 mice, an outbred strain, are often used in transgenic research. They experience a high mortality rate, which is related to their high susceptibility to some immunopathologies and the high incidence of systemic amyloidosis [28]. Therefore, CD-1 mice may develop premature cognitive decline and could be useful as a model of aging. However, only a few studies have been devoted to age-related behavioral performance in CD-1 mice. These studies indicated that middle-aged (12 months old) CD-1 mice exhibited premature deficits in cognitive function measured in 3D. They also exhibited a great deal of anxiety in both non-social and social situations, spending less time in the open arms of the plus-maze and performing more freezing behavior in response to aggression [29-32]. In another study [33], CD-1 mice displayed decreased exploratory activity in a T-maze task as they aged. Our present study comprehensively evaluated behavioral changes in the middle-aged CD-1 mice, and found that they displayed decreased species-typical behaviors, spontaneously exploratory activity, and activities of sensorimotor, learning and memory, and increased anxiety, some of which (including exploratory activity, anxiety and cognitive function) were consistent with these previous studies [26-29]. These indicated the normal middle-aged CD-1 mouse used in the present study was a qualified control.

Increasing evidence supports the "fetal origins of adult disease" hypothesis, which holds that disadvantageous events during the fetal period increase the risk of later adult diseases, including coronary artery disease, hypertension, obesity, and insulin resistance, as well as degeneration diseases of the nervous system (such as AD) [2, 34]. Experimental evidence indicates that prenatal exposure to some pesticides causes long-term hormonal and behavioral alteration [32, 35, 36], which could eventually lead to AD. FV is particularly toxic to neurons, and it has been demonstrated to have endocrine disruptive effects [18]. A great deal of evidence indicates that endocrine disruption can affect cognitive, sexual, or anxiety-related behaviors in humans and animals, especially during the critical period of development [37]. However, only few studies have been conducted to explore the effects of prenatal exposure to FV on the long-term behaviors, which mainly focusing on exploration, anxiety, and sexual behaviors [18, 19].

- In the present study, the pregnant CD-1 mothers were administrated 7.5 mg/kg of FV by gavage daily during gestation (GD 8-18). Maternal FV exposure did not cause fetal external anomalies or malformations. Moreover, maternal treatment with FV did not influence the physical development of the pups, *i.e.*, no differences were observed in body weight when compared to the controls during adulthood. In addition, our battery of behavioral tests showed that compared to the same-age controls, there were no changes in species-typical behaviors, locomotor activity, or sensorimotor abilities in the FV-treated offspring at the age of 13 months. Thus, we assumed that this pesticide had no toxic effects on general development during adulthood. Studies on laboratory rodents have indicated that pesticide exposure during development, even at low doses, can induce long-term changes in anxiety-like behaviors. For instance, chronic perinatal exposure to low doses of chlorpyrifos led to an increase in anxiety-like behaviors in adult offspring of female mouse [38, 39]. Our present study found that maternal exposure to FV could increase anxiety in middle-aged offspring mice, according to the latency of the open field, a parameter clearly related to anxiety in this task. However, the FV-treated mice and the control mice showed similar anxiety levels in the elevated plus and black-white alley tests. This finding might suggest that the open field task could be more sensitive in assessing FV-treated anxiety than the other two tests.

The acute effects of pyrethroids on learning and memory have been examined in a number of studies [14], but little evidence is available of their long-term effects on learning and memory. For instance, low-level exposure to deltamethrin in utero during GD 14-20 decreased learning and memory performance in rats, and this change could persist even up to 12 weeks postnatal [34]. Low permethrin exposure from postnatal day 6 to 21 in rats impaired spatial working memory during late adulthood [37]. Mosquito repellent exposure during prenatal (GD 1-20) in rats could decrease learning and memory performance at the age of postnatal day 31 [36]. FV is a type-II pyrethroid, and to our knowledge, there have been no reports about the effects of chronic FV exposure in utero on learning and memory in adults. In this study, we simultaneously used NOR and RAWM tasks to assess whether non-spatial and spatial learning and memory abilities were affected by maternal exposure to FV in middle-aged offspring of mice. In the NOR task, the middle-aged CD-1 mice exhibited impaired NOR after both 10-min and 24-h delays, and the FV treatment might aggravate this memory decline in the same tests. These results suggested that maternal exposure to FV declined hippocampus-dependent non-spatial memory in middle-aged offspring. For the RAWM task, our data showed that reduced performance was observed in the FV-treated offspring at 13 months when compared to the control mice of the same age, suggesting that an age-related decline in spatial learning and memory was accelerated by the maternal FV exposure during gestation.

In summary, we reported that CD-1 offspring whose mothers received 7.5 mg/kg FV gavage daily during GD 8–18 showed a relatively normal duration of development, but they displayed accelerated the impairment in hippocampus-dependent learning and memory (spatial and non-spatial) and perhaps anxiety in the middle age. These results meet the behavioral criteria of AD. Further research is needed to explore the underlying mechanism by which FV influences these behavioral changes.

CONFLICT OF INTEREST

The authors have indicated no financial conflicts of interest.

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