

Hippocampal ER Stress and Learning Deficits Following Repeated Pyrethroid Exposure

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ABSTRACT

Endoplasmic reticulum (ER) stress is implicated as a significant contributor to neurodegeneration and cognitive dysfunction. Previously, we reported that the widely used pyrethroid pesticide deltamethrin causes ER stress-mediated apoptosis in SK-N-AS neuroblastoma cells. Whether or not this occurs *in vivo* remains unknown. Here, we demonstrate that repeated deltamethrin exposure (3 mg/kg every 3 days for 60 days) causes hippocampal ER stress and learning deficits in adult mice. Repeated exposure to deltamethrin caused ER stress in the hippocampus as indicated by increased levels of C/EBP-homologous protein (131%) and glucose-regulated protein 78 (96%). This was accompanied by increased levels of caspase-12 (110%) and activated caspase-3 (50%). To determine whether these effects resulted in learning deficits, hippocampal-dependent learning was evaluated using the Morris water maze. Deltamethrin-treated animals exhibited profound deficits in the acquisition of learning. We also found that deltamethrin exposure resulted in decreased BrdU-positive cells (37%) in the dentate gyrus of the hippocampus, suggesting potential impairment of hippocampal neurogenesis. Collectively, these results demonstrate that repeated deltamethrin exposure leads to ER stress, apoptotic cell death in the hippocampus, and deficits in hippocampal precursor proliferation, which is associated with learning deficits.

Key words: deltamethrin; ER stress; adult neurogenesis; water maze; hippocampus

Pyrethroid insecticides are one of the most widely used agricultural and household pesticides and their use has been increasing over recent years since the use of many organophosphates has been restricted by the United States Environmental Protection Agency (Morgan, 2012). Although exposure of the general population to pyrethroids is generally low, significant levels of pyrethroid metabolites, including those of deltamethrin, have been found in human urine and high levels of exposure have been observed in pesticide applicators (Aprea *et al.*, 1997; Berkowitz *et al.*, 2003; Calvert *et al.*, 2003; Kimata *et al.*, 2009; Trunnelle *et al.*, 2014; Ueyama *et al.*, 2009). In general,

pyrethroid toxicity is thought to be low in humans because of efficient metabolic detoxication. Pyrethroids are rapidly hydrolyzed by serum carboxylesterases (Crow *et al.*, 2007); however, human serum lacks carboxylesterase activity, suggesting humans may have a reduced capacity to metabolize pyrethroids. In addition, there are other species differences in deltamethrin metabolism based on hepatic P450s that may influence susceptibility (Godin *et al.*, 2007). Most recently, a physiologically based pharmacokinetic study demonstrated that exposure to the pyrethroid deltamethrin was predicted to generate a 2-fold greater peak brain concentration in humans compared with rats

(Godin *et al.*, 2010). Taken together, these data suggest that humans may be at greater risk of the toxic effects of pyrethroids than previously thought.

Residual neurological symptoms, such as disruption of cognitive function, have been reported in individuals who are highly exposed to pyrethroids (Muller-Mohnssen, 1999). However, the mechanism by which this occurs is not clear. Higher level acute exposure to the type II pyrethroid deltamethrin causes apoptosis both *in vivo* (Wu and Liu, 2000) and *in vitro* (Elwan *et al.*, 2006; Wu *et al.*, 2003). Recent work from our laboratory identified that deltamethrin-induced apoptosis *in vitro* was through activation of the endoplasmic reticulum (ER) stress pathway (Hossain and Richardson, 2011). Persistent activation of the ER stress pathway is linked to progressive loss of neurons leading to neurodegeneration and cognitive dysfunction (Hotamisligil, 2010; Salminen *et al.*, 2009), which may be the result of reduced synaptic plasticity and neuronal viability in the hippocampus (Sama and Norris, 2013). To date, there are no data regarding the ability of pyrethroids to produce ER Stress *in vivo* and whether this would result in neurobehavioral consequences.

Here, we report that repeated deltamethrin exposure elicits hippocampal ER stress and learning deficits in adult mice. Further, these effects were associated with decreased proliferation of cells in the dentate gyrus (DG) of the hippocampus, suggesting a potential effect on adult hippocampal neurogenesis. As these effects occurred at a dose (3 mg/kg) near the lowest observed adverse effect level (LOAEL) of 2.5 mg/kg established by the EPA (2010) in risk assessment of deltamethrin, these findings underscore the need for additional research to determine whether hippocampal neurogenesis is affected and whether or not these effects might occur in the human population.

MATERIALS AND METHODS

Animals. Male C57BL/6 mice, 10 weeks of age, were purchased from Jackson Laboratory (Bar Harbor, ME) and housed 5 per cage on a 12-h light-dark cycle with food and water available *ad libitum*. Animal handling and experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the animal care committee of Rutgers-Robert Wood Johnson Medical School.

Treatment. Deltamethrin (purity 99%, Chem Service Inc., West Chester, PA) was dissolved in corn oil and administered at 0 ($n = 17$) or 3 mg/kg ($n = 17$) to mice via oral gavage every 3 days for 60 days. The dose of deltamethrin used in this study is near the LOAEL of 2.5 mg/kg/day derived from subchronic rat, subchronic dog, and chronic dog studies (Environmental Protection Agency (EPA), 2010) and did not cause any signs of toxicity such as tremor, salivation, ataxia, or decreased body weight gain throughout the experiment. This dosing paradigm was chosen to represent a subchronic exposure, while allowing animals 3 days for clearance of deltamethrin from the body. This was based on recent physiologically based pharmacokinetic (PBPK) study demonstrating that deltamethrin is rapidly metabolized and almost completely eliminated within 48 h of an oral administration at a dose of 3 mg/kg (Godin *et al.*, 2010). At the end of the dosing paradigm, the 34 mice ($n = 17$ control and 17 deltamethrin) were randomly divided into 4 groups for behavioral ($n = 6$ per group), biochemical ($n = 5$ per group), immunohistochemical ($n = 3$ per group) or neurogenesis ($n = 3$ per group) studies. Three days after the last dose of deltamethrin, animals

for biochemical analysis were euthanized with CO₂ and hippocampi were dissected out, frozen on dry ice and stored at -80°C until processing for Western blots.

Western blot analysis. Western blots were performed as described previously (Hossain and Richardson, 2011). Hippocampal tissues were homogenized in tissue homogenization buffer (0.32 M sucrose, 5 mM HEPES; pH 7.4) supplemented with 0.1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Samples were centrifuged at 3500 rpm for 5 min at 4°C . Supernatants were collected and re-centrifuged at 14 000 rpm for 45 min at 4°C . Resulting pellets were re-suspended in 100 μl of homogenization buffer supplemented with 0.1% protease inhibitor. Protein concentrations were quantified using the bicinchoninic acid (BCA) assay (Smith *et al.*, 1985), and 20 μg of protein sample was loaded per lane on 4–12% NuPAGE[®] Novex[®] Bis-Tris Mini Gels (Invitrogen, Carlsbad, CA). After electrophoresis, proteins from the gels were transferred to polyvinylidene difluoride (PVDF) membranes (Invitrogen, Carlsbad, CA). The membranes were incubated in 7.5% non-fat milk in 0.1% Tween-20 containing Tris buffered saline (TTBS) for 1 h at room temperature to block non-specific protein binding sites. The membranes were then incubated overnight at 4°C with anti-caspase-3 (1:500; cat # 9661, Cell Signaling, Danvers, MA), caspase-12 (1:1000; cat # 2202, Cell Signaling, Danvers, MA), anti-GRP78 (glucose-regulated protein 78) (1:1000; cat # 3177, Cell Signaling) and anti-CHOP (C/EBP-homologous protein) (1:500, cat # sc 575; Santa Cruz, CA) primary antibodies. After being washed 3 times with TTBS, the membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The specific antibody-bound protein was detected by SuperSignal[®] West Dura Extended Duration Substrate (Thermo Scientific, Rockford, IL) using Alpha Innotech Fluorochem (San Leandro, CA) imaging system and stored as a digital image. Membranes were then stripped for 15 min at room temperature with Pierce Stripping Buffer (Thermo Scientific, Rockford, IL) and re-probed with a monoclonal α -tubulin antibody to confirm equal protein loading.

Immunohistochemistry. Three days after the final deltamethrin dose, mice were anesthetized with sodium pentobarbital (50 mg/kg) and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS (pH 7.4). Brains were removed and post-fixed in 4% paraformaldehyde at 4°C for 24 h, and then transferred into 30% sucrose with 0.1% sodium azide in PBS. Coronal sections through the entire hippocampus were cut (30 μm) on a freezing, sliding microtome and stored at -20°C in cryoprotectant solution (25% sucrose + 25% ethylene glycol in PBS).

Caspase-3 immunohistochemistry was performed on free-floating sections as described previously (Falluel-Morel *et al.*, 2007). Briefly, every sixth section was taken for immunohistochemical analysis and each section was 180 μm apart. Sections were washed in PBS to remove cryoprotectant; and heat-induced antigen retrieval was performed by steaming sections in 0.1 M citrate buffer (pH 6.0) for 10 min at 97°C . Sections were then incubated with 0.3% hydrogen peroxide in methanol for 10 min to inactivate endogenous peroxidases. Sections were rinsed, blocked in PBS containing 10% normal goat serum and 0.3% Triton X-100 for 1 h and then incubated with rabbit monoclonal anti-caspase-3 (1:250; cat # 9661, Cell Signaling) in PBS containing 2% normal goat serum and 0.3% Triton X-100 overnight at 4°C . After rinsing 3 times with PBS, sections were incubated with biotinylated horse anti-mouse or goat

anti-rabbit IgG (Vector Laboratories, Burlingame, CA) secondary antibody for 1 h at room temperature, followed by incubation in avidin–biotin peroxidase complex (ABC kit; Vector Laboratories) for 1 h at room temperature for amplification. Diaminobenzidine (DAB) fast-tab solution (Sigma, St. Louis, MO) was applied as a chromogen to localize the peroxidase. Sections were then rinsed in PBS, mounted onto slides (VWR, West Chester, PA) and counterstained with 0.25% Toluidine Blue (Sigma, St. Louis, MO).

Caspase-3 positive cell visualization and quantification. Following immunohistochemistry, dark brown caspase-3 positive cells from every sixth coronal section containing hippocampus were visualized using a Carl Zeiss Axiophot El- Einsatz microscope (Zeiss Inc., Germany) with a ProgRes[®] C14plus camera (Jenoptik Optical Systems GmbH) and ProgRes[®]CapturePro 2.8 software (Jenoptik Inc., Easthampton, MA). The positive cells from bilateral hippocampi were manually counted at higher magnification ($\times 40$) from a total of 6 sections per animal and 3 animals per group. The mean for each animal (from the 6 sections) then was averaged to obtain the group mean, and compared by an unpaired t-test with Welch's correction.

Morris water maze. Three days after withdrawal of deltamethrin, mice ($n=6$ per group) underwent Morris water maze training (Morris, 1984). Data were acquired and analyzed with a live video tracking system (Ethovision, Noldus). The maze consisted of a circular pool (100 cm diameter \times 30 cm height) filled with water at $23 \pm 1^\circ\text{C}$ which was made white opaque with powdered milk (Cat # 902887, MP Biomedicals, Solon, OH). The hidden platform (5 cm diameter \times 18 cm height) was submerged 1.5 cm below the water surface. The maze was located in a 2.5×1.4 m room and the geometric signs of triangle, square, and circle were used on the wall as spatial cues. The maze was divided into four quadrants (north, south, east, and west). The platform was placed in the middle of the east quadrant and remained in the same position during acquisition. During acquisition, each animal had 4 trials daily at 1 min intervals for 7 days. Each animal was released from 1 of the quadrants facing the wall of the pool. The order of the start location was rotated every trial as south-west-north-south and each trial lasted until the animal found the platform within 60 s and remained on the platform for 30 s. If the animal failed to find the platform within 60 s, it was placed on the platform for 30 s by the researcher. At the end of each trial, mice were removed, towel dried, and placed in a cage under a heating lamp until the next trial.

A visible platform (2.5 cm above the water surface) test was performed at the beginning of training (day 1) and end of the last session (day 7) to identify whether there were any visual or motor deficits with mice that impaired their performance. Similar results were observed in both tests and data from the last test are presented.

BrdU administration and cell proliferation assay. To assess cell proliferation, mice received daily 100 mg/kg IP injections of 5-bromo-2-deoxyuridine (BrdU) for 6 consecutive days starting 24 h after the last dose of deltamethrin (Figure 6A). BrdU is an analog of thymidine, and incorporates into the newly synthesized DNA in S-phase of the mitosis and labels proliferating cells (Falluel-Morel et al., 2007; Mishra et al., 2012; Winocur et al., 2006). Mice were killed 6 h after the last BrdU injection. Brains were then removed, fixed in 4% paraformaldehyde as above, and $30\ \mu\text{m}$ coronal sections were cut through the entire hippocampus. Free floating sections were incubated in 2N HCl for

30 min at room temperature to denature DNA, followed by incubation with mouse monoclonal anti-BrdU (1:200; cat # 5292, Cell Signaling) according to the procedure described above.

BrdU⁺ cell quantification. Following immunohistochemical labeling, BrdU⁺ cells in the bilateral dentate gyrus subgranular zone (SGZ) of each brain were counted from a total of 6 sections per animal and 3 animals per group. Then the average number of BrdU⁺ cells from the 6 sections for each animal was the averaged to obtain the group mean, and compared by t-test.

Statistical analysis. Statistical analyses were performed using Prism 5.01 software (GraphPad, San Diego, CA). Data are presented as mean \pm SEM. Data for the neurochemical and histological assays, data were analyzed by an unpaired t-test with Welch's correction. Body weights were analyzed by 2-way ANOVA. For the Morris maze, daily blocks from the average of 4 trials within each day were used for analysis. A 2-way repeated measures ANOVA was then used to determine main effects (treatment) with day as the repeated measure and latency as the dependent variable.

RESULTS

General Appearance and Body Weight

Mice treated with deltamethrin for 60 days showed no signs of toxicity such as tremor, salivation, and ataxia. Likewise, deltamethrin did not cause deficits in weight gain, as animals in both groups gained weight over time at a similar rate (Figure 1).

Activation of the ER Stress Pathway in Hippocampus Following Repeated Exposure to Deltamethrin

To determine whether deltamethrin induced ER stress *in vivo*, ER stress-related proteins in the hippocampus were quantified by Western blot analysis 3 days after final exposure. Repeated exposure to deltamethrin significantly increased the ER stress-related proteins CHOP (131%) and GRP78 (96%) in hippocampus (Figure 2A and B).

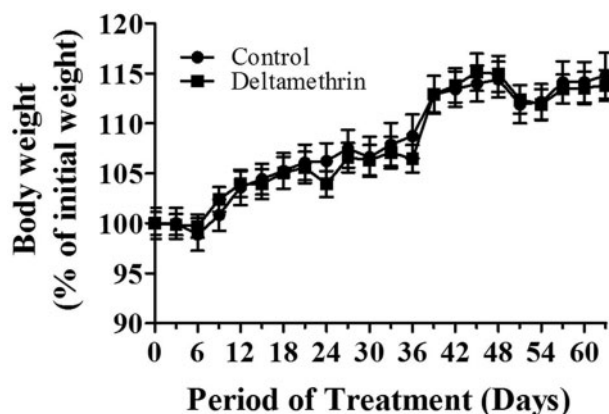


FIG. 1. Repeated deltamethrin exposure does not cause changes in weight gain during treatment. Body weight was recorded prior to dosing. The initial average body weight for control animals was 30.08 ± 0.47 (g) and for deltamethrin animals was 28.68 ± 0.17 (g). The values represent mean \pm SEM from 12 to 14 animals per group.

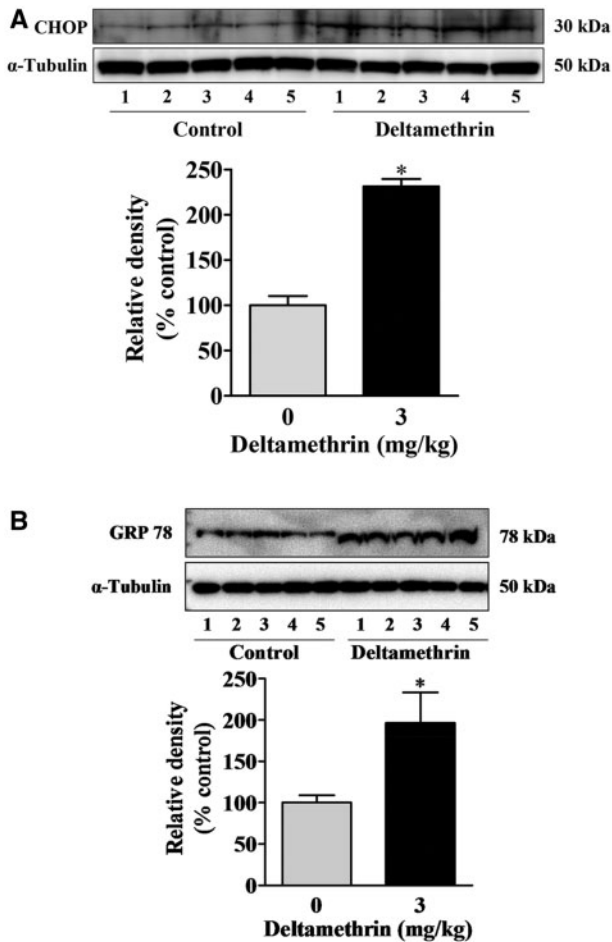


FIG. 2. Repeated deltamethrin exposure causes ER stress in the hippocampus of adult mice. Induction of CHOP (A) and GRP78 (B) proteins at 3 days following last deltamethrin exposure was determined by Western blot analysis. α -Tubulin was used as loading control. Relative densities are presented in bar graphs and representative blots are provided above the graphs. The values represent mean \pm SEM from 5 animals per group. Asterisk indicates significantly different from control ($t=10.01$; $df=8$; $P<.001$ for CHOP and $t=2.53$; $df=8$; $P=.02$ for GRP78).

Repeated Exposure to Deltamethrin Causes ER-Stress Mediated Apoptosis in the Hippocampus

We measured protein levels of hippocampal caspases, as ER stress-mediated apoptotic death signaling can occur through the sequential activation of caspase-12 and subsequently caspase-3. A 2-fold increase in caspase-12 was found in the hippocampus of deltamethrin-treated animals (Figure 3). Deltamethrin exposure also significantly increased the protein levels of activated caspase-3 by 50% in the hippocampus as compared with control (Figure 4A). To further define the localization of cells undergoing apoptosis, we performed immunohistochemistry to examine the dentate gyrus SGZ. There was a 4-fold increase in the number of cleaved caspase-3 immunoreactive cells observed in the dentate gyrus following deltamethrin exposure, primarily localized to the SGZ (Figure 4B).

Impairment of Learning in Adult Mice Following Repeated Treatment with Deltamethrin

To determine whether deltamethrin exposure and ER stress were associated with cognitive dysfunction, hippocampal-dependent learning was evaluated in mice using the Morris

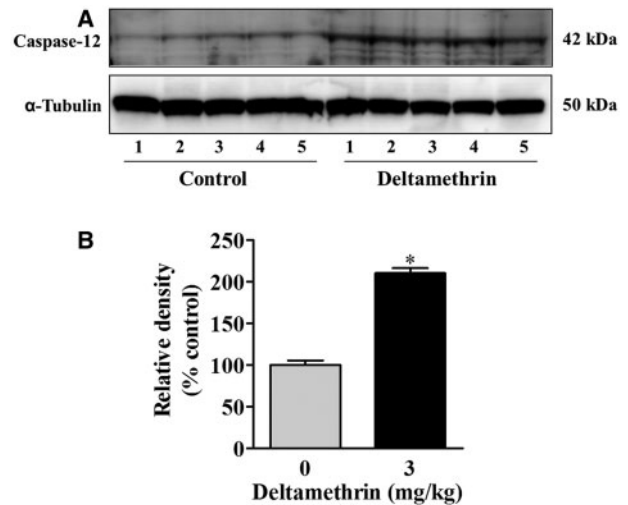


FIG. 3. Repeated deltamethrin exposure activates caspase-12 in the hippocampus of adult mice. A, Deltamethrin increased protein levels of caspase-12 in the hippocampus. B, Relative densities from representative blots are presented in bar graphs. α -Tubulin was used as loading control for Western blot analysis. The values represent mean \pm SEM from 5 animals per group. Asterisk indicates significantly different from control ($t=12.98$; $df=8$; $P<.0001$).

water maze. In this task, animals were trained to find the hidden platform in a pool of opaque water. Mice learned the location of the platform by using visual cues outside the maze for 7 days. Control animals showed daily improvements in their abilities to find the hidden platform during the acquisition phase, whereas deltamethrin-treated animals showed an impaired acquisition process (Figure 5A). The difference between groups was not the result of motor or visual impairment, because neither swim speed (Figure 5B) nor visual acuity (Figure 5C) was affected by deltamethrin treatment.

Repeated Deltamethrin Exposure Decreases Cell Proliferation in the Dentate Gyrus

To examine the effects of deltamethrin on cell proliferation, mice were given a single BrdU injection daily for 6 days and sacrificed 6 h after the last BrdU injection (Figure 6A). Deltamethrin significantly reduced the number of BrdU-positive cells (30%) in the dentate gyrus (Figure 6B).

DISCUSSION

ER stress is a significant contributor to neurodegeneration and cognitive dysfunction in a variety of diseases, including Alzheimer's disease, Parkinson's disease, and Amyotrophic lateral sclerosis (Hoozemans et al., 2009; Lindholm et al., 2006; Prell et al., 2014; Salminen et al., 2009; Zhang et al., 2013). Previously, we reported that the pyrethroid pesticide deltamethrin causes ER stress and apoptosis *in vitro* (Hossain and Richardson, 2011). In this study, we demonstrate that repeated exposure to low levels of deltamethrin causes hippocampal ER stress and deficits in learning in adult mice. These deficits were accompanied by a marked reduction of BrdU⁺ cells and increased cell death in the hippocampal dentate gyrus, suggesting that repeated deltamethrin exposure may inhibit hippocampal neurogenesis by reducing neuronal progenitor cell proliferation.

To evaluate ER stress following long-term deltamethrin exposure to mice, we measured the ER-specific chaperone GRP78 and the transcription factor CHOP in the hippocampus.

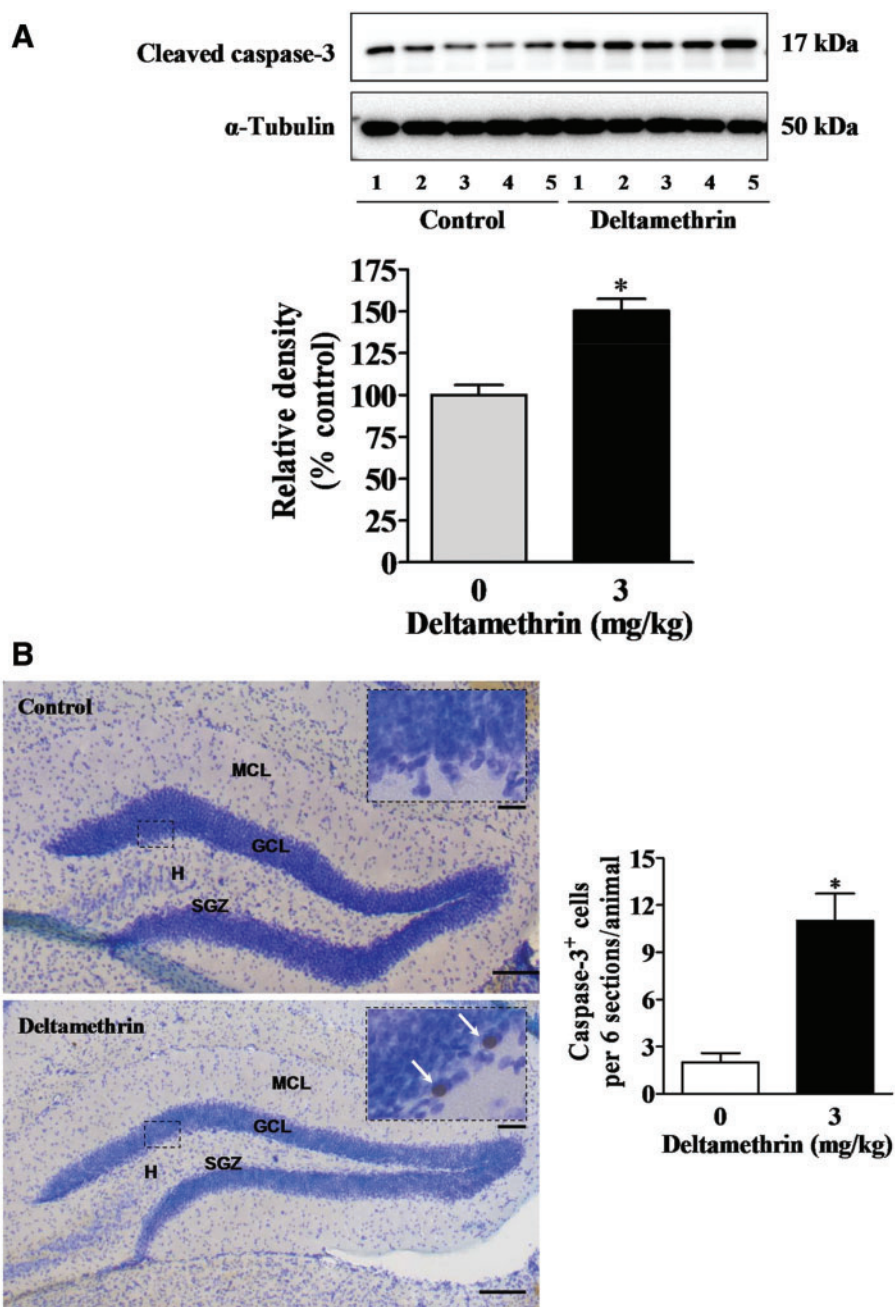


FIG. 4. Repeated deltamethrin exposure activates caspase-3 in the hippocampus of adult mice. **A**, Western blot analysis was used to assess cleaved caspase-3 protein levels in the whole hippocampus 3 days after the last deltamethrin exposure. α -Tubulin was used as loading control. Relative densities are presented in bar graphs and a representative blot is provided above the graph. The values represent mean \pm SEM from 5 animals per group. Asterisk indicates significantly different from control ($t = 5.35$; $df = 8$; $P = .003$). **B**, Caspase-3 immunoreactive cells were visualized by immunostaining in the dentate gyrus (DG) of hippocampus. Arrows point to caspase-3⁺ labeled cells. H, Hilus of the dentate gyrus; GCL, granule cell layer; SGZ, sub granular zone; MCL, molecular cell layer. Scale bar = 1600 μ m (B) and 200 μ m in inset. Data are from 3 animals per group. Asterisk indicates significantly different from control ($t = 4.93$; $df = 4$; $P = .008$).

GRP78 and CHOP are normally expressed at very low levels under physiological conditions but they are increased under conditions of ER stress (Oyadomari and Mori, 2004). CHOP decreases expression of the anti-apoptotic molecule Bcl-2 (Xu *et al.*, 2005) and increases the expression of various pro-apoptotic molecules including DR5, TRB3, and GADD34 in ER-stressed cells (Zhang and Kaufman, 2008). The balance between pro-apoptotic and anti-apoptotic molecules plays a role in regulating the transition from a protective to an apoptotic response.

Our results demonstrate that repeated exposure to low levels of deltamethrin caused ER stress as both GRP78 and CHOP were significantly increased in the hippocampus.

Activation of caspase-3 is a critical event in the execution phase of apoptosis involving pesticide exposure and occurs through mitochondrial dysfunction or ER stress pathways, depending on the origin of the death stimulus (Choi *et al.*, 2010; Hossain and Richardson, 2011; Kitazawa *et al.*, 2003; Ramachandiran *et al.*, 2007; Tait and Green, 2010). In our

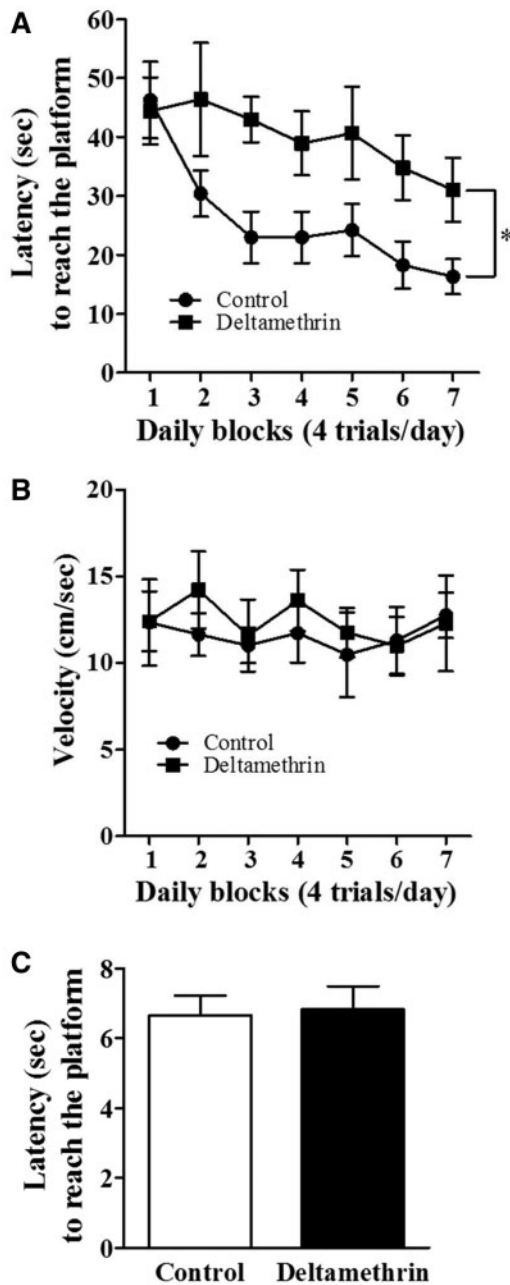


FIG. 5. Repeated deltamethrin exposure causes deficits in hippocampal-dependent learning in adult mice. A, Latency to find hidden platform; B, Swim speed; and C, Visual platform test. Data represent mean \pm SEM of 6 individual animals. Asterisk indicates significant treatment effect ($F=23.70$; $df=1,6$; $P=.0001$).

previous study, we found that deltamethrin caused apoptosis in neuroblastoma cells through the ER stress pathway (Hossain and Richardson, 2011). Caspase-12 is an up-stream cysteine protease normally found in an inactive form in the ER and activated during ER stress (Nakagawa et al., 2000). In the ER stress pathway, apoptotic cell death is different from the intrinsic pathway because caspase-12 can directly activate the downstream caspase-9 without release of cytosolic cytochrome c from mitochondria (Hossain and Richardson, 2011, Morishima et al., 2002). In this study, an increase in activated caspase-3 was accompanied by up-regulation of caspase-12 in the hippocampus of deltamethrin-treated mice, supporting the hypothesis

that deltamethrin causes apoptotic neuronal cell death in hippocampus through activation of the ER stress pathway, similar to that observed *in vitro* (Hossain and Richardson, 2011).

Cumulative exposure to pesticides is thought to pose considerable health risks, including behavioral dysfunction (Rohlman et al., 2007), yet few data are available regarding the potential effects of pyrethroid pesticides. There are two previous studies involving deltamethrin exposure of adult rats that reported cognitive effects in the Y-maze (Husain et al., 1996) and Morris Water Maze (Chen et al., 2012), but both used much higher doses, 7 and 12.5 mg/kg/day. Here, we found that mice repeatedly exposed to deltamethrin exhibited deficits in hippocampal-dependent learning as assessed by the Morris Water Maze. Our results further demonstrated that animals were not impaired in the visible platform task, indicating that the deficit in learning was not the result of impaired vision or motor function following deltamethrin treatment. Taken together with our finding of increased ER stress, this finding is consistent with recent literature data reporting that sustained ER stress is associated with learning deficits (Hotamisligil, 2010; Salminen et al., 2009). Additionally, Zhang et al. (2013) reported that ER stress-mediated apoptosis in hippocampus produces a deficit in learning in diabetic rats. Thus, our data suggest that persistent hippocampal ER stress following long-term low-levels of pyrethroids exposure may contribute to neuronal dysfunction leading to learning deficits in mice.

It is becoming increasingly recognized that exposure to environmental toxicants, including pesticides, can lead to disruption of neurogenesis and cognitive deficits, particularly following developmental exposures (Falluel-Morel et al., 2007; Mishra et al., 2012; Ojo et al., 2014; Sokolowski et al., 2013). Further, disruption of hippocampal neurogenesis is an important mediator of cognitive function (Winocur et al., 2006; Zhao et al., 2008). The adult-born cells in DG migrate to the granule cell layer and differentiate into mature neurons whose projections along the mossy fibers extend to the CA3 region in the hippocampus (Cameron et al., 1993; Stanfield and Trice, 1988; Thuret et al., 2009). Other studies demonstrate that adult-born neurons in the DG are functionally integrated into the existing neuronal circuitry and contribute to hippocampal-dependent learning and memory (Lemaire et al., 2000; Lu and Chang, 2004; Snyder et al., 2005; Thuret et al., 2009; Zhao et al., 2008). Here, we found that deltamethrin decreased the number of BrdU⁺ cells, those engaged in mitotic S-phase, in the DG of hippocampus, suggesting that deltamethrin may reduce hippocampal neurogenesis by damaging the neuronal progenitor cells (NPCs) which are thought to be involved in learning processes in the adult hippocampus (Falluel-Morel et al., 2007; Shors et al., 2002; Snyder et al., 2005). However, additional studies will be required to determine the relative contributions of reduced cell proliferation and/or enhanced cell death in the hippocampal abnormalities that follow deltamethrin exposure.

In conclusion, this study provides evidence that long-term exposure to relatively low levels of deltamethrin causes ER stress and apoptotic cell death in the hippocampus of adult mice. This was accompanied by a significant reduction of cell proliferation and increased apoptosis in the dentate gyrus of deltamethrin-treated mice. Therefore, it is conceivable that the impairment of hippocampal neurogenesis by deltamethrin may contribute to the observed deficits in learning in mice. In addition, the hippocampus seems may be particularly vulnerable to pyrethroids, as Na⁺ current and Na⁺ channel density is higher in the hippocampus than other brain regions (Xia et al., 2003). Taken together, these findings raise a concern that long-term

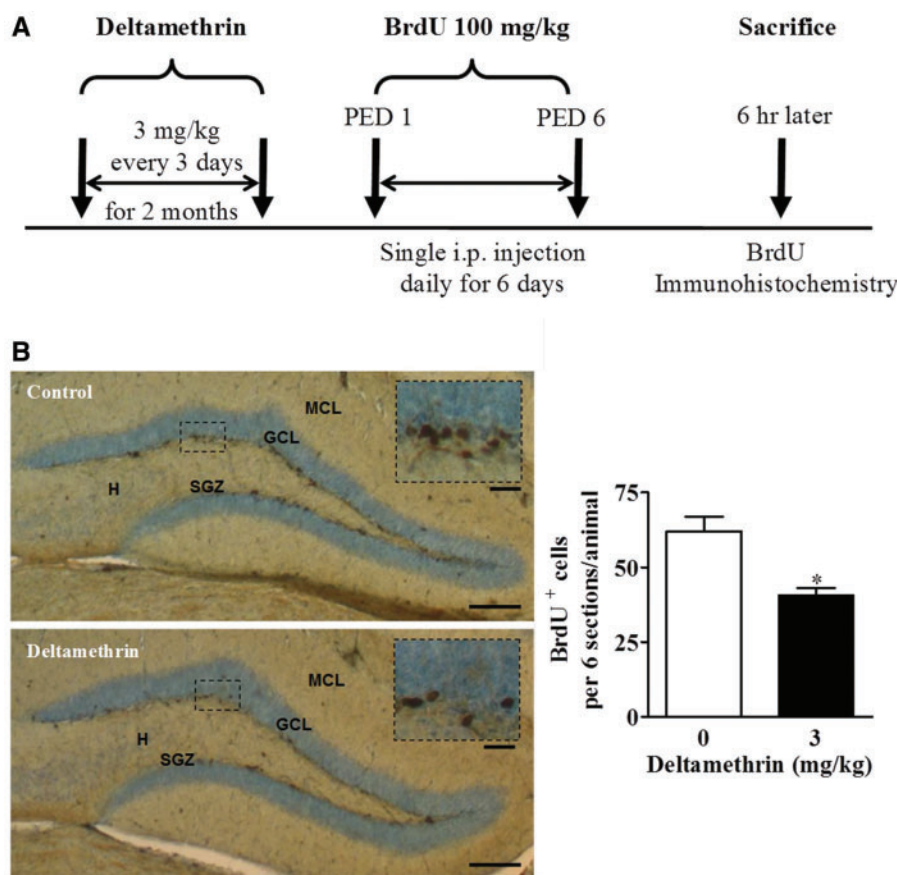


FIG. 6. Repeated deltamethrin exposure reduced the number of BrdU⁺ cells in the dentate gyrus (DG) in hippocampus of adult mice. A, Experimental paradigm used to examine proliferation; PED, post-exposure day, B, Immunostaining of BrdU (dark brown) in the DG region of the hippocampus. BrdU-positive cells in DG were significantly decreased following long-term low-level deltamethrin exposure to adult mice. The values represent mean cell number \pm SEM per section from 6 sections per animal and 3 animals per group. Asterisk indicates significantly different from control ($t = 3.91$; $df = 4$; $P = .02$). H, hilus; GCL, granule cell layer; SGZ, sub granular zone; MCL, molecular cell layer. Scale bar = 1600 μ m (B) and 200 μ m in inset.

human exposure to pyrethroid pesticides could contribute to deficits in learning. Further studies are required to confirm impairment of hippocampal neurogenesis and identify the precise mechanism(s) by which long-term adult exposure to low levels of pyrethroid pesticides contribute to deficits in learning.

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