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Dietary cholesterol concentration affects synaptic plasticity and dendrite spine morphology of rabbit hippocampal neurons

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Abstract

Previous studies have shown dietary cholesterol can enhance learning but retard memory which may be partly due to increased cholesterol levels in hippocampus and reduced afterhyperpolarization (AHP) amplitude of hippocampal CA1 neurons. This study explored the dose-dependent effect of dietary cholesterol on synaptic plasticity of rabbit hippocampal CA1 neurons and spine morphology, the postsynaptic structures responsible for synaptic plasticity. Field potential recordings revealed a low concentration of dietary cholesterol increased long-term potentiation (LTP) expression while high concentrations produced a pronounced reduction in LTP expression. Dietary cholesterol facilitated basal synaptic transmission but did not influence presynaptic function. DiI staining showed dietary cholesterol induced alterations in dendrite spine morphology characterized by increased mushroom spine density and decreased thin spine density, two kinds of dendritic spines that may be linked to memory consolidation and learning acquisition. Dietary cholesterol also modulated the geometric measures of mushroom spines. Therefore, dietary cholesterol dose-dependently modulated both synaptic plasticity and dendrite spine morphologies of hippocampal CA1 neurons that could mediate learning and memory changes previously seen to result from feeding a cholesterol diet.

Keywords

Cholesterol; Hippocampus; Synaptic plasticity; Dendritic spine; Morphology

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1. Introduction

Cholesterol is crucial for maintaining normal brain function by regulating synapse formation, neurotransmission, and synaptic plasticity. A significant body of research has demonstrated dietary cholesterol can modulate learning and memory in different behavioral paradigms including water maze (Dufour et al., 2006), radial arm maze (Granholt et al., 2008), fear conditioning (Heyward et al., 2012), avoidance learning (Thorne et al., 1990), and eyeblink conditioning (Darwish et al., 2010; Sparks and Schreurs, 2003). Some of these studies show dietary cholesterol improved learning and memory (Dufour et al., 2006; Schreurs et al., 2003) while others show a cognitive deficit (Darwish et al., 2010; Sparks and Schreurs, 2003) that is accompanied by neuropathological changes especially when copper was added to the drinking water (Sparks and Schreurs, 2003; Woodruff-Pak et al., 2007). Further studies confirmed that the effects of dietary cholesterol on learning and memory may be dependent on the amount and duration of dietary cholesterol (Schreurs et al., 2007a; Schreurs et al., 2012), animal age and species (Dufour et al., 2006; Granholt et al., 2008). Therefore, there is evidence for an effect of dietary cholesterol on behavior although the consequences may be either beneficial or detrimental and the mechanisms are still unclear.

The hippocampus plays a critical role in learning and memory and the effects of dietary cholesterol on learning and memory may be related to its actions on the hippocampus. Dietary cholesterol can model hypercholesterolemia and data from epidemiological, clinical, and animal studies have shown an association between cholesterol levels and cognitive performance in Alzheimer's disease (Lesser et al., 2009), the elderly (West et al., 2008), healthy middle-aged women (Henderson et al., 2003), and rabbits fed with cholesterol (Schreurs et al., 2012). However, the relationship between cholesterol levels and cognitive function has been inconsistent. For example, if cholesterol has a detrimental effect on learning and memory, lowering cholesterol levels would be of benefit, but there is no good evidence that lowering cholesterol with statins ameliorates the failure of learning and memory in dementia (Sparks et al., 2010; Stuart et al., 2013).

In order to explain the mixed results of dietary cholesterol on cognitive function, it is necessary to explore how dietary cholesterol regulates cellular properties that are involved in the process of learning and memory. To date there are no reports showing dietary cholesterol concentrations influence synaptic plasticity and dendritic spine morphology of hippocampal CA1 neurons, which may underlie the complicated cognitive changes observed in animal and clinical studies.

2. Results

In order to investigate the effect of dietary cholesterol concentrations on short-term and long-term synaptic plasticity of hippocampal CA1 pyramidal neurons, field potential recordings were made in hippocampal slices from rabbits fed with either normal chow or cholesterol chow.

2.1. Input-output curve and Pre-synaptic function

An input-output curve was constructed with stimulus intensity versus the slope of EPSP elicited in response to increasing intensities of stimulation. Figure 1A exhibited the typical traces of EPSP, which were evoked at a stimulus intensity of 100 μ A, from Chol 0, Chol 0.5, and Chol 2.0 groups, respectively. As shown in Figure 1B, the mean slope of EPSP increased with stronger intensity of stimulus, and rabbits fed with dietary cholesterol exhibited greater EPSP slope. This was confirmed by an analysis of variance that revealed group differences at stimulus intensity of 50 μ A [$F(2,66) = 21.81, p < 0.001$] and 100 μ A [$F(2,66) = 8.17, p < 0.01$]. A trend analysis indicated the data were fit by a linear model at these two stimulus intensities [all $p < 0.001$]. Post Hoc comparisons revealed the group effects were attributable to a difference between Chol 2.0 group and both Chol 0 and Chol 0.5 groups [all $p < 0.05$]. This suggests dietary cholesterol may be able to facilitate basal synaptic transmission in the Schaffer collateral commissural pathway of rabbit hippocampus. Similar results were seen in Figure 1C showing functions for stimulus intensity versus the amplitude of evoked fiber volley, but statistical analysis did not reveal any group differences. This indicates dietary cholesterol may not affect presynaptic responses.

PPF was used to assess pre-synaptic short-term synaptic plasticity. Figure 1D shows the slope ratio of EPSP₂/EPSP₁ versus the interstimulus intervals was maximal at an interval of 50ms and recovered at an interval of 350ms. When compared to Chol 0, the Chol 0.5 group showed a slight increase in the slope ratio of EPSP₂/EPSP₁ at 50ms interstimulus interval, whereas Chol 2.0 group exhibited a slightly decrease. However, statistical analysis of EPSP₂/EPSP₁ revealed no group differences. This supports the contention that dietary concentrations may not significantly influence presynaptic function in hippocampus and thus pre-synaptic function may not be the major contributor to the altered behavioral performance induced by dietary cholesterol concentrations (Schreurs et al., 2003; Schreurs et al., 2007b; Schreurs et al., 2012; Schreurs, 2013).

2.2. Post-synaptic function

LTP was used to assess the long-term synaptic plasticity mediated by post-synaptic function. Field potential recordings were made in the stratum radiatum of CA1 hippocampus in response to stimulation of the Schaffer collateral-commissural pathway.

As shown in Figure 2, rabbits in Chol 2.0 group showed reduced LTP expression (average level of LTP was $26.63 \pm 6.5\%$) compared to Chol 0 ($67.27 \pm 4.4\%$) whereas rabbits in Chol 0.5 group demonstrated an increase in LTP expression ($94.64 \pm 4.5\%$). This was confirmed by an analysis of variance which revealed group differences [$F(2,290) = 15.10, p < 0.001$]. A trend analysis indicated the data were fit by a quadratic model [$p < 0.001$]. Post Hoc comparisons revealed the group effects were attributable to a significant difference between Chol 2.0 group and both Chol 0 and Chol 0.5 groups [all $p < 0.001$]. Therefore, our data suggest dietary cholesterol concentrations can modulate the expression of LTP in the hippocampal CA1 neurons, with a low dose increasing LTP expression while high doses leading to a pronounced reduction in LTP expression.

2.3. Dendrite spine morphology

In addition to the synaptic functional changes, we used DiI staining to explore whether there were structural changes in dendrite spine morphologies induced by dietary cholesterol concentrations. Table 1 summarizes the spine morphological changes in apical dendrites of hippocampal CA1 pyramidal neurons. As can be seen in Table 1, there was no significant difference in total spine density among groups, however, as shown in Figure 3 and Table 1, rabbits fed cholesterol exhibited prominent density alterations in mushroom and thin spines, which are two main types of dendritic spines that are associated with memory consolidation and learning acquisition, respectively (Dumitriu et al., 2010; Matsuzaki et al., 2001). This observation was confirmed by an analysis of variance that yielded significant group effects in Mushroom spine density [$F(2,176) = 7.624, p < 0.01$] and Thin spine density [$F(2,176) = 5.234, p < 0.01$]. A trend analysis indicated the data for Mushroom spine density were well fit by a linear model [$p < 0.001$], and the data for Thin spine density were fit by a quadratic model [$p < 0.05$]. Post Hoc comparison revealed the group effects in Mushroom spine density were attributable to significant differences between the Chol 0 and Chol 2.0 group [$p < 0.01$], and between Chol 0.5 and Chol 2.0 [$p < 0.05$]. The group effects in Thin spine density were attributable to a significant difference between the Chol 0 and either of Chol 0.5 and Chol 2.0 groups [$p < 0.01$, and 0.05 , respectively]. The data suggest dietary cholesterol concentrations increased mushroom spine density but decreased thin spine density. These changes in dendrite spine morphology may underlie the alterations in synaptic plasticity and behavioral performance caused by dietary cholesterol.

Interestingly, focal or varicose swellings of dendritic arbors were found in rabbit dendritic segments from the Chol 2.0 group. Figure 4 shows typical images of numerous varicosities with or without prominent spine loss. This indicates dietary cholesterol caused the dendritic beading that may contribute to the dysfunction of synaptic transmission and neuronal circuit function underlying the changes in behavioral performance.

In addition to the above spine morphological changes, we also made geometric measurements of mushroom spines. As shown in Table 2 and Figure 5, dietary cholesterol concentration bi-directionally altered the geometric measures, a slight increase at low dose but a decrease at higher dose. These were confirmed by an analysis of variance that yielded significant group effects in head width [$F(2,1435) = 9.728, p < 0.001$], neck width [$F(2,1435) = 16.86, p < 0.001$], neck length [$F(2,1435) = 9.963, p < 0.001$], and head volume density [$F(2,1435) = 11.77, p < 0.001$]. A trend analysis indicated the data for head width, neck width, and head volume were well fit by a quadratic model [all $p < 0.001$], and the data for neck length were fit by a linear model [$p < 0.001$]. Post Hoc comparisons revealed the group effects in: (1) head width were attributable to significant differences between the Chol 0.5 group and both the Chol 0 and Chol 2.0 groups [$p < 0.05, < 0.001$, respectively]; (2) neck width were attributable to significant differences between the Chol 2.0 group and the Chol 0 and Chol 0.5 groups [$p < 0.05, < 0.001$, respectively]; (3) neck length were attributable to significant differences between the Chol 2.0 group and both the Chol 0 and Chol 0.5 groups [$p < 0.001, < 0.001$, respectively]; (4) head volume were attributable to significant differences between the Chol 0.5 group and both the Chol 2.0 and Chol 0 groups [$p < 0.001, < 0.01$,

respectively]. These suggest dietary cholesterol concentration altered the geometry of dendritic spines that may contribute to changes in synaptic plasticity.

3. Discussion

The present study shows dietary cholesterol dose-dependently affected basal synaptic transmission and long-term potentiation, which is a basic form of synaptic plasticity that may be involved in learning and memory (Berger, 1984; Sastry et al., 1986). More importantly, dietary cholesterol dose-dependently increased mushroom spine density and reduced thin spine density in dendrites of hippocampal CA1 neurons, two kinds of spines that are associated with the memory consolidation and learning acquisition (Dumitriu et al., 2010; Kasai et al., 2003), and affected the geometric measures of mushroom spines. Taken together, dietary cholesterol may affect learning and memory by affecting synaptic plasticity and dendritic spine morphology of hippocampal CA1 neurons, and excessive dietary cholesterol could be detrimental to behavioral performance as a result of its negative effect on synaptic plasticity and spine morphology in hippocampal CA1 neurons.

PPF has been widely regarded as a form of short-term synaptic plasticity, which is a kind of pre-synaptic plasticity that reflects the release probability of the presynaptic cell (Fontan-Lozano et al., 2007; Tan et al., 2006) characterized by a transient increase in glutamate transmitter release and a greater postsynaptic response to the 2nd presynaptic spike (Frank et al., 2008). Therefore, PPF changes are an indicator of pre-synaptic function alteration. Unlike the dietary cholesterol-induced increase in paired-pulse ratio of glutamatergic synapses in rats (Dufour et al., 2006), our data showed no group PPF difference in rabbits fed with either normal chow or cholesterol chow, suggesting pre-synaptic function does not change as a function of dietary cholesterol concentrations. This is consistent with a previous study that reported no PPF changes in cholesterol depleted rat hippocampal CA1 neurons (Frank et al., 2008). Because synaptic transmission and synaptic plasticity in cholesterol depleted neurons are probably mediated by glutamatergic receptors rather than GABAergic receptors, the membrane microdomains on postsynaptic structures in Schaffer collateral-commissural pathway of hippocampus (Frank et al., 2008), dietary cholesterol induced changes in learning and memory could be attributed to the functional and structural alterations in postsynaptic structures rather than the presynaptic cell. LTP, a basic form of synaptic plasticity in a variety of neural structures including hippocampus, has been widely considered as one of the major cellular mechanisms that underlies learning and memory (Gruart et al., 2006; Tsien et al., 1996). Cholesterol is vital to synaptic transmission and plasticity as cholesterol depletion by M β CD can reduce LTP at hippocampus Schaffer collateral synapses (Koudinov and Koudinova, 2001; Maggo and Ashton, 2014) or even block LTP expression induced by HFS (Frank et al., 2008; Maggo and Ashton, 2014). Our data revealed dietary cholesterol may increase LTP expression at a low concentration but lead to a pronounced LTP reduction at higher concentrations which may contribute to the cognitive impairments in cholesterol fed animals (Darwish et al., 2010; Schreurs et al., 2012; Schreurs, 2013; Schreurs et al., 2013). These findings are consistent with a previous report showing decreased N-methyl-D-aspartate related postsynaptic currents in hippocampal CA1 neurons from cholesterol fed rats (Dufour et al., 2006). Though cholesterol cannot cross the BBB and dietary cholesterol does not directly affect the total levels of cholesterol and

sulfatides in the brain (Darwish et al., 2010), cholesterol accumulation in the hippocampus is indeed promoted by both dietary cholesterol (Stranahan et al., 2011) and experience (Darwish et al., 2010). Therefore, the changes in hippocampal synaptic plasticity may not be directly modulated by hypercholesterolemia in cholesterol fed rabbits, but the gamut of modulators for LTP impairments in cholesterol fed rabbits could range from brain cholesterol metabolism (Darwish et al., 2010; Martin et al., 2014; Stranahan et al., 2011), accumulated beta-amyloid and senile plaque-like structures (Gengler et al., 2010; Sparks and Schreurs, 2003), down regulation of postsynaptic protein and dendritic spine-specific protein (Bhat and Thirumangalakudi, 2013) and dendritic microtubule associated protein (Freeman et al., 2011), alterations in dendrite morphology as shown in this manuscript, to synaptic ultrastructural changes in synapses of hippocampus Schaffer collateral pathway (Ya et al., 2013). Previous studies have shown copper can affect both the sensitivity and the facilitation capability of hippocampal excitatory glutamatergic synapses (Gaier et al., 2014; Goldschmith et al., 2005; Peters et al., 2011) either directly by impaired copper homeostasis in the brain (Gaier et al., 2014; Goldschmith et al., 2005; Peters et al., 2011) or by increased beta amyloid accumulation (Sparks and Schreurs, 2003), therefore, copper may produce additive effect on these altered synaptic properties in cholesterol fed rabbits, the interaction between copper and cholesterol could exacerbate the neurological damage and degeneration and thus contribute to the retarded learning and memory loss (Arnal et al., 2013; Schreurs, 2013; Sparks and Schreurs, 2003).

Dendritic spines are specialized structures for the regulation of synaptic function. Based on relative length of spine necks and the diameter of spine heads containing N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5methyl-4-isoxazolepropionic acid (AMPA) receptors, they are classified into five groups including Mushroom, Thin, Stubby, Filopodia, and Bifurcation. Mushroom spines have larger heads and are considered as highly stable spines and not vulnerable to aging (Dumitriu et al., 2010). They may be the more effective excitatory synaptic sites as their heads contain larger numbers of glutamate receptors and have been proposed to be the locus of long term memories (Kasai et al., 2003). On the other hand, thin spines have smaller heads that contain a lower density of glutamate receptors (Matsuzaki et al., 2001), are generally assumed to be functionally weaker, and thought to be particularly plastic and linked to learning (Dumitriu et al., 2010; Matsuzaki et al., 2001). Our data showed dietary cholesterol affected gross dendritic structure, specifically it increased mushroom and decreased thin dendrite spines, which are the two postsynaptic elements that would potentially determine excitatory transmission and synaptic plasticity during high frequency repetitive stimulation of Schaffer collateral-commissural pathway in rabbit hippocampus. Based on this anatomic evidence and a report that shows aging related cognitive impairment is associated with selective changes in thin spine density and morphology (Dumitriu et al., 2010), we conclude the dietary cholesterol-induced changes in mushroom spines may be helpful in preserving EPSP properties following plasticity-driven changes in synaptic strength required for memory consolidation and those changes in thin spines may be responsible for modifying the amplitude of EPSPs required for learning a new task. Our data also demonstrated that dietary cholesterol concentration affected geometric measures of mushroom spines, for example, dietary cholesterol led to an increase in the width of spine head and neck at the low dose but a decrease in the width of spine neck at the

highest dose. Since the reduced neck width of mushroom spines resulting from dietary cholesterol is negatively associated with spine neck resistance and synaptic efficacy, it is very likely dietary cholesterol induced LTP changes may be mediated by morphologic alterations in thin spines. This is supported by a recent report from computational models that shows that EPSPs amplitude in the spine head are heavily dependent on spine neck resistance rather than spine head diameters or synaptic conductances (Gulledge et al., 2012) as the spine neck can act as an electrical filter to reduce amplitudes of EPSPs received by parent dendrites and soma (Araya et al., 2006).

The dendritic beading, focal or varicose swellings of dendritic arbors, has been regarded as a morphological hallmark of neuronal injury and dendrotoxicity (Ahlgren et al., 2014; Al-Noori and Swann, 2000) and regularly-spaced and spherical dendritic varicosities may be Ca²⁺-independent (Hoskison et al., 2007). Our data showed this dendritic beading was frequently observed in hippocampus of rabbits fed with 2% cholesterol but absent in normal chow fed rabbits. Therefore, it is conceivable anatomical remodeling may be accompanied by functional alterations in dendrites that could contribute to the dysfunction of synaptic transmission and neuronal circuit function induced by dietary cholesterol.

In summary, this is the first report to show dietary cholesterol concentrations affect both the electrophysiological properties and dendrite spine morphology in rabbit hippocampal CA1 pyramidal neurons, which can account for dietary cholesterol facilitated learning of a new task and degraded memory recall of acquired learning task in our previous experiments (Darwish et al., 2010; Schreurs et al., 2007a; Schreurs et al., 2007c; Schreurs et al., 2012; Schreurs et al., 2013).

4. Experimental Procedure

4.1. Subjects

Three months old male New Zealand rabbits (*Oryctolagus cuniculus*) weighing 2.0–2.2 kg were supplied by Harlan (Indianapolis, IN), housed in individual cages, given free access to food and ultra pure water (18 M Ω , Millipore Academic) and maintained on a 12 h light/dark cycle. Rabbits were randomly assigned to three groups, normal chow (Chol 0, $n = 8$), 0.5% cholesterol chow (Chol 0.5, $n = 9$), and 2% cholesterol chow (Chol 2.0, $n=8$). Cholesterol-fed rabbits received cholesterol with different concentrations incorporated into Purina rabbit chow (Dyets Inc., Bethlehem, PA) for 8 weeks before electrophysiological experiments and normal chow fed rabbits received standard Purina rabbit chow (0% added cholesterol). Rabbits were maintained in accordance with National Institutes of Health guidelines, and the research was approved by the West Virginia University Animal Care and Use Committee. Rabbits in each group were allocated to either field potential recording or DiI staining.

4.2. Slice preparation

Procedures identical to those previously published (Schreurs et al., 1998; Wang and Schreurs, 2010; Wang and Schreurs, 2014) were used for brain slices and electrophysiological recordings and data analysis. Briefly, rabbits were anesthetized with 30 mg/kg sodium pentobarbital and then transcardially perfused with chilled sucrose modified artificial cerebrospinal fluid (S-ACSF) containing (in mM) Sucrose 200, KCl 2.5, MgCl₂

1.2, CaCl_2 0.5, NaH_2PO_4 1.25, NaHCO_3 26 and Dextrose 20. After 2 min of perfusion, rabbits were decapitated and the hippocampus was isolated and sliced into 350 μm transverse slices on a Leica VT1200S Vibratome. Slices were incubated in O_2 - CO_2 -saturated ACSF containing (in mM) NaCl 125, KCl 3.0, MgSO_4 1.2, CaCl_2 2.0, NaH_2PO_4 1.2, NaHCO_3 26 and Dextrose 10 for a minimum of 1 h at 34 °C and then kept at room temperature until electrophysiological recording. All recordings were performed at room temperature.

4.3. Synaptic plasticity

Hippocampal CA1 neurons were visualized using an Olympus BX50WI microscope. Field EPSPs (fEPSPs) were recorded extracellularly from the stratum radiatum of CA1 hippocampus using an ACSF-filled electrode (1-5 $\text{m}\Omega$) in response to stimulation of the Schaffer collateral-commissural pathway using a tungsten bipolar electrode, which was placed 400-500 μm away from the recording electrode in the stratum radiatum. The stimulation was delivered by an A360 Stimulus Isolator (WPI) driven by a computer-controlled Master-8 Stimulator (AMPI, Jerusalem, Israel). Initially, recordings were carried out with lower frequency stimulation (0.05 Hz) at intensities of 100-300 μA to determine the maximal excitatory potential, then stimulation intensity was adjusted to produce 50% of the maximal response for baseline recordings and LTP induction. fEPSPs were confirmed by bath-application of 20 μM Bicuculline methochloride (Tocris) at the end of experiment.

4.3.1. Paired pulse facilitation (PPF)—The paired pulse slope ratio of evoked fEPSPs to pairs of stimuli (interpulse interval between 50 ms and 350 ms with 50 ms increments) delivered to the stratum radiatum at 0.05 Hz was assessed for short-term synaptic plasticity that was mainly attributed to a presynaptic effect. Paired responses were averaged, and the paired pulse slope ratio was defined as $\text{EPSP}_2/\text{EPSP}_1$.

4.3.2. LTP induction and assessment—After a 5-10 min stable baseline period, LTP was induced by high frequency stimulation (HFS), which consisted of a total of 400 stimuli that were delivered as 4 discrete bursts with burst intervals of 20 sec, stimuli within bursts were always delivered at 10 ms intervals (100 Hz). The strength of fEPSPs was assessed by measuring the slope (initial 20 - 80%) of the fEPSPs rising phase. LTP was quantified by comparing the mean of fEPSPs slope between 20 min and 30 min post-HFS period with the mean fEPSPs during the baseline and expressed as the percentage change from baseline.

All electrophysiological data were recorded online using the Clampex 10.0 software (Axon Instruments). For fEPSPs recording, signals were amplified (gain 200-500), filtered (6 kHz), acquired at a sampling rate of 10 kHz and 20 s intervals with MultiClamp 700B amplifier and pClamp 10.0 and stored on computer for further analysis. Standard off-line analyses were conducted using Clampfit 10.0.

4.4. Dil staining and confocal microscopy

Right dorsal hippocampus was sectioned with a vibratome (LEICA VT1000S) at 300 μm thickness and further fixed with 4% paraformaldehyde in PBS at room temperature for 2 h

for DiI (1, 1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanineperchlorate, Invitrogen) staining to visualize the dendrite spines with a Zeiss confocal microscope (LSM 710).

Glass electrodes made from borosilicate glass (catalog #: BF150-86-10; 1.5 mm OD, 0.86 mm ID; Sutter Instrument Company, Novato, CA) were pulled with a P97 Brown-Flaming micropipette puller (Sutter Instrument Company, Novato, CA). The electrode tip was immersed in 5% (w/v) DiI in dichloromethane (Sigma-Aldrich) for 10 s (2–3 times) and air dried at room temperature for 1 h, and then inserted, broken, and left in the strata oriens of the CA1 area of hippocampal slices (3–4 electrode tips/slice) overnight in PBS at 4°C to allow DiI to diffuse along the plasma membrane of CA1 neurons, then resectioned to 35 μ m and mounted on glass slides with distilled water.

Dendritic spines stained with DiI (>510 nm/568 nm; excitation/emission) in the strata radiatum were imaged with the LSM710, using a 100 $\times$ Plan-Apochromat oil-immersion objective (1.4 numerical aperture). Dendritic spines of individual dendritic shafts were counted offline with the ImageJ program based on a series of randomized confocal images (1024 $\times$ 1024 pixels) taken every 0.45 μ m with line scan mode and a pinhole of about 1.00 Airy unit. Individual spines identified on one image were also verified on adjacent images to approximate the three-dimensional structure of the spines. Spines that had head diameters more than three times larger than the diameter of their necks and appeared in more than three planes of the images were identified as mushroom spines. 3–4 hippocampal slices from each animal were used for DiI staining and approximately 9–12 stacked image sets were obtained from the proximal one-third of the stratum radiatum of CA1 hippocampus where receives input from the CA3 Schaffer collaterals, and therefore most closely parallels the area of CA1 analyzed physiologically. To avoid biased sampling and biased counting of labeled spines, persons who were involved in tissue preparation for DiI staining, spine count and analysis for all image sets were blinded to animal group allocation.

Geometric measurements of Mushroom spines including head length, head width, neck length and width, were done on raw images of single sections in Image J as reported (Tonnesen et al., 2014), head volume was also calculated based on the description by Tonnesen et al. as volume = $4/3\pi(\text{head radius})^2 \times (0.5 \times \text{head length})$.

Data from both field potential recordings and all graphic are represented as means $\pm$ SEM. One-way ANOVA followed by trend analysis and LSD Post Hoc comparisons were calculated in SPSS (version 21, SPSS Inc., Chicago, IL, USA) with $p < 0.05$ as a criterion for significance.

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Highlights

- Dietary cholesterol affects learning and memory but its mechanisms remain elusive
- Dietary cholesterol dose-dependently modulated hippocampal synaptic plasticity
- Dietary Cholesterol shaped the morphology and geometry of hippocampal dendritic spines
- These indices of hippocampal plasticity could mediate learning and memory changes from feeding a cholesterol diet

Fig. 1. Dietary cholesterol concentration affected input-output measures of both EPSP and fiber volley and PPF in rabbit hippocampal slices

A represents the typical traces of EPSP, which were evoked at a stimulus intensity of 100 μ A, from Chol 0, Chol 0.5, and Chol 2.0 groups, respectively.

B represents the input-output curves for the Chol 0, Chol 0.5, and Chol 2.0 groups. Note that the Chol groups showed a slight increase in the slope of fEPSPs and a statistical difference between Chol 2.0 group and both Chol 0 and Chol 0.5 groups.

C represents the amplitude of fiber volley recorded in response to increasing stimulus intensities. Note that there is no significant difference between the groups.

D represents PPF evoked in the Chol 0, Chol 0.5, and Chol 2.0 groups. Data shown are the slope ratio (slope₂/slope₁) of fEPSP. Note that there was no statistical significance between the groups. N-number for slices is 12, 14, and 10 for Chol 0, Chol 0.5 group, and Chol 2.0 group, respectively.

Fig. 2. Dietary cholesterol concentrations modulated LTP in rabbit hippocampal slices

A represents the raw fEPSP recordings from Chol 0, Chol 0.5, and Chol 2.0 groups, respectively. Note that fEPSPs were evoked before and after LTP induction through HFS. B represents LTP for Chol 0, Chol 0.5, and Chol 2.0 groups. Data shown are the percentile changes in fEPSP slope $\pm$ S.E.M. * indicates there is a statistical significance of $p < 0.001$ if compared to Chol 0 group. Note that Chol 2.0 group showed impaired LTP. N-number for slices is 11, 13, and 8 for Chol 0, Chol 0.5 group, and Chol 2.0 group, respectively.

Fig. 3. Dietary cholesterol increased Mushroom spine density but decreased Thin spine density of apical dendrites of hippocampal CA1 pyramidal neurons

Top panel represents typical confocal images of rabbit dendritic segments for Chol 0, Chol 0.5 group, and Chol 2.0 group, respectively. **Bottom left panel** is a bar graph for Mushroom spine density in rabbit dendritic segments for Chol 0, Chol 0.5 group, and Chol 2.0 group. Mushroom spine density increased with dietary cholesterol concentrations. **Bottom right panel** is a bar graph for Thin spine density in rabbit dendritic segments for Chol 0, Chol 0.5 group, and Chol 2.0 group. Thin spine density decreased with dietary cholesterol concentrations.

T: Thin spine; M: Mushroom spine

Figure 4. abnormal-shaped dendrites exhibited in hippocampus of rabbits fed with cholesterol
Left image represents a typical confocal image of rabbit dendritic segments from Chol 2.0 group showing numerous varicosities and long thin spines, but no prominent spine loss. Right image represents a typical confocal image of rabbit dendritic segments from Chol 2.0 group showing numerous varicosities and spine loss.

Figure 5. Dietary cholesterol dose-dependently altered the geometric measures of mushroom spines of rabbit hippocampal CA1 pyramidal neurons

A-D represent the average measures taken from head width, neck width, neck length, and head volumes, respectively.

Table 1

Dietary cholesterol concentrations significantly increased mushroom spine density but reduced thin spine density

Group	Density (Spine number/100μm)					Total
	Mushroom	Thin	Stubby	Filopodia	Bifurcation	
Chol 0	22.91±1.26	50.68±2.36	57.64±2.69	9.42±1.09	3.26±0.69	143.90±3.78
Chol 0.5	27.02±1.44	39.78±2.06**	67.02±2.48	6.19±0.66	1.84±0.30	141.84±3.23
Chol 2.0	32.55±2.0**	41.79±2.81*	63.31±2.50	10.22±1.17	2.94±0.55	150.82±4.71

n (dendrite segments/slices/animals) is 45/37/4, 78/61/5, 55/47/5 for Chol 0, Chol 0.5, and Chol2.0, respectively.

* indicated p<0.05 and <0.01, respectively, if compared to Control

** indicated p<0.05 and <0.01, respectively, if compared to Control

Dietary cholesterol concentrations significantly altered geometric measures of mushroom spines

Table 2

Group	Head length (μm)			Head width (μm)			Neck length (μm)			Neck width (μm)			Head Volume (μm ³)			N (spines/slices/animals)
	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	
Chol 1.0	0.37-1.44	0.75	0.76	0.36-1.36	0.68	0.6	0.7-1.86	0.45	0.38	0.04-0.31	0.14	0.1	0.03-0.97	0.21	0.18	278/37/4
Chol 0.5	0.37-1.62	0.81	0.78	0.31-1.38	0.72	0.7	0.08-1.42	0.43	0.38	0.04-1.06	0.15	0.1	0.02-1.26	0.25	0.24	653/61/5
Chol 1.2	0.35-1.56	0.79	0.76	0.35-1.24	0.68	0.6	0.05-1.68	0.38	0.3	0.05-0.34	0.13	0.1	0.04-0.90	0.28	0.17	507/47/5