Published in final edited form as:

J Alzheimers Dis. 2019 ; 68(3): 1257–1271. doi:10.3233/JAD-190043.

Tumor Necrosis Factor-Alpha Alters Electrophysiological Properties of Rabbit Hippocampal Neurons

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Abstract

Previous studies have shown tumor necrosis factor-alpha (TNF- α) may impact neurodegeneration in Alzheimer's disease (AD) by regulating amyloid- β and tau pathogenesis. However, it is unclear whether TNF- α has a role in a cholesterol-fed rabbit model of AD or TNF- α affects the electrophysiological properties of rabbit hippocampus. This study was designed to investigate whether long-term feeding of cholesterol diet known to induce AD pathology regulates TNF- α expression in the hippocampus and whether TNF- α would modulate electrophysiological properties of rabbit hippocampal CA1 neurons. TNF- α ELISA showed dietary cholesterol increased hippocampal TNF- α expression in a dose-dependent manner. Whole-cell recordings revealed TNF- α altered the membrane properties of rabbit hippocampal CA1 neurons, which was characterized by a decrease in after-hyperpolarization amplitudes; Field potential recordings showed TNF- α inhibited long-term potentiation but did not influence presynaptic function. Interestingly, TNF- α did not significantly affect the after-hyperpolarization amplitudes of hippocampal CA1 neurons from cholesterol fed rabbits compared to normal chow fed rabbits. In conclusion, dietary cholesterol generated an *in vivo* model of chronic TNF- α elevation and TNF- α may underlie the learning and memory changes previously seen in the rabbit model of AD by acting as a bridge between dietary cholesterol and brain function and directly modulating the electrophysiological properties of hippocampal CA1 neurons.

Keywords

Cholesterol; Hippocampus; membrane properties; synaptic plasticity; tumor necrosis factor-alpha

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, which is characterized by memory loss and other cognitive deficits induced by progressive neuropathology in specific brain regions. Though many risk factors including high cholesterol [1–6], age [7, 8],

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The author's disclosure is available online (<https://www.j-alz.com/manuscript-disclosures/19-0043r1>).

genetics and family history [9, 10], hypertension and heart disease [7, 11, 12], and infection [13, 14] have been identified, the cause of sporadic AD remains unknown.

Tumor necrosis factor- α (TNF- α), a proinflammatory cytokine produced by many types of cells including macrophages and neurons, has been implicated in a wide range of biological processes including cell proliferation, differentiation, apoptosis, and lipid metabolism. For example, TNF- α may play a crucial physiological role in regulating the strength of normal synaptic transmission by its actions on excitatory neurotransmission and long-term potentiation [15–17], and synaptic scaling [18]. However, prolonged excessive TNF- α in the brain may play a role in pathophysiological processes and structural alterations in AD such as amyloid- β (A β) plaque deposition [19] and tau phosphorylation [20]. This has been supported by findings of excessive TNF- α in cerebrospinal fluid [21, 22], serum/plasma, and brain tissue of patients with AD [23, 24], and anti-TNF- α treatment can rescue A β deposition and behavioral impairments in animals [25–27] and AD patients [28, 29]. Though it is clear that TNF- α can result in A β production and plaque in transgenic mice [19] probably via modulating gamma-secretase activity [30], and TNF- α may mediate the inhibitory effects of A β on synaptic plasticity in mutant mice null for TNF receptor type I [31], it remains unclear whether TNF- α affects the electrophysiological properties of rabbit hippocampal neurons.

The cholesterol-fed rabbit has been widely regarded as a unique, non-transgenic, animal model of AD [1, 4, 32–34] because high dietary cholesterol can induce many of the pathological hallmarks of AD including A β accumulation and plaque formation [1, 2, 33, 35–37], neurovascular changes [38–40], and cognitive changes [1, 4, 41, 42]. The alterations in behavioral performance may be mediated by the changes in intrinsic excitability [43], synaptic plasticity and spine morphology of hippocampal neurons [44], brain cholesterol levels [45], brain cholesterol metabolism and neuronal estrogen receptors expression [34], neurovascular deterioration and compromise [38, 39, 46], or A β production and plaques [1, 32, 35]. Though dietary cholesterol cannot cross the blood-brain barrier, a high-cholesterol diet does increase the expression of cytokines including TNF- α and interleukin-6 in the hippocampus of transgenic mice [47–49]. This suggests a potential role for inflammation and cytokines in the altered behavioral performance observed in the cholesterol-fed rabbit model of AD [50–53]. However, there are no reports about whether feeding dietary cholesterol induces a chronic persistent TNF- α elevation in rabbit brain and whether TNF- α can modulate the electrophysiological properties of hippocampal neurons. In order to address these questions, the current project was designed to explore whether dietary cholesterol regulates TNF expression in the hippocampus and whether *in vitro* TNF- α treatment modifies the electrophysiological properties of hippocampal neurons in rabbits fed dietary cholesterol.

MATERIALS AND METHODS

Subjects

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. After one week of

acclimation to the facility, rabbits were divided into three different cohorts. The first cohort of male rabbits was randomly assigned to three groups fed with special diet for 8 weeks, normal chow fed rabbits (Chol 0, n = 12) received standard Purina rabbit chow (0% added cholesterol), cholesterol-fed rabbits (Chol 1.0, n = 11) received 1% cholesterol incorporated into Purina rabbit chow (Dyets Inc., Bethlehem, PA, USA), and cholesterol-fed rabbits (Chol 2.0, n = 7) received 2% cholesterol incorporated into Purina rabbit chow (Dyets Inc.). These rabbits were used for detecting hippocampal TNF- α levels with TNF- α ELISA. The second cohort of 8 male rabbits was used for *in vitro* hippocampal slice recording to explore the effect of TNF- α treatment. The third cohort of female rabbits was randomly assigned to two groups, normal chow fed rabbits (Chol 0, n = 16) received standard Purina rabbit chow (0% added cholesterol) or cholesterol-fed rabbits (Chol 1.0, n = 8) received 1% cholesterol incorporated into Purina rabbit chow (Dyets Inc.) for 20 weeks before electrophysiological experiments. The dose of 1% cholesterol was chosen not only because a modest increase of TNF- α was expected based on pilot TNF- α ELISA but also because the rabbits may be able to reach the experiment endpoints without intolerable hypercholesteremic adverse effects. 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.

TNF- α protein extraction and ELISA

Rabbit hippocampus was homogenized in 8 volumes of ice-cold homogenizing phosphate buffer containing 50 mM Tris-HCl, 2 mM EDTA, one tablet complete protease inhibitor, and 0.5% NP40 (pH 7.4) by using Sonicator S-4000 (Misonix Ultrasonic liquid processors, Newtown, CT, USA), then centrifuged at 4°C and 10000'g for 30 min. The supernatant was collected as a homogenate for TNF- α ELISA test using Rabbit TNF- α DuoSet ELISA development kit from R&D systems (Minneapolis, MN, USA). The homogenate's protein concentration was measured with a protein assay reagent kit to ensure an equal amount of protein from each sample.

Slice preparation

Procedures identical to those previously published [43, 44, 54–56] were used for brain slices and electro-physiological 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, CaCl₂ 0.5, NaH₂PO₄ 1.25, NaHCO₃ 26, and Dextrose 20. After 1–2 min of perfusion, rabbits were decapitated and the hippocampus was isolated and sliced into 350- μ m transverse slices on a Leica VT1200S Vibratome. Slices were incubated in O₂–CO₂-saturated ACSF containing (in mM) NaCl 125, KCl 3.0, MgSO₄ 1.2, CaCl₂ 2.0, NaH₂PO₄ 1.2, NaHCO₃ 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.

Whole-cell patch clamp recordings

Hippocampal CA1 neurons were visualized using an Olympus BX50WI microscope. Whole-cell patch-clamp recordings were performed at room temperature using an Axon MultiClamp 700B. Patch pipettes with tip resistance between 2 and 5 M Ω were filled with a

solution containing (in mM) potassium gluconate ($\text{C}_6\text{H}_{11}\text{O}_7\text{K}$) 140, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 4.6, HEPES 10, EGTA 10, Na_2ATP 4.0 (pH 7.3). Data were low-pass filtered at 2 kHz and acquired at 5–10 kHz. Membrane properties were measured when the neuron had stabilized for five minutes after whole-cell configuration was achieved. Quantitative analysis included resting membrane potential measured directly upon breakthrough in whole-cell configuration, input resistance based on membrane potential changes to depolarizing current injections immediately after whole cell configuration, action potential (AP) threshold, current required for eliciting the first AP, half-width of elicited AP including rising and falling phases, amplitude of elicited AP, the number of elicited APs, latency to the first AP elicited by a 250-ms duration depolarizing current injection, and peak amplitude of the after-hyperpolarization (AHP). To obtain an accurate measurement of neuronal excitability independent of membrane potential changes, continuous direct current was applied through the recording electrode to hold the cell at a -70 mV baseline.

Synaptic plasticity

Field EPSPs (fEPSPs) were recorded extracellularly from the stratum radiatum of CA1 hippocampus using an ACSF-filled electrode ($1\text{--}5$ m Ω) in response to stimulation of the Schaffer collateral-commissural pathway using a tungsten bipolar electrode, which was placed $400\text{--}500$ 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 $50\text{--}500$ μA to determine the maximal excitatory potential, then stimulation intensity was adjusted to produce 50% of the maximal response for baseline recordings and long-term potentiation (LTP) induction. fEPSPs were confirmed by bath-application of 20 μM Bicuculline methochloride (Tocris) at the end of experiment.

Paired pulse facilitation (PPF)

The paired pulse slope ratio of evoked fEPSPs to pairs of stimuli (interpulse interval between 50 ms and 500 ms with 50 ms increments) delivered to the stratum radiatum at 0.05 Hz was used to assess short-term synaptic plasticity that was mainly attributable to a presynaptic effect. Paired responses were averaged, and the paired pulse slope ratio was defined as $\text{EPSP}_2/\text{EPSP}_1$.

LTP induction and assessment

After a $5\text{--}10$ 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 s, stimuli within bursts were always delivered at 10 ms intervals (100 Hz). The strength of fEPSPs was assessed by measuring the slope (initial $20\text{--}80\%$) of the fEPSPs rising phase. LTP was quantified by comparing the mean of fEPSPs slope between 20 min and 60 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 (Molecular Devices). For fEPSPs recording, signals were amplified (gain $200\text{--}500$), 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.

TNF- α treatment

Recombinant Human TNF- α protein was purchased from R&D systems (Minneapolis, MN, USA) and was used either by bath application for 30 min during slice recording or adding it to the incubation solution more than 1 h before slice recording.

Statistics

Data are represented as means $\pm$ SEM. One-way ANOVA followed by *Post Hoc* comparisons were calculated in SPSS (version 24, SPSS Inc., Chicago, IL, USA) with $p < 0.05$ as a criterion for significance.

RESULTS

TNF ELISA

In order to confirm whether dietary cholesterol influenced endogenous TNF- α expression in rabbit hippocampus, TNF- α ELISA was used to measure hippocampal TNF- α levels. As shown in Fig. 1, dietary cholesterol concentrations increased hippocampal TNF- α levels [22.21 ± 0.91 pg/mL, 24.01 ± 1.91 pg/mL, and 29.35 ± 1.87 pg/mL for Chol 0 ($n = 12$), Chol 1.0 ($n = 11$), and Chol 2.0 group ($n = 7$), respectively.], and ANOVA confirmed a significant group difference [$F(2, 29) = 4.736$, $p < 0.05$]. *Post Hoc* analysis showed the group difference was attributable to a significant difference between Chol 2.0 and both Control and Chol 1.0 ($p < 0.01$, and < 0.05 , respectively). A trend analysis indicated the data were well fit by a linear model ($p < 0.05$), indicating dietary cholesterol affected hippocampal TNF- α levels in a dose-dependent manner. Our data showed dietary cholesterol concentrations led to a chronic and persistent elevation of endogenous TNF- α levels in hippocampus, which may be a factor in the altered behavior performance observed in the rabbit model of AD that was created by feeding dietary cholesterol.

Membrane properties

To assess the effects of TNF- α on membrane properties of rabbit hippocampal CA1 neurons, whole-cell current clamp recordings were performed on hippocampal CA1 neurons with or without TNF- α treatment. Similar to our previous observation [43], hippocampal CA1 neurons were silent at resting membrane potential but showed action potentials in response to depolarizing current.

Table 1 shows the membrane properties of rabbit hippocampal CA1 neurons pretreated with TNF- α for more than one hour. As seen in Table 1 and Fig. 2, TNF- α pretreatment produced extensive significant effects on membrane properties of hippocampal CA1 neurons, and ANOVA confirmed the changes in threshold [$F(1, 36) = 4.529$, $p < 0.05$] and current required for evoked AP [$F(1, 36) = 19.647$, $p < 0.01$], latency [$F(1, 36) = 9.652$, $p < 0.01$], AHP amplitude [$F(1, 36) = 6.545$, $p < 0.05$], and S_1S_2 interval [$F(1, 32) = 12.99$, $p < 0.05$]. These findings suggest TNF- α pretreatment caused a range of effects on intrinsic membrane properties of hippocampal CA1 neurons including increased excitability featured by reduced

AHP amplitude, shorten latency for evoked AP, and shorten S_1S_2 interval, and decreased excitability characterized by slightly higher threshold and slightly larger current required to evoke APs.

Since the TNF- α ELISA revealed an increase of endogenous TNF- α in the hippocampus of rabbits fed with dietary cholesterol, it suggests the hippocampus from rabbits fed with cholesterol may have been exposed to the elevated endogenous TNF- α for a prolonged time. In order to confirm whether exogenous TNF- α treatment has additive effects on membrane properties of hippocampal CA1 neurons from rabbits fed with dietary cholesterol, whole-cell current clamp recordings were made in hippocampal slices from rabbits given a five-month diet of either 0% or 1% cholesterol. As shown in Table 2 and Fig. 3, if compared to Control group fed with normal chow, five-months of 1% cholesterol produced significant changes in membrane properties of hippocampal CA1 neurons. ANOVA analysis confirmed the difference in AHP amplitude [$F(1,80) = 6.090, p < 0.01$] and threshold for evoked AP [$F(1,80) = 7.658, p < 0.05$]. This suggests dietary cholesterol is capable of regulating intrinsic excitability of hippocampal CA1 neurons, which is in line with our previous report [43].

Table 3 shows a comparison of measures of evoked APs from hippocampal CA1 neurons in rabbits fed with either 0% or 1% dietary cholesterol, which were taken prior to and 30 min post bath application of TNF- α . Unlike the findings of hippocampal slices pretreated with TNF- α for more than 1 h, 30 min bath application of TNF- α only exhibited an effect on the AHP amplitude in Fig. 4. ANOVA analysis revealed a significant group effect in the AHP amplitude [$F(3, 33) = 7.658, p < 0.01$] and *post hoc* analysis confirmed the statistical difference was only present between Pre and Post TNF- α treatment in rabbits fed with 0% dietary cholesterol and not in slices from rabbits fed with 1% cholesterol. Our data demonstrate that exogenous TNF- α treatment had no additional effects on membrane properties of hippocampal CA1 neurons from cholesterol-fed rabbits likely due to the increased levels of endogenous TNF- α . These results suggest exogenous TNF- α elevation may mediate the changes in membrane properties of hippocampal CA1 neurons from chow-fed rabbits but increased endogenous TNF- α in hippocampus of cholesterolfed rabbits may partly explain why exogenous TNF treatment did not further modulate the membrane properties of hippocampal CA1 neurons from rabbits fed dietary cholesterol.

Synaptic properties

To investigate TNF- α effects on short-term and long-term synaptic plasticity in rabbit hippocampal CA1 neurons, field potential recordings were performed on hippocampal slices treated with TNF- α .

Input-output curve and pre-synaptic function

An input-output curve was constructed with stimulus intensities versus the slope of EPSP elicited in responding to increasing stimulus intensities. Figure 5A shows typical examples of EPSPs which were evoked at a stimulus intensity of 100 μ A from Control and TNF- α groups, respectively. As shown in Fig. 5B, the mean EPSP slopes for both the Control and TNF- α groups increased with stimulus intensity and confirmed by statistical analysis that

revealed significant differences across stimulus intensities [$F(10,160) = 15.01, p < 0.001$]. Although TNF- α treatment exhibited a smaller EPSP slope than in Controls, statistical analysis revealed no group effect [$F(1,16) = 0.589, p = 0.45$]. This suggests that TNF- α treatment may not significantly affect basal synaptic transmission in the Schaffer collateral pathway.

Pre-synaptic function was assessed by paired pulse facilitation, which is thought to be short-term synaptic plasticity representing the release probability of presynaptic neurons. Figure 5C shows the slope ratio of EPSP₂/EPSP₁ versus the interstimulus intervals was maximal at an interval of 50 ms and then decreased with longer interstimulus intervals, which was confirmed by statistical analysis that revealed significant differences across interstimulus intervals [$F(9, 207) = 15.41, p < 0.001$] but without a main group effect [$F(1, 23) = 0.009, p = 0.92$]. This suggests that TNF- α did not significantly influence pre-synaptic function in the Schaffer collateral pathway of rabbit hippocampus.

Post-synaptic function

Post-synaptic function was assessed by LTP. As illustrated in Fig. 5D, TNF- α pretreatment showed less LTP expression (average LTP level was $253.57 \pm 5.49\%$) than Control (average LTP level was $315.85 \pm 9.54\%$) which was confirmed by ANOVA that revealed a significant main group effect [$F(1, 639) = 37.00, p < 0.001$]. Therefore, our data suggest TNF treatment can reduce LTP expression in hippocampal CA1 neurons.

DISCUSSION

The principal findings of the current experiment were that: 1) Dietary cholesterol increased TNF- α levels in the rabbit hippocampus, 2) TNF- α had a range of effects on the intrinsic excitability of rabbit hippocampal CA1 neurons with increased excitability featured by a reduced AHP amplitude, shortened latency for evoked APs, and shortened S1S2 interval whereas decreased excitability was characterized by a larger current required for an evoked AP, 3) TNF- α did not significantly affect the membrane properties of hippocampal CA1 neurons from rabbits fed with dietary cholesterol, and 4) TNF- α altered the synaptic properties in rabbit hippocampal Schaffer collateral pathway characterized by reduced LTP expression. Taken together, dietary cholesterol may comprise an *in vivo* model for chronic inflammation in the hippocampus through persistent TNF- α elevation, and thus TNF- α signaling pathways may be involved in cognitive changes previously seen in this rabbit model of AD by acting as a bridge between dietary cholesterol and brain function through its regulation of electrophysiological properties of hippocampal CA1 neurons.

Dietary cholesterol has been widely recognized as one of the best approaches to modeling sporadic AD in rabbits [1, 4, 35], rats [57–59], mice [48, 60], and guinea pigs [61]. Previous studies show that high cholesterol diet can increase TNF- α expression in mice [48, 49] and rats [62], our data revealed dietary cholesterol concentrations increased TNF- α expression in the rabbit hippocampus, suggesting dietary cholesterol can model a persistent TNF- α elevation in hippocampus. Since the TNF- α protein we detected by ELISA is a transmembrane protein whose biological functions mainly derive from activation of TNF- α receptor II that is limited to neurons, microglia, and astrocytes [63], this increased

endogenous transmembrane TNF- α may cause an imbalance between neuronal protection and neurotoxicity or neuronal damage initiated by TNF- α receptor I and II [64], and eventually lead to loss of homeostasis in vulnerable sites such as synapses that are responsible for learning and memory [65]. Most importantly, hippocampal neuroinflammation in cholesterol-fed rabbits occurred in the absence of neurodegenerative changes and was independent of amyloid accumulation in both cerebral cortex and hippocampus [66], and thus cholesterol diet-associated endogenous TNF- α elevation and neuroinflammation in hippocampus may be involved in learning and memory changes observed in this rabbit model of AD [1, 41, 45, 67]. This has been corroborated by the correlation between neuroinflammation and working memory loss in mice [48] and by the relationship between the brain region-specific and time-dependent TNF- α increase, which happened from 7 days onward in the hippocampus and frontal cortex but only at 10 months in cerebellum of rats treated with a single intraperitoneal injection of endotoxin, and behavioral alterations observed at 7 days and 10 months post endotoxin injection [68].

TNF- α plays a pivotal role in the physiological and pathophysiological function of the central nervous system [18, 69, 70] though the concentration is very low or even undetectable under physiological conditions [71, 72]. For example, the continual presence of endogenous TNF- α is required for the preservation of synaptic strength at glutamatergic excitatory synapses and endogenous TNF- α blockade can decrease synaptic strength in the hippocampus via a reduced surface expression of glutamate receptors [69]. Application of exogenous TNF- α can increase synaptic efficacy through an increase in glutamatergic receptors [69, 73] and reduce the excitotoxic injury elicited by intracerebral injection of N-methyl-D-aspartate (NMDA) in the neonatal rat brain [74]. TNF- α is also involved in homeostatic synaptic scaling, another form of activity-dependent synaptic plasticity that entails uniform adjustment in the strength of all synapses of a cell in response to prolonged changes in the cell's electrical activity [18]. In this study, we used a low dose of TNF- α , which is slightly higher than physiological concentrations [72] but far below those concentrations that can cause neuronal toxicity [75–78], and found that TNF- α demonstrated a range of effects on the membrane properties of hippocampal CA1 neurons. The increased neuronal excitability induced by TNF- α treatment is in line with previous findings in rat cerebellar Purkinje cells [79], cultured cortical neurons [80], dorsal root ganglion [81, 82], rat suprachiasmatic nuclear neurons [83], neurons from rat subfornical organ [84], and in excised guinea pig spinal cord tissue [78]. This increased excitability may be attributed to the changes in neuronal membrane properties [83], including an increase in calcium current observed in cultured rat hippocampal neurons [85] and cultured sympathetic neurons from neonatal rat superior cervical ganglia [86], in transient outward potassium currents in cultured cortical neurons from embryonic rats [75], in sodium current observed in cultured mouse cortical neurons [80] and isolated rat dorsal root ganglion [81], or the suppression of sustained potassium currents in rat small dorsal root ganglion neurons [87]. Interestingly, TNF- α treatment did not significantly affect the membrane properties of hippocampal CA1 neurons from rabbits fed with dietary cholesterol, which may be attributed to an increased endogenous TNF- α in cholesterol-fed rabbits which was confirmed by our data and previous studies in mice [48, 49]. This indicates the hippocampus from cholesterol-fed rabbits has been exposed for a long time to increased endogenous TNF-

α and thus chronic hippocampal neuroinflammation and a TNF- α signaling pathway may be involved in behavioral alterations observed in rabbit model of AD created by dietary cholesterol [1, 41, 45, 67].

Unlike previous study showing high-dose TNF- α treatment increased basal synaptic transmission in rat hippocampal slices [15], we found TNF- α treatment at a relatively low concentration did not significantly influence either basal synaptic transmission or presynaptic function in the Schaffer collateral pathway of rabbit hippocampus, which is in line with a recent report showing TNF- α did not affect the baseline synaptic transmission at Schaffer collateral-CA1 synapse in mice [88] and in 8-week-old transgenic rats that overexpress a murine TNF α gene [89]. This suggests a presynaptic mechanism via the regulation of presynaptic transmitter release may be not the main contributor to TNF- α induced electrophysiological alterations in rabbit hippocampus. Therefore, the effects of TNF- α on basal neural transmission and presynaptic function may be partly attributed to neuronal toxicity and hyper-excitability induced by a large dose of TNF- α [15, 90] and may be brain region specific [74] or tissue specific [91]. However, TNF- α treatment did reduce LTP expression in rabbit hippocampal slices, indicating postsynaptic changes may be a major contributor to the electrophysiological alterations in rabbit hippocampus. This is in line with the limited electrophysiological reports about impaired LTP in TNF- α treated acute hippocampal slices from rats [15, 92–94] and mice [88], and suppression of synaptosomal chemically-induced LTP in mice [95]. This is also supported by other studies showing a soluble TNF- α inhibitor can rescue impaired LTP in 5xFAD mice [96] and systemic inflammation impaired LTP at mouse Schaffer collateral-CA1 synapses [97]. However, there are also some contradictory reports showing an increased LTP in transgenic rats that overexpress a murine TNF- α gene [89] and enhanced excitatory field potentials in hippocampal CA 1 stratum radiatum from rat colonic inflammation induced by 2,4,6-trinitrobenzenesulfaonic acid [98] and mouse peritoneal inflammation [99]. These suggest the effects of TNF- α on the central nervous system may be bidirectional and this complexity may depend on many factors including dose [75, 100], exposure duration [75, 100], distribution of different TNF- α receptors among different tissues and cells [63], and the cross-talk between signaling pathways initiated by TNF- α receptor 1 and receptor 2 [64]. Therefore, in addition to an increase in neuronal content of cholesterol in hippocampus [35, 45], A β and phosphorylated tau [1, 35, 101], damaged blood-brain barrier from cholesterol-fed rabbits [102, 103], cholesterol metabolites including 27-hydroxycholesterol [33, 34], cytokines may be another form of interaction between dietary cholesterol and brain cellular changes, and cognitive deficit observed in cholesterol-fed rabbits [1, 34, 104].

In summary, dietary cholesterol can induce a persistent hippocampal TNF- α elevation and model chronic hippocampal inflammation. TNF- α alters membrane properties and synaptic plasticity. These findings provide another explanation for the cellular mechanisms underlying the behavioral changes observed in the rabbit model of AD by dietary cholesterol feeding.

ACKNOWLEDGMENTS

This project was supported by NIH Grant AG023211, Transition Grant Support: Office of Research and Graduate Education, WVU Health Sciences Center, and BRNI funds to Bernard G. Schreurs. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the NIA. I am grateful to Dr. Bernard G. Schreurs for critical comments on the manuscript and to Carrie A. Smith-Bell for animal care.

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Fig. 1.

Dietary cholesterol dose-dependently increased endogenous TNF- α expression in rabbit hippocampus. Each point in the Figure represents the result from a single rabbit and dash line illustrates the average for each group. Note that dietary cholesterol increased TNF- α expression in dose-dependent manner in rabbit hippocampus. The statistical difference was between Chol 2.0 group and both Chol 0 and Chol 1.0 groups.

Fig. 2.

Exogenous TNF- α treatment produced changes in membrane properties of rabbit hippocampal CA1 neurons. A represents the typical traces of evoked APs from hippocampal CA1 neurons with and without exogenous TNF- α treatment. B-F represent those significant changes induced by TNF- α treatment in AP threshold, current required for evoked AP, latency, AHP amplitude, and S₁S₂ interval, respectively. Note that TNF- α treatment exhibited a range of changes in intrinsic membrane excitability of hippocampal CA1 neurons, increased excitability featured by shortened latency for evoked AP, reduced AHP amplitude, and shortened S₁S₂ interval; decreased excitability characterized by slightly higher AP threshold and slightly larger current required for evoked AP.

Fig. 3. Dietary cholesterol induced changes in membrane properties of rabbit hippocampal CA1 neurons. A-B represent the changes induced by dietary cholesterol in AP threshold and AHP amplitude, respectively. Note that dietary cholesterol increased intrinsic membrane excitability of hippocampal CA1 neurons featured by reduced AHP amplitude and slightly lower AP threshold.

Fig. 4.

TNF- α treatment altered AHP amplitude of evoked AP in hippocampal CA1 neurons from rabbits fed with normal chow but not dietary cholesterol. A represents the changes induced by TNF- α treatment in AHP amplitude of evoked APs from hippocampal CA1 neurons. Note that there was a significant change between post and prior to TNF- α treatment in AHP amplitude of evoked APs from hippocampal CA 1 neurons of rabbits fed with normal chow but not dietary cholesterol. B represents the percentile changes between post and prior to TNF- α treatment in AHP amplitude of evoked AP from hippocampal CA 1 neurons. Note that the percentile change in AHP amplitude was significant between normal chow-fed rabbits and cholesterol-fed rabbits.

Fig. 5.

TNF- α altered the synaptic properties in the Schaffer collateral pathway of rabbit hippocampus. A represents the typical traces of fEPSP, which were evoked at a stimulus intensity of 100 μ A, from Control and TNF- α groups, respectively. B-D represent input-output curves, PPF curves, and LTP curves from Control and TNF- α groups, respectively. Note that the input-output curves (B) showed the slope of fEPSP increased in response to increasing stimulus intensities, and TNF- α treatment slightly decreased the slope of fEPSP. PPF curves (C) showed the slope ratio of EPSP₂/EPSP₁ decreased in response to prolonged interstimulus interval but there is no difference between Control and TNF- α groups. LTP curves (D) showed the percentile changes in slope ratio of fEPSP before and after LTP induction through HFS. Note that TNF- α treatment reduced the level of LTP expression. N-number for slices is 6 and 10 for Control and TNF- α groups, respectively.

TNF- α pretreatment altered membrane properties of rabbit hippocampal CA1 neurons

Table 1

	V _m (mV)	Input Res (M Ω)	AP threshold (mV)	Current required for evoked AP (nA)	Latency (ms)	Amplitude (mV)	APD ₅₀ (ms)	APD ₅₀ rising (ms)	APD ₅₀ falling (ms)	AHP (mV)	SIS2 interval (ms)
Control	-63.66 $\pm$ 0.65	55.79 $\pm$ 3.15	-49.87 $\pm$ 0.72	0.14 $\pm$ 0.01	92.06 $\pm$ 12.27	107.91 $\pm$ 1.76	2.37 $\pm$ 0.08	0.62 $\pm$ 0.03	1.75 $\pm$ 0.05	-0.83 $\pm$ 0.86	55.81 $\pm$ 7.41
TNF	-65.53 $\pm$ 0.61	55.46 $\pm$ 3.42	-47.29 $\pm$ 0.84*	0.19 $\pm$ 0.01**	57.51 $\pm$ 4.50**	102.60 $\pm$ 1.63	2.14 $\pm$ 0.08	0.58 $\pm$ 0.03	1.55 $\pm$ 0.05	1.96 $\pm$ 0.67*	32.03 $\pm$ 2.70*

Control, No exogenous TNF- α treatment; TNF, Exogenous TNF- α treatment. Cell number (n) for Control and TNF was 14, and 23, respectively. Note that there were significant group differences in AP threshold, Current required for evoked AP, latency for evoked AP, AHP amplitude, and SIS2 interval. (*, ** indicate $p < 0.05$, 0.01, respectively). AP, Action potential; AP duration was measured at the width of 50% AP duration (APD50); AHP, Afterhyperpolarization; Input Res, Input resistance.

Membrane properties of hippocampal CA1 pyramidal neurons from rabbits fed with dietary cholesterol

Table 2

	V _m (mV)	Input Res (MΩ)	AP threshold (mV)	Current required for evoked AP (nA)	Latency (ms)	Amplitude (mV)	APD ₅₀ (ms)	APD ₅₀ rising (ms)	APD ₅₀ falling (ms)	AHP (mV)	SIS2 interval (ms)
Control	-66.75±0.58	70.77±2.99	-47.56±0.54	0.18±0.01	42.79±4.54	92.53±1.43	2.34±0.06	0.72±0.02	1.63±0.04	1.83±0.50	76.82±5.19
TNF	-66.57±0.64	71.41±4.87	-49.85±0.73*	0.16±0.01	46.36±5.84	93.38±1.90	2.34±0.07	0.72±0.03	1.62±0.04	4.32±0.79**	84.29±5.89

Control, Normal chow; Chol, 1% cholesterol diet. Cell number (n) for Control and TNF was 55, and 26, respectively. Note that there were significant group differences in AP threshold and AHP amplitude (*, ** indicate $p < 0.05$, 0.01, respectively). AP, Action potential; AP duration was measured at the width of 50% AP duration (APD₅₀); AHP, Afterhyperpolarization; Input Res: Input resistance.

TNF- α posttreatment altered membrane properties of rabbit hippocampal CA1 neurons

Table 3

	V _m (mV)	Input Res (M Ω)	AP threshold (mV)	Current required for evoked AP (nA)	Latency (ms)	Amplitude (mV)	APD ₅₀ (ms)	APD ₅₀ rising (ms)	APD ₅₀ falling (ms)	AHP (mV)	SIS2 interval (ms)
ControlPre	-67.75 $\pm$ 0.46	65.23 $\pm$ 6.35	-47.86 $\pm$ 0.74	0.18 $\pm$ 0.02	41.45 $\pm$ 4.86	98.44 $\pm$ 2.18	2.12 $\pm$ 0.09	0.61 $\pm$ 0.04	1.51 $\pm$ 0.05	2.76 $\pm$ 0.76	90.77 $\pm$ 11.05
ControlPost	-66.93 $\pm$ 0.40	53.61 $\pm$ 4.48	-48.07 $\pm$ 0.69	0.18 $\pm$ 0.02	48.43 $\pm$ 5.52	99.80 $\pm$ 2.01	2.28 $\pm$ 0.07	0.60 $\pm$ 0.03	1.68 $\pm$ 0.05	7.17 $\pm$ 0.99*	90.87 $\pm$ 6.29
Chol Pre	-68.68 $\pm$ 0.80	77.32 $\pm$ 15.66	-48.07 $\pm$ 1.71	0.16 $\pm$ 0.02	63.02 $\pm$ 13.57	91.63 $\pm$ 4.28	2.31 $\pm$ 0.20	0.74 $\pm$ 0.09	1.57 $\pm$ 0.11	5.26 $\pm$ 2.06	109.49 $\pm$ 20
Chol Post	-67.90 $\pm$ 0.71	54.24 $\pm$ 9.58	-48.61 $\pm$ 1.50	0.16 $\pm$ 0.02	62.44 $\pm$ 13.68	101.17 $\pm$ 2.86	2.22 $\pm$ 0.17	0.56 $\pm$ 0.06	1.66 $\pm$ 0.12	4.31 $\pm$ 2.04	76.56 $\pm$ 13.53

Control Pre, Control group prior to TNF treatment; Control Post, Control group post TNF treatment; Chol Pre, Chol group prior to TNF treatment; Chol Post, Chol group post TNF treatment. Cell number (n) for Control Pre, Control Post, Chol Pre, and Chol Post groups was 12, 12, 5, and 5, respectively. Note that there was significant group difference in AHP amplitude and the difference was between Control Pre and Control Post (* indicates $p < 0.05$). AP, Action potential; AP duration was measured at the width of 50% AP duration (APD50); AHP, Afterhyperpolarization; Input Res, Input resistance.