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Classical conditioning of the rabbit's nictitating membrane response is a function of the duration of dietary cholesterol

BERNARD G. SCHREURS¹, CARRIE A. SMITH-BELL¹, DEYA S. DARWISH², GORAN STANKOVIC³, and D. LARRY SPARKS³

¹Department of Physiology and Pharmacology, Blanchette Rockefeller Neurosciences Institute, West Virginia University, Morgantown, WV, USA

²Department of Neurobiology and Anatomy, West Virginia University, Morgantown, WV, USA

³Sun Health Research Institute, Sun City, AZ, USA

Abstract

Modifying dietary cholesterol may improve learning and memory but very high cholesterol can cause pathophysiology and death. Rabbits fed 2% cholesterol for 8, 10 or 12 weeks with 0.12 ppm copper added to distilled water and rabbits fed a normal diet without copper added to distilled water (0 weeks) were given a difficult trace classical conditioning task and an easy delay conditioning task pairing tone with corneal air puff. The majority of cholesterol-fed rabbits survived the deleterious effects of the diet but survival was an inverse function of the diet duration. Compared to controls, the level of classical conditioning and conditioning-specific reflex modification were an inverted "U"-shaped function of diet duration. Highest levels of responding occurred in rabbits on cholesterol for 10 weeks and trace conditioning was negatively correlated with the number of hippocampal β -amyloid-positive neurons. Rabbits on the diet for 12 weeks responded at levels comparable to controls. The data provide support for the idea that dietary cholesterol may facilitate learning and memory but there is an eventual trade off with pathophysiological consequences of the diet.

Keywords

β -Amyloid; cholesterol; classical conditioning; nictitating membrane response; learning; rabbit

Introduction

The cholesterol-fed rabbit has a long history of being used as an animal model of atherosclerosis as a result of demonstrations by Anitschkow (1913) that a cholesterol diet can induce vascular lesions (Finking and Hanke 1997; Bocan 1998; Fan and Watanabe 2000; Moghadasian 2002). Based on Sparks' observation that rabbits fed cholesterol have β -amyloid staining in the brain (Sparks et al. 1994; Sparks 1997b), the cholesterol-fed rabbit has also been proposed as an animal model of β -amyloid accumulation in the brain (Sparks et al. 1994, 2000; Sparks 1997b; Zatta et al. 2002; Ghribi et al. 2006; Sjogren et al. 2006).

Our initial studies examining the ability of cholesterol-fed rabbits to learn found that although a cholesterol diet elevated the number of β -amyloid labeled neurons in the cortex,

it actually improved classical conditioning of the nictitating membrane response (NMR) compared to normal-diet control rabbits (Schreurs et al. 2003). It was only when cholesterol-fed rabbits displayed β -amyloid plaque-like structures induced by adding copper to the drinking water that classical conditioning of the NMR was retarded (Sparks and Schreurs 2003). The addition of copper to the drinking water was based on the finding that copper was the critical component in tap water that increased the number of β -amyloid immunoreactive neurons in cholesterol-fed rabbits (Sparks et al. 2002, 2006; Sparks and Schreurs 2003). When plaque-like structures are not induced by supplementation with copper, cholesterol-fed rabbits show classical conditioning of the NMR at levels as high as or higher than normal-diet controls (Schreurs et al. 2007a).

We have shown previously that feeding rabbits cholesterol can facilitate classical conditioning and conditioning-specific reflex modification (CRM) of the rabbit NMR (Schreurs et al. 2003) as well as classical conditioning and CRM of rabbit heart rate (Schreurs et al. 2007b). CRM describes changes in reflexive responding to an unconditioned stimulus (US) following classical conditioning when the US is tested by itself (Schreurs et al. 1995). In the case of classical conditioning of the rabbit NMR, CRM involves an increase in the size (NMR amplitude) and shape (peak latency and topography) of the response (Schreurs et al. 1995; Schreurs 2003). This facilitation of learning and CRM occurs despite the pathophysiology caused by a high-cholesterol diet that includes atherosclerosis, coronary heart disease and liver dysfunction (Sparks 1997a). The purpose of the present experiment was to determine whether the facilitative effects of cholesterol on classical conditioning of the rabbit NMR as well as NMR CRM were a function of the duration of the cholesterol diet.

Methods and materials

Subjects

Forty two male, New Zealand white rabbits (*Oryctolagus cuniculus*) supplied by Harlan (Indianapolis, IN) weighed approximately 2.0–2.2 kg upon arrival. Animals were housed in individual cages, given one cup of food (140 g) daily and ultra pure water (18 M Ω , Millipore Academic) and maintained on a 12 h light/dark cycle. Rabbits were assigned to four groups comprising access to a normal diet and distilled water for 12 weeks ($n = 6$, 0 week group) or access to 2% cholesterol diet and distilled water supplemented with 0.12 ppm copper as copper sulfate for 8 weeks ($n = 10$, 8 week group), 10 weeks ($n = 13$, 10 week group), or 12 weeks ($n = 13$, 12 week group) prior to classical conditioning. The classical conditioning experiments took place over a period of 19 days following the 12-week dietary manipulation and rabbits continued on their normal or 2% cholesterol diet and their appropriate water throughout the behavioral procedures. Five rabbits in the 12 week group died as a result of cholesterol-induced pathology before classical conditioning could begin and a sixth was euthanized before classical conditioning due to a back injury. Three rabbits in the 10 week group died of cholesterol-induced pathology during trace conditioning and their data were not included in the behavioral analyses. Another two rabbits in the 10 week group died during delay conditioning and their data were included. Histological analysis was only conducted on the brains of rabbits included in the behavioral analyses except for one rabbit in the 8 week group whose brain was not available. In sum, behavioral data were collected from 100% of rabbits fed the normal diet, from 100% of rabbits fed cholesterol for 8 weeks, from 77% of rabbits fed cholesterol for 10 weeks and from 58% of rabbits fed cholesterol for 12 weeks.

Cholesterol-fed rabbits received 2% cholesterol incorporated into Purina rabbit chow (Purina 5321; Dyets Inc., Bethlehem, PA) for 8, 10 or 12 weeks prior to behavioral experiments. The rabbits all arrived at the same time to equate for age and duration in the

facility. Rabbits in the 12 week group were fed the 2% cholesterol diet and given copper-supplemented ultra pure water upon arrival. Rabbits in the 10 week group were fed standard Purina rabbit chow (Purina 5321, 0% added cholesterol) and given ultra pure water for two weeks before being switched to the cholesterol diet and copper supplemented water and rabbits in the 8 week group were fed normal chow and given ultra pure water for four weeks before being switched to the cholesterol diet and copper supplemented water. Rabbits in the 0 week group were fed standard rabbit chow (Purina 5321, 0% added cholesterol) and given ultra pure water for 12 weeks before the behavioral experiment. Each diet was maintained throughout the 19 days of the behavioral experiments. Cholesterol was supplied as lanolin that was dissolved in ether and mixed with Purina 5321 rabbit chow meal. The ether was allowed to evaporate, and the meal was then formed into pellets (Alissa et al. 2004). Blood cholesterol levels were not assessed in the present study but a 2% cholesterol diet invariably elevates rabbit cholesterol to levels approaching 2000 mg/dl within 6 weeks of starting the diet (Bocan 1998). Rabbits were maintained in accordance with guidelines issued by the National Institutes of Health and the research was approved by the West Virginia University Animal Care and Use Committee.

Apparatus

The apparatus has been detailed by Schreurs and Alkon (1990) who modeled their apparatus after those developed and described by Gormezano (1966) (Coleman and Gormezano 1971). Each rabbit was restrained in a Plexiglas box and trained in a sound-attenuating, ventilated chamber (Coulbourn Instruments, Allentown, PA; Model EI0-20). A stimulus panel containing a speaker and a house light (10-W, 120-V incandescent lamp) was mounted at a 45° angle, 15 cm anterior to and 15 cm above the subject's head. An ambient noise level of 65 dB was provided by an exhaust fan. A programmable air pressure delivery system (Model ER-3000, Tescom Corp., Elk River, MN) was used to deliver a puff of air through a tube (1 mm internal diameter) positioned 5 mm from and perpendicular to the center of the cornea.

Details of transducing nictitating membrane (NM) movements have been reported previously (Gormezano and Gibbs 1988; Schreurs and Alkon 1990). A 1 mm hook connected to an L-shaped lever containing a freely moving ball and socket joint was attached to a 6-0 nylon loop sutured into, but not through, the NM. The other end of the lever was attached to a potentiometer (Novotechnik US Inc., Southborough, MA; Model P2201) that, in turn, was connected to a 12-bit analog-to-digital converter (5 ms sampling rate; 0.05 mm resolution). Individual analog-to-digital outputs were stored on a trial-by-trial basis for subsequent analysis. Data collection, analysis and stimulus delivery were accomplished using a LabVIEW system (National Instruments, Austin, TX).

Procedure

Rabbits received one day of adaptation, one 60-trial session of air puff US pretesting (pretest), 10 daily sessions of tone and air puff trace conditioning, a 60-trial session of US post-testing (post test), four daily sessions of tone and air puff delay conditioning and two days of tone intensity testing. Adaptation was designed to habituate the rabbits to restraint and the training chambers. US pretest enabled an assessment of sensory and motor effects of the cholesterol diets on the NMR to air puff as well as providing the baseline reflexive responding against which to compare any conditioning-specific effects on the NMR during US post test. Trace conditioning provided an opportunity to assess the effects of cholesterol duration on the acquisition of a difficult learning task created by the temporal gap or trace between the tone and air puff (Solomon et al. 1986; Moyer et al. 1990) and delay conditioning enabled us to ensure that rabbits could all learn a relatively simple conditioning task (Schneiderman 1966). Finally, tone intensity testing allowed us to determine whether

the cholesterol diet duration had any effects on the rabbits' ability to hear (Schindler et al. 1984). Following four days of delay conditioning, hearing of those rabbits responding above 80% CRs during delay conditioning was tested by pairing tones that were softer and louder than the training intensity (82 dB) with the air puff. An 80% CR criterion was used to ensure that all rabbits would respond to the tone reliably during tone intensity testing.

On adaptation day, the rabbits were prepared for recording of NM movement and then adapted to the training chambers for the length of time of subsequent training sessions (60 min). On both the pretest and post test day all subjects received a total of 60 trials of air puff presented, on average, every 60 s (50–70 s range). Each trial involved the presentation of 1 of 15 possible combinations of stimulus intensity (0.5, 1.0, 2.0, 4.0, 8.0 psi) and stimulus duration (25, 50, or 100 ms). Four separately randomized sequences of the 15 stimulus combinations were presented, with the restriction that the same intensity or duration could not occur on more than three consecutive trials. Each of the 10 paired trace conditioning sessions consisted of 60 presentations of a 100 ms, 1 kHz, 82 dB, tone conditioned stimulus (CS) that was followed by a 500 ms trace interval and then a 100 ms, 4 psi air puff US (i.e. 600 ms interstimulus interval). Each of the four days of paired delay conditioning consisted of 60 presentations of a 400 ms, 1 KHz, 82 dB tone CS that coterminated with a 100 ms, 4 psi air puff US (i.e. 300 ms interstimulus interval). Stimulus presentations were delivered, on average, every 60 s (50–70 s range). The two tone intensity testing sessions consisted of the presentation of one of seven 100 ms tone intensities (60, 65, 70, 75, 80, 85, 90 dB) or a zero intensity (0 dB) followed by a 200 ms trace interval and then a 100 ms, 4 psi air puff US (i.e. 300 ms interstimulus interval). Each tone intensity-air puff pairing was presented eight times as a randomized sequence with a trial delivered, on average, every 60 s (50–70 s range). To facilitate the delivery of the air puff US, the eyelids were loosely held open during each session with tailor's hooks attached to an adjustable elastic strap (Buck et al. 2001).

An NM conditioned response (CR) was defined as any extension of the NM exceeding 0.5 mm that was initiated after CS onset but prior to US onset. A NM unconditioned response (UR) was defined as any extension of the NM exceeding 0.5 mm that occurred within 300 ms of US onset (Schreurs et al. 2000). In order to overcome the statistical limitations of empty data cells produced by subthreshold US intensities during US testing without introducing the potential biases of data imputation, we used dependent variable measures of magnitude of the response and magnitude of the response area. These measures included all amplitudes and areas above the baseline regardless of whether or not a response met the 0.5 mm criterion (Gracia et al. 2003). Response topographies for each of the US intensities were averaged across subjects and US durations and examined for differences by comparing the shape of the averaged response (Seager et al. 2003; Schreurs et al. 2006).

Histology

In order to determine the effects of the cholesterol diet on the hippocampus and cortex—areas previously found to show β -amyloid immunoreactive neurons (Sparks et al. 1994, 1995; Sparks 1996), rabbits were anesthetized deeply with a cocktail of Ketamine (640 mg/kg)/Xylazine (130 mg/kg) and perfused transcardially with 0.5% paraformaldehyde in 0.1 M phosphate-buffered saline. Brains were extracted and post-fixed for 14 days in 4% paraformaldehyde. Fifty-micron vibratome sections of the hippocampus and surrounding cortex were immunostained with an antibody to β -amyloid (10D5, 1:100 dilution; provided by Dale Schenk of Elan Pharmaceuticals) using published peroxidase–antiperoxidase immunohistochemical methods (Sparks et al. 1994). The cells positively stained for the 10D5 antibody within a 0.5×0.5 mm square grid were counted in and averaged across at least eight randomly selected areas within the inferior and superior temporal cortex and at least four randomly selected areas within the hippocampus including CA1 and dentate gyrus

within a randomly selected section using a 20× objective as previously described (Schreurs et al. 2003; Sparks et al. 2002, 2006; Sparks and Schreurs 2003). Counts were made by a researcher who was blind to the treatment conditions (GS).

Statistical analysis—Analysis of variance was used to test for main effects and interactions and follow up comparisons between groups were based on statistical tests of contrasts between group means (Bird 2004).

Results

Body weight

Figure 1 shows body weights for rabbits in the 0, 8, 10 and 12 week groups across the 12 weeks prior to classical conditioning. The figure shows that whereas weight gain for the 0 week group was linear, weight gain for the cholesterol-fed rabbits was lower and reached a plateau within 4 weeks of starting the diet. Weight gain for the cholesterol-fed rabbits was an inverse function of cholesterol diet duration but body weights for the cholesterol-fed rabbits were comparable by the beginning of behavioral training. Analysis of body weights yielded a main effect of groups ($F(3,28) = 7.96, p < 0.01$, weeks ($F(11,308) = 334.13, p < 0.001$) and an interaction of group by weeks ($F(33,253) = 12.46, p < 0.001$) confirming group differences during the course of the diet. Follow-up group comparisons indicated that rabbits fed no cholesterol gained more weight from week 3 on than the cholesterol-fed rabbits (smallest $F(1,28) = 6.05, p < 0.05$). Rabbits fed cholesterol for 12 weeks gained less weight than rabbits fed cholesterol for 8 weeks particularly from week 5 to week 10 of the diet (smallest $F(1,23) = 4.50, p < 0.05$). There were no significant differences between the weights of rabbits on the cholesterol diet for 10 weeks and 12 weeks at any point during the diet (largest $F(1,23) = 2.57, p > 0.12$). Cholesterol-fed rabbit body weights converged during the last weeks before classical conditioning and there were no significant differences between the groups at 12 weeks (largest $F(1,23) = 2.48, p > 0.12$).

Behavior

Classical conditioning—Figure 2 shows mean percent CRs ($\pm$ SEM) for rabbits in the 0, 8, 10 and 12 week groups across the 10 days of trace conditioning and the four days of delay conditioning. The figure shows very similar acquisition functions for the four groups during trace conditioning with the suggestion that rabbits in the 10 week group responded at somewhat higher levels than rabbits in the 0, 8, and 12 week groups. This impression was even more striking on the first day of delay conditioning but with continued delay conditioning all groups acquired high levels of conditioning. Analysis of mean percent CRs during trace conditioning yielded a significant main effect days ($F(9,252) = 26.95, p < 0.001$), but no overall significant main effect or interaction of group (F 's < 1.2). However, a specific comparison between the 10 week group and the other groups across the 10 days of trace conditioning did yield a significant interaction of group by days ($F(9,270) = 2.19, p < 0.05$). Analysis of mean percent CRs during delay conditioning yielded a significant main effect of days ($F(3,84) = 86.07, p < 0.001$) and a significant interaction of group $\times$ days ($F(9,84) = 2.33, p < 0.05$). A follow-up comparison of mean percent CRs across the days of delay conditioning showed that there was a significant difference between the rabbits in the 10 week group and the other three groups ($F(3,90) = 4.81, p < 0.005$).

Conditioning-specific reflex modification—Figure 3 shows averaged topographies and Figure 4 shows mean ($\pm$ SEM) UR frequency, magnitude and magnitude of the UR area for rabbits in the 0, 8, 10 and 12 week groups on the first 15 trials of pretest and on the first 15 trials of Post Test across five US intensities collapsed across US duration. Examination of the figures shows that URs increased as a function of US intensity and there was a change

in responding from pretest to post test. The two largest and most consistent changes from pretest to post test were the decrease in UR topographies in the 0 week group and the increase in UR topographies in the 10 week group. Changes from pretest to post test were more variable in the 8 and 12 week groups. These data suggest that the level of CRM was higher in the 10 week group than the 0, 8 or 12 week groups. Indeed, it appears that the level of CRM reflected the terminal level of responding following trace conditioning.

Analysis of UR frequency, magnitude and magnitude of the UR area yielded main effects of US intensity (smallest $F(4,92) = 44.54, p < 0.001$), and main effects or interactions of test (pretest vs. post test) (smallest $F = 3.36, p < 0.05$) confirming both the increase in URs as a function of US intensity and the significant changes in UR topography from pretest to post test. More importantly, there were significant interactions of test with group in UR Frequency ($F(3,36), p < 0.05$), UR magnitude ($F(2,23) = 6.03, p < 0.01$) and magnitude of the UR area ($F(2,23) = 4.50, p < 0.05$), confirming the differences in URs, and hence, evidence of CRM, that was shown by rabbits in the 10 week group but not by rabbits in the 0, 8 and 12 week groups. Finally, despite apparent between-group differences in URs on pretest, there were no significant main effects or interactions of group on any pretest UR measure suggesting that sensory and motor aspects of rabbit responding to air puff were not differentially affected by manipulation of the duration of the cholesterol diet (largest $F(12,112) = 1.42, p > 0.16$).

Tone intensity—Figure 5 shows mean percent CRs to eight intensities of the tone CS for rabbits in the 0, 8, 10 and 12 week groups collapsed across two additional days of trace conditioning. The data were restricted to rabbits showing levels of responding during delay conditioning that were greater than or equal to 80% CRs to ensure that all rabbits could respond reliably to the tone during intensity testing. As a result, two rabbits from the 0 week group, one rabbit from the 8 week group and another from the 12 week group were dropped. The figure shows that the level of responding was a function of tone intensity and that rabbits in the 12 week group appeared to be responding at lower levels than rabbits in the 0, 8 or 10 week groups. These data suggest an increase in auditory threshold. It is unlikely that these differences were due to changes in motor performance because only rabbits with a level of responding of at least 80% CRs were included in the analysis and there were no differences in the frequency or amplitude of responding to the US during post test between rabbits in the 0 and 12 week groups.

Analysis of responding during tone intensity testing revealed main effects of intensity ($F(7,168) = 159.80, p < 0.001$), group ($F(3,24) = 4.54, p < 0.05$), and an interaction of intensity by group ($F(21,168) = 2.19, p < 0.005$). Follow-up comparisons indicated that rabbits in the 12 week group had lower levels of responding to tones from 65 to 90 dB than rabbits in the 0, 8 and 10 week groups (smallest $F(1,24) = 4.31, p < 0.05$).

Histology—Figure 6 shows mean number of (β -amyloid immunoreactive (labeled) cells in the hippocampus and temporal cortex for rabbits in the 0, 8, 10, and 12 week groups. Examination of Figure 6 shows the number of β -amyloid-labeled cells in the hippocampus and the cortex increased as a result of the presence of cholesterol in the diet (0 week group vs. the rest). Figure 6 also shows the number of β -amyloid-labeled cells in the cortex but not in the hippocampus varied as a function of cholesterol duration. Specifically, there seemed to be fewer labeled cells in the 8 week group than in the 10 and 12 week groups. Analysis of the number of β -amyloid-labeled cells in the hippocampus and cortex revealed significant differences between groups for both the hippocampus ($F(3,27) = 5.20, p < 0.01$) and cortex ($F(3,27) = 11.26, p < 0.001$). Follow-up comparisons for the hippocampus showed that the 0 week group had fewer labeled cells than the cholesterol-fed groups ($F(1,27) = 13.33, p < 0.005$) which, in turn did not differ from each other (p 's > 0.13). Follow-up comparisons for

the cortex showed that the 0 week group had fewer labeled cells than the cholesterol-fed groups ($F(1,27) = 24.98, p < 0.001$) and that the 8 week group had lower levels than the 10 and 12 week groups ($F(1,27) = 4.79, p < 0.05$). Although there was a clear elevation in the number of β -amyloid immunoreactive cells in cholesterol-fed rabbits, there was no evidence of β -amyloid plaque-like structures in the cortex or hippocampus in any of the rabbits.

Finally, the correlation between the number of labeled cells in the hippocampus and terminal levels of responding during trace conditioning was significant for the 10 week group ($-0.83, p < 0.01$) but not for the 0 week ($-0.41, p > 0.21$), 8 week ($0.00, p > 0.50$) or 12 week groups ($0.19, p > 0.31$). The significant negative correlation in the 10 week group suggests that performance on the hippocampally-dependent trace conditioning task for these rabbits was better the fewer the number of labeled hippocampal neurons. There were no significant correlations between the number of labeled cells in the cortex and responding at the end of trace conditioning.

Discussion

The principal findings of the current experiment were that: (1) compared to rabbits fed a normal diet, both the level of classical conditioning and CRM in rabbits were an inverted “U”-shaped function of the duration of a cholesterol diet; (2) performance on a hippocampally-dependent trace conditioning task was negatively correlated with the number of β -amyloid-labeled cells in the hippocampus; and (3) the cholesterol diet may eventually produce sensory deficits evidenced by an increase in auditory threshold.

We have previously shown that cholesterol can enhance NMR conditioning and CRM (Schreurs et al. 2003), and the present results extend this finding to show that the level of facilitation is a function of cholesterol duration. There appears to be an optimal period on the cholesterol diet when the facilitative effects of cholesterol on learning and memory outweigh the deleterious physical effects of the diet. These deleterious effects include reduced weight gain, increased auditory thresholds, and eventually death. However, the fact that rabbits fed cholesterol for 12 weeks performed as well as those fed for only 8 weeks and those fed no cholesterol at all suggests that performance was not totally attributable to the deleterious physical effects of the diet. The data cannot be explained by differential mortality either because two rabbits that succumbed to the effects of cholesterol at the end of delay conditioning performed at levels during trace conditioning and post test that were comparable to those that survived.

Given that dietary cholesterol does not normally cross the blood brain barrier (BBB) directly in any appreciable amount (Dietschy and Turley 2001, 2004; Tuma and Hubbard 2003; Bjorkhem and Meaney 2004), it remains to determine how classical conditioning and CRM were facilitated by the cholesterol diet. One possible explanation for these facilitative effects is that cholesterol does cross the BBB to influence neurons in the brain but only after prolonged exposure to a high cholesterol diet (Sparks 1997b). For example, Ghribi et al. (2006) reported that a 1% cholesterol diet produced a significant elevation in the cholesterol content of rabbit hippocampal neurons without increasing the overall level of hippocampal cholesterol concentration. Sparks (1997b) found a significant increase in cholesterol concentration in the rabbit cortex adjacent to the hippocampus following an 8-week 2% cholesterol diet. Another possible explanation is that metabolites that cross the blood brain barrier, particularly 27-hydroxysterol, may have had some influence on learning (Heverin et al. 2005). There is evidence, albeit indirect, for the involvement of 27-hydroxysterol in learning and memory because levels of this oxysterol are significantly

reduced in patients with mild cognitive impairment, Alzheimer's disease and vascular dementia (Kolsch et al. 2004).

The significant negative correlation between trace conditioning performance and the number of (β -amyloid-labeled neurons in the hippocampus of rabbits in the 10 week group suggests there may not be a positive role for intracellular (β -amyloid in learning and memory. Although there is good evidence for the normal function of (β -amyloid in cellular processes (Plant et al. 2003; Pearson and Peers 2006) and that it exists in the brain from infancy to old age (Wegiel et al. 2007), it has been shown to retard learning both in plaque (Chen et al. 2000; Morgan 2003; Sparks and Schreurs 2003) and soluble forms (Koistinaho et al. 2001). Triple knockout transgenic mice that develop β -amyloid plaques, neurofibrillary tangles and tau protein pathology show age-related deficits in spatial learning in the Morris water maze that correlate with accumulation of intraneuronal β -amyloid prior to the appearance of β -amyloid plaques or tangle pathology (Billings et al. 2005). Ewers and colleagues have reported that in an amyloid precursor protein + presenilin1 double knockout mice, the level of eyeblink conditioning using an electrodermal US was no different from controls, but was inversely related to the β -amyloid burden (Ewers et al. 2005).

The low density lipoprotein receptors (LDLRs) may provide another mechanism for dietary effects of cholesterol on learning and memory (Qiu et al. 2005; Herz and Chen 2006). These receptors are known to regulate cholesterol transport and are capable of transducing a number of extracellular signals across the blood brain barrier and may play a role in synaptic plasticity via NMDA receptors (Herz and Chen 2006). The most well known of the LDLR ligands is apolipoprotein E—a protein implicated in Alzheimer's disease (Harris-White and Frautschy 2005). Recent research suggests that LDLRs may be involved in learning and memory because a transgenic mouse model of Alzheimer's disease lacking the LDLR developed larger spatial learning deficits than a transgenic mice with the receptor (Cao et al. 2006).

A final explanation of the effects of cholesterol on learning and memory involves pro-inflammatory cytokines (Yehuda et al. 2005). Cytokines including interleukin 1α and 1β , tumor necrosis factor α , and interleukin-6 known to be induced by a high cholesterol diet (Fazio and Linton 2001; Kaul 2001; Rahman et al. 2005) cross the blood brain barrier to affect learning and memory. The role of cytokines in learning and memory has been explored by a number of groups including Maier and colleagues who found that interleukin 1β can adversely affect fear conditioning (Maier and Watkins 2003; Thomson and Sutherland 2005; Barrientos et al. 2006). Specifically, it was found that interleukin 1β interferes with the consolidation of hippocampally dependent contextual fear conditioning (Maier and Watkins 2003). Using a T-maze avoidance paradigm, Banks and colleagues found that interleukin 1α crossed the blood brain barrier to impair memory. On the other hand, Brennan and colleagues have found evidence that the pro-inflammatory cytokine tumor necrosis factor α facilitated performance of avoidance learning (Brennan et al. 2004; Brennan and Tieder 2006). Finally, the systemic injection of interleukin 1β has been shown to facilitate classical eyeblink conditioning in male rats (Servatius and Beck 2003). It is conceivable that the balance between the facilitating and retarding effects of pro-inflammatory cytokines in the present experiment was a function of the cholesterol duration.

In conclusion, it is clear that the negative health effects of high cholesterol outweigh its potential positive effects on learning and memory. However, what remains to be determined is how, despite the pathophysiology caused by a high-cholesterol diet including atherosclerosis, coronary heart disease and liver dysfunction (Sparks 1997a), rabbits fed cholesterol for 10 weeks showed better classical conditioning of the NMR and NMR CRM than rabbits fed cholesterol for 8 or 12 weeks. One answer may lie in the separation of the

peripheral and central cholesterol pools. Dietary cholesterol may have a negative effect on the body but an indirectly positive effect on the brain via cholesterol metabolites, LDL receptors, or pro-inflammatory cytokines. There are a number of examples where high cholesterol damages the body but may have a positive effect on the brain. For example, dietary cholesterol may provide protection from seizures (Alexander and Kopeloff 1971), hypoxia (Xi et al. 2006) and stroke (Vauthey et al. 2000; Zuliani et al. 2004). A recent study by Cui and colleagues shows that although total serum cholesterol is positively correlated with coronary heart disease, it is negatively correlated with stroke (Cui et al. 2006). Low serum cholesterol levels have also been associated with mood disorders and suicide (Beasley et al. 2005). Nevertheless, the present experiment demonstrates that there is a fine line between cholesterol facilitating learning and memory and having deleterious and eventually fatal effects on the body.

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Figure 1. Mean (SEM) body weight for rabbits in the 0, 8, 10, and 12 week groups across the 12 weeks prior to the classical conditioning experiments. White bars indicate the starting point and duration of each diet for the cholesterol fed rabbits (8, 10, 12 weeks).

Figure 2. Mean (SEM) percent conditioned nictitating membrane responding (CRs) to the CS for rabbits in the 0, 8, 10, and 12 week groups across the 10 days of trace conditioning and the four days of delay conditioning.

Figure 3. Average topographies for rabbits in the 0, 8, 10, and 12 week groups on the first 15 trials of pretest and on the first 15 trials of the post test across five US intensities collapsed across US duration.

Figure 4.

Average UR frequency, magnitude and magnitude of the UR area on the first 15 trials of pretest and on the first 15 trials of the post test across five US intensities collapsed across US duration for rabbits in the 0, 8, 10, and 12 week groups.

Figure 5. Mean (SEM) percent conditioned nictitating membrane responding to eight intensities of the tone CS (0, 60, 65, 70, 75, 80, 85 and 90 dB) for rabbits in the 0, 8, 10, and 12 week groups collapsed across two days of tone intensity presentations following delay conditioning.

Figure 6.

Mean (SEM) number β -amyloid immunoreactive (labeled) cells in the inferior and superior temporal cortex and hippocampus of rabbits in the 0, 8, 10, and 12 week groups. Fifty-micron vibratome sections of the hippocampus and surrounding cortex were immunostained with an antibody to β -amyloid (10D5, 1:100 dilution; provided by Dale Schenk of Elan Pharmaceuticals). Cells positively stained for the 10D5 antibody within a 0.5×0.5 mm square grid were counted in at least eight randomly selected areas in the cortex and four randomly selected areas of the hippocampus within a randomly selected section using a 20x objective. Counts were made by a researcher (GS) who was blind to the treatment conditions. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.