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High dietary cholesterol facilitates classical conditioning of the rabbit's nictitating membrane response

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Abstract

Studies have shown that modifying dietary cholesterol may improve learning and that serum cholesterol levels can be positively correlated with cognitive performance. Rabbits fed a 0, 0.5, 1 or 2% cholesterol diet for eight weeks and 0.12ppm copper added to their drinking water received trace and then delay classical conditioning pairing tone with corneal air puff during which movement of the nictitating membrane (NM) across the eye was monitored. We found that the level of classical conditioning and conditioning-specific reflex modification (CRM) as well as the number of beta amyloid-labeled neurons in the cortex and hippocampus were a function of the concentration of cholesterol in the diet. The data provide support for the idea that dietary cholesterol may facilitate learning and memory.

Keywords

Beta amyloid; cholesterol; classical conditioning; nictitating membrane response; learning; rabbit

Introduction

Research with animals suggests that modifying dietary cholesterol can improve learning and memory. Increasing cholesterol in young DBA/2 mutant mice improves performance on the Morris water maze—a spatial learning task that is normally impaired in this mutant (Upchurch and Wehner 1988; Miller and Wehner 1994). Feeding cholesterol to young, normal rats also improves performance on the Morris water maze (Dufour et al. 2006). Feeding cholesterol to rats that are either deficient in cholesterol or have cholesterol synthesis blocked reverses problems with learning and memory (Endo et al. 1996; Xu et al. 1998; O'Brien et al. 2002; Voikar et al. 2002). We have shown previously that feeding rabbits cholesterol can facilitate classical conditioning and conditioning-specific reflex modification (CRM) of the rabbit nictitating membrane response (NMR) (Schreurs et al. 2003). More specifically, we have shown that cholesterol facilitates classical conditioning of the rabbit NMR during trace conditioning with pairings of a tone conditioned stimulus (CS) and a corneal air puff unconditioned stimulus (US). We have also shown that cholesterol can facilitate CRM of the rabbit NMR following trace conditioning with a tone and prioribital

electrical stimulation (Schreurs et al. 2003). CRM describes changes in reflexive responding to a US following classical conditioning when the US is tested by itself (Schreurs et al. 1995). In the case of NMR conditioning, CRM involves an increase in the size (NMR amplitude) and shape (peak latency and topography) of the response (Schreurs et al. 1995; Schreurs 2003).

Many of the positive effects of cholesterol on learning and memory may be due to the crucial role cholesterol plays in forming new synapses, normal receptor function and synaptic plasticity (Koudinov and Koudinova 2001; Mauch et al. 2001; Sooksawate and Simmonds 2001a,b; Goritz et al. 2002). Cholesterol is so crucial to normal neuronal function that it is synthesized *de novo* in the CNS and 25% of the body's total unestrified cholesterol is found in the brain (Dietschy and Turley 2001, 2004). Despite segregation between the peripheral and central cholesterol pools (Dietschy and Turley 2001), there is substantial cross-talk between these pools through a number of cholesterol metabolites (oxysterols) including 24S-hydroxycholesterol (Bjorkhem and Meaney 2004; Dietschy and Turley 2004) and 27-hydroxycholesterol (Heverin et al. 2005)—compounds that are capable of crossing the blood brain barrier. Studies of hypercholesterolemic patients treated with cholesterol-lowering statin drugs showed not only lowered levels of cholesterol in the blood but also lowered levels of the brain-derived cholesterol metabolites (Locatelli et al. 2002). Analysis of brain tissue in hypercholesterolemic rabbits shows that cholesterol concentrations in the cerebral cortex increased significantly over control rabbits (Sparks 1997b). Clearly, modifying peripheral cholesterol levels affects the brain (Bjorkhem 2002).

As noted above, we have shown that feeding cholesterol can result in improved acquisition or the classically conditioned rabbit NMR as well as enhanced CRM (Schreurs et al. 2003). The purpose of the present experiment was to determine whether the facilitative effects of cholesterol are a function of cholesterol concentration.

Methods and materials

Subjects

Twenty four male, New Zealand white rabbits (*Oryctolagus cuniculus*) supplied by Harlan (Indianapolis, IN, USA) weighed approximately 2.0–2.2 kg upon arrival. Animals were housed in individual cages, given free access to food and ultra pure water (18 MΩ, Millipore Academic) and maintained on a 12-hour light/dark cycle. Rabbits were assigned to four groups ($n = 6$) comprising the concentration of cholesterol added to their food: 0, 0.5, 1, or 2%. One rabbit from the 1% group showed excessively low levels of responding to air puff and was excluded from behavioral assessment and only used for histological analysis.

Cholesterol-fed rabbits received 0.5, 1 or 2% cholesterol incorporated into Purina rabbit chow (Purina 5321; Dyets Inc., Bethlehem, PA, USA) for eight weeks prior to behavioral experiments and normal chow control rabbits (0% cholesterol) received standard Purina rabbit chow (Purina 5321, 0% added cholesterol). 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). Numerous reports have shown that rabbits fed a 2% cholesterol diet for eight weeks have serum cholesterol levels approaching 2000 mg/dl whereas a 1% cholesterol diet results in levels of approximately 1400 mg/dl and a 0.5% cholesterol diet results in levels of 700 mg/dl compared to rabbits fed normal chow that have levels of less than 40 mg/dl (Henry and Bentley 1981; Sparks 1997b; Lamb et al. 2001; Huang et al. 2004). Although rabbits on the cholesterol diets did not gain as much weight as controls (see Results), they continued to eat the same amounts of food (100–150g/day). All rabbits had drinking water supplemented with 0.12 ppm copper as copper sulfate previously shown to increase the number of beta

amyloid labeled cells in the brain (Sparks et al. 2006b). 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 described by Gormezano (1966) and 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 E10-20). A stimulus panel containing a speaker and a house light (10-W, 120-V incandescent lamp) was mounted at a 45° angle, 15cm anterior to and 15cm 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, USA).

Procedure

All rabbits received one day of adaptation, one 60-trial session of air puff US pretesting (pretest), eight 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 concentration on the acquisition of a difficult learning task and delay conditioning enabled us to ensure that rabbits could all learn a relatively simple conditioning task. Finally, tone intensity testing allowed us to determine whether the cholesterol diets had any effects on the rabbits' ability to hear. Following four days of delay conditioning, all rabbits were responding at a reliable level to the tone CS and we probed their hearing by pairing tones that were softer and louder than the training intensity (82 dB) with the air puff.

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 60s (50-70s 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 eight paired trace conditioning sessions consisted of 60 presentations of a 100-ms, 1-kHz, 82-dB, tone 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 60s (50–70s 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, 90dB) or a zero intensity (0 dB) followed by a 200-ms trace interval and then a 100-ms, 4-psi air puff US. Each tone intensity–air puff pairing was presented eight times as a randomized sequence with a trial delivered, on average, every 60s (50–70s range). To facilitate 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 U.S 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 brain, rabbits were anesthetized deeply with a cocktail of ketamine (640 mg/kg)/xylazine (130mg/kg) and perfused transcardially with 0.9% 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 beta amyloid (10D5, 1:100 dilution; provided by Dr 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 at least eight randomly selected areas of the cortex and at least four areas of the hippocampus within a randomly selected section using a 20X objective as previously described (Sparks et al. 2002, 2006b; Sparks and Schreurs 2003; Schreurs et al. 2003). Counts were made by a researcher who was blind to the treatment conditions (GS).

Copper levels—Copper levels in serum were measured using the Association of Official Analytical Chemists (AOAC) Method 983.24—atomic absorption spectrophotometric method for copper in serum with the modification that samples were diluted 1 + 4 in water rather than 1 + 1. Copper levels in brain were measured using an adaptation of AOAC Method 985.40—atomic absorption spectrophotometric method for copper in liver with the modification that samples were diluted 1 + 9 instead of 1 + 24.

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

Results

Body weight

Figure 1 shows and analysis of rabbit weights confirmed that after eight weeks on the diet, rabbits on the 0.5, 1 and 2% cholesterol diets had gained less weight (2.82 kg) than the 0% cholesterol control group (3.44 kg, $p < 0.001$). There were no differences in the weights of rabbits on the 0.5, 1 and 2% cholesterol diets.

Behavior

Classical conditioning—Figure 2 shows mean percent CRs for rabbits in the four cholesterol groups across the eight days of trace conditioning and the four days of delay conditioning. The figure shows very clear differences in the acquisition of NMR conditioning between the four groups as a function of days of trace conditioning. The 2% cholesterol group showed higher levels of responding than the remaining groups and the 1% cholesterol group reached a comparable terminal level of responding to the 2% group at the end of the trace-conditioning phase. Analysis of mean percent CRs during trace conditioning yielded a significant main effect of cholesterol concentration [$F(3,19) = 3.91$, $p < 0.05$], a significant effects of days [$F(7,133) = 20.67$, $p < 0.001$] and a significant interaction of cholesterol concentration by days [$F(21,133) = 3.14$, $p < 0.001$]. *Post hoc* orthogonal contrasts (Bird 2004) confirmed that the 2% group was significantly higher than the other three groups across the eight days of trace conditioning ($p < 0.01$) and that the 1% group was different from the 0.5 and 0% groups ($p < 0.05$) which, in turn, were not different from each other.

Following US post test (see below), rabbits were shifted to delay conditioning and all groups showed rapid acquisition of CRs to levels at or near 90%. The relationship among the groups from the trace-conditioning phase was retained during delay conditioning with the 2 and 1% cholesterol groups responding at higher levels than the 0.5 and 0% cholesterol groups. Analysis of mean percent CRs during delay conditioning yielded a significant effects of days [$F(3,57) = 84.86$, $p < 0.001$] and an interaction of cholesterol concentration by days [$F(9,57) = 2.07$, $p < 0.05$]. *Post hoc* contrasts confirmed that the 2 and 1% cholesterol groups showed higher levels of response acquisition than the 0.5 and groups ($p < 0.05$) which, in turn, were not different from each other.

Conditioning-specific reflex modification—Figure 3 shows averaged topographies and Figure 4 shows average UR frequency, magnitude and magnitude of the UR area for the four 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 figures shows that the level CRM was higher in the 2 and 1% groups than the 0.5 and 0% cholesterol groups. In fact, it appears that CRM was a function of the terminal level of responding following trace conditioning. Specifically, there was an increase in UR topographies from pretest to post test in the 2 and 1% groups but not in the 0.5 and 0% groups. If anything, there was actually a decrease in UR topographies from pretest to post test in the 0.5 and 0% groups. These effects are most clearly illustrated with UR frequency where analysis of variance yielded a significant interaction of test (pretest vs. post test) and cholesterol concentration [$F(1,19) = 7.07$, $p < 0.01$] as well as a main effect of US intensity [$F(4,76) = 22.95$, $p < 0.001$]. A *post hoc* comparison confirmed that the changes in responding from pretest to post test for the 2 and 1% cholesterol groups were different from those for the 0.5 and 0% groups ($p < 0.001$). A similar *post hoc* comparison of UR magnitude was also significant ($p < 0.05$). An analysis of magnitude of the UR area yielded a significant pretest–post test difference [$F(1,19) = 4.87$, $p < 0.05$], a main effect in US intensity [$F(4,76) = 24.05$, $p < 0.001$] and an interaction of test and US intensity [$F(4,76) = 2.86$, $p < 0.05$]. Finally, there were no significant effects

of cholesterol concentration on any pretest measures suggesting that sensory and motor aspects of the rabbit responding to air puff were not differentially affected by manipulation of the cholesterol concentration.

Tone intensity—Figure 5 shows mean percent CRs to eight intensities of the tone CS for rabbits in the 0, 0.5, 1 and 2% cholesterol groups collapsed across two additional days of trace conditioning. The figure shows that the level of responding was a function of tone intensity and that all groups responded at about the same levels. Despite some suggestion in the figure that the 0.5% cholesterol group may have responded less at the higher tone intensities, analysis only yielded a significant main effect of tone intensity [$F(7,133) = 106.32, p < 0.001$]. There were no significant effects of cholesterol concentration even when each of the tone intensities was analyzed separately (all F 's < 1).

Histology—Figure 6 shows mean number and Figure 7 shows low-power photomicrograph examples of beta amyloid immunoreactive (labeled) cells in the cortex and hippocampus. Examination of Figure 6 shows the number of beta amyloid-labeled cells in the cortex and, to a lesser extent in the hippocampus, was a function of the cholesterol concentration. Figure 7 shows there was no evidence of extracellular plaque-like structures in the cortex or hippocampus at any cholesterol concentration. Analysis of the number of beta amyloid labeled cells in the cortex revealed a significant effect of cholesterol concentration [$F(3,20) = 77.14, p < 0.001$] and *post hoc* comparisons of the number of labeled cells in the cortex revealed that they were a function of the cholesterol concentration. The smallest difference between groups in the number of labeled cells was between the 0 and 0.5% cholesterol groups (3.35 ± 0.25 vs. $5.03 \pm 0.44, p < 0.05$). Analysis of the number of beta amyloid labeled cells in the hippocampus also revealed a significant effect of cholesterol concentration [$F(3,20) = 16.62, p < 0.001$] but in this case *post hoc* comparisons revealed that only the 2% cholesterol group had significantly higher numbers of beta amyloid-labeled cells than the remaining groups ($p < 0.001$). This significantly higher number of beta amyloid-labeled cells in the hippocampus is consistent with the higher overall level of trace conditioning in the 2% cholesterol group and with the essential role of the hippocampus in trace conditioning.

Copper levels—An analysis of copper levels in serum and brain as a result of 0.12 ppm copper in the drinking water revealed no significant differences between the groups in either serum or brain copper levels (F 's < 1.8).

Discussion

The principal findings of the current experiment were that the level classical conditioning and CRM and the numbers of beta amyloid-labeled cells were all a function of cholesterol concentration. Furthermore, the conditioning effects appear to be associative in nature because separate tests of responding to the tone and air puff showed no nonassociative effects of cholesterol concentration. 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 concentration. In addition to the present results, a recent report shows that feeding cholesterol to young, normal rats also improves learning—in this case spatial, learning and memory in the Morris water maze (Dufour et al. 2006). Taken together, the results from a number of different species including transgenic mice, normal and cholesterol-deficient rats and normal rabbits subjected to a number of different behavioral paradigms including spatial learning and classical conditioning suggest that dietary cholesterol may improve learning and memory.

Given that dietary cholesterol does not cross the blood brain barrier directly in any appreciable amount (Dietschy and Turley 2001, 2004; Tuma and Hubbard 2003; Bjorkhem and Meaney 2004), it remains to determine how trace conditioning, which is controlled in part by the hippocampus, was facilitated by cholesterol-rich diet. The same is true for the effects of cholesterol on rat water maze learning (Dufour et al. 2006) and previous research showing that addition of cholesterol to the diet of animals deficient in cholesterol or that have cholesterol synthesis blocked reverses learning and memory deficits (Endo et al. 1996; Xu et al. 1998; O'Brien et al 2002; Voikar et al 2002).

One possible explanation for the facilitative effects of dietary cholesterol on the brain is that cholesterol metabolites that do 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-hydroxycholesterol in learning and memory because levels of this oxysterol are significantly reduced in patients with mild cognitive impairment, Alzheimer's disease vascular dementia (Kolsch et al. 2004).

Another possible explanation is the effect of dietary cholesterol on the number of beta amyloid-labeled cells in the brain. The significant increase in the number of beta amyloid immunoreactive neurons as a function of cholesterol concentration particularly in the hippocampus in the 2% cholesterol group, suggests a role for intracellular beta amyloid in learning and memory. Indeed, there is good evidence for the normal function of beta amyloid in cellular processes (Plant et al. 2003; Pearson and Peers 2006) and in fact, beta amyloid modulates potassium channels (Plant et al. 2006)—an important mediator of many forms of learning and memory (Giese et al. 1998; Schreurs et al. 1998; Nelson et al. 1999; Schrader et al. 2002). It is certainly possible that by increasing the number of beta amyloid-labeled neurons, the cholesterol diet facilitated NMR conditioning by modulating cellular potassium channels and affecting neuronal excitability—a mechanism known to be involved in learning and memory (Zhang and Linden 2003; Johnston et al. 2003; Xu and Kang 2005). The significantly higher levels of trace conditioning and higher numbers of beta amyloid-labeled neurons in the hippocampus (a structure essential for trace conditioning) for the 2% cholesterol group are consistent with such a hypothesis.

The highly conserved low density lipoprotein receptors (LDLR) are yet another potential mechanism by which cholesterol can affect 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). Perhaps the most well known of the LDLR ligands is apolipoprotein E—a protein known to be implicated in Alzheimer's disease. Recent research suggests that this class of receptors 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 possibility is that pro-inflammatory 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 most notably Maier and colleagues who have found that interleukin 1β can 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). More important for the present results, is the finding that systemic injections of interleukin 1β facilitate classical eyeblink conditioning in male rats (Servatius and Beck 2003). Consequently, it is possible that a high cholesterol diet may facilitate learning via pro-inflammatory cytokines.

Taken together, the results of the present experiment as well as other animal experiments suggest that cholesterol may facilitate learning and memory. However, there is also a considerable body of evidence from animal experiments on the negative effects of cholesterol on learning and memory. For example, decreasing cholesterol in aged animals improves learning in the Morris water maze (Kessler et al. 1986; Yehuda and Carasso 1993; Yehuda et al. 1998). Similarly, rats, mice and rabbits given calcium channel blockers that, among other things, decrease the level of cholesterol by reducing the esterification of cholesterol and increasing hydrolysis of existing cholesterol esters (Schachter 1997; Nayler 1999), demonstrate improvements in learning and memory in a number of tasks including the Morris water maze and classical conditioning of the rabbit NMR (Deyo et al. 1989; Woodruff-Pak et al. 1997; Kane and Robinson 1999; Quartermain 2000).

In addition to differences among animal studies on the effects of cholesterol on learning and memory, human studies also appear to be mixed. On one hand, recent studies show that language (Reitz et al. 2005) and cognitive performance (Elias et al. 2005) are a function of *increasing* plasma lipid levels in the healthy elderly. On the other hand, a larger number of studies show that elevated serum cholesterol is a significant risk factor for mild cognitive impairment (Näslund et al. 2000; Kivipelto et al. 2001; Yaffe et al. 2002) and Alzheimer's disease (Sparks et al. 1990; Evans et al. 2000; Hartmann 2001; Simons et al. 2001; Schonknecht et al. 2002; Yanagisawa 2003; Poirier 2005; Papassotiropoulos et al. 2005; Whitmer et al. 2005; Wood et al. 2005) and that cholesterol levels may be negatively correlated with measures of intelligence (Reitan and Shipley 1963; Muldoon et al. 1997; Atzmon et al. 2002; van Exel et al. 2002; Yaffe et al. 2002). Moreover, epidemiological evidence is mixed on the relationship between cholesterol levels and the cognitive deficits characteristic of Alzheimer's disease (Jarvik et al. 1995; Notkola et al. 1998; Evans et al. 2000; Hartmann 2001; Simons et al. 2001; Stewart et al. 2001; Wood et al. 2005). Finally, there is controversy about whether lowering cholesterol with statins may ameliorate some of the cognitive symptoms of Alzheimer's disease (Shobab et al. 2005). For example, some retrospective epidemiological studies have suggested that statin treatment has an effect on Alzheimer's disease (Wolozin et al. 2000; Rockwood et al. 2002) as have some prospective studies (Simons et al. 2002; Sparks et al. 2006a) whereas others have not (Rockwood 2006). In a recent animal study, lowering cholesterol with a statin produced improvements in learning and memory independently of beta-amyloid levels in the brain (Li et al. 2006). Specifically, Li et al. (2006) found that simvastatin reversed spatial maze learning deficits not only in a transgenic mouse model of Alzheimer's diseases that showed high levels of beta amyloid but also improved spatial maze learning in their non-transgenic littermates that did not have high levels of beta amyloid.

In conclusion, it is almost certainly true 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 severe pathophysiology caused by a high-cholesterol diet including atherosclerosis, coronary heart disease and liver dysfunction (Sparks 1997a), many cholesterol fed animals learn better than normal controls. 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, increased neuronal beta amyloid, the LDL receptor, or pro-inflammatory

cytokines. There are a number of other, examples where high cholesterol damages the body but still has positive effects on the brain. For example, dietary cholesterol has been shown to provide protection from seizures (Alexander and Kopeloff 1971), hypoxia (Xi et al. 2006) and Stroke (Vauthey et al. 2000; Zuliani et al. 2004). In fact, a recent study 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, at some point, a high cholesterol diet begins to have deleterious effects on the brain—beta amyloid plaque-like structures form and learning and memory are debilitated (Sparks and Schreurs 2003). Clearly, we need to eliminate the negative effects of high cholesterol while determining the nature of its positive effects on learning and memory.

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Figure 1. Mean ($\pm$ SEM) body weight for rabbits in the 0, 0.5, 1 and 2% cholesterol groups across the eight weeks of the cholesterol diet.

Figure 2. Mean percent conditioned NM responding (CRs) to the CS for rabbits in the 0, 0.5, 1 and 2% cholesterol groups across the eight days of trace conditioning and the four days of delay conditioning.

Figure 3.

Average topographies for rabbits in the 0, 0.5, 1 and 2% cholesterol 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, 0.5, 1 and 2% cholesterol groups.

Figure 5. Mean percent conditioned NM responding (CRs) to eight intensities of the tone CS (0, 60, 65, 70, 75, 80, 85 and 90 dB) for rabbits in the 0, 0.5, 1 and 2% cholesterol groups collapsed across two days of tone intensity presentations following delay conditioning.

Figure 6.

Mean number beta amyloid immunoreactive (labeled) cells in the cortex and hippocampus of rabbits in the 0, 0.5, 1 and 2% cholesterol groups. Fifty-micron vibratome sections of the hippocampus and surrounding cortex were immunostained with an antibody to beta amyloid (10D5, 1:100 dilution; provided by Dr 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.

Figure 7. Low power photomicrographs of representative beta amyloid immunoreactive (labeled) cells in the cortex (left) and hippocampus (right) of a rabbit from the 0, 0.5, 1 and 2% cholesterol groups. Scale bar 100 μ m.