Published in final edited form as:

Behav Neurosci. 2010 February ; 124(1): 115–123. doi:10.1037/a0018253.

Dietary Cholesterol Impairs Memory and Memory Increases Brain Cholesterol and Sulfatide Levels

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Abstract

Cholesterol and sulfatides play many important roles in learning and memory. To date, our observations about the effects of cholesterol on learning have been assessed during response acquisition i.e., the learning of a new memory. Here we report for the first time on the effect of a cholesterol diet on a previously formed memory. Rabbits were given trace conditioning of the nictitating membrane response for ten days, then fed a 2% cholesterol diet for eight weeks, and then assessed for memory recall of the initially learned task. We show that dietary cholesterol had an adverse effect on memory recall. Second, we investigated whether dietary cholesterol caused an increase in brain cholesterol and sulfatide levels in four major brain structures (hippocampus, frontal lobe, brainstem, and cerebellum) using a technique for analyzing myelin and myelin-free fractions separately. Although our data confirm previous findings that dietary cholesterol does not directly affect cholesterol and establish that it does not affect sulfatide levels in the brain, these levels did increase rather significantly in the hippocampus and frontal lobe as a function of learning and memory.

Keywords

Learning; memory; cholesterol; sulfatide; diet

Introduction

The effect of dietary cholesterol on learning and memory remains unclear. On the one hand, a significant body of research has demonstrated a positive effect of dietary cholesterol on learning in several animal species including rats, mice, and rabbits (Dufour et al., 2006; Miller & Wehner, 1994; Schreurs et al., 2003; Voikar et al., 2002; Upchurch & Wehner,

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1988). Feeding cholesterol to rats that are either deficient in cholesterol or have cholesterol synthesis suppressed ameliorates learning and memory impairments (Endo et al., 1996; O'Brien et al., 2000; Voikar et al., 2002; Xu et al., 1998). Dufour and colleagues (2006) have shown feeding rats 2% cholesterol enhances water maze learning. Our recent studies have also consistently shown an increase in the level of rabbit classical conditioning following an 8-week 2% cholesterol diet (Schreurs et al., 2007b; Schreurs et al., 2003). On the other hand, there is evidence that dietary cholesterol can have a negative effect on learning and memory (Yehuda & Carasso, 1993; Yehuda et al., 1998; Kessler et al., 1986). In a recent study, feeding mice a 2% cholesterol diet for 8 weeks resulted in loss of working memory (Thirumangalakudi et al., 2008). Moreover, reducing cholesterol levels with statins results in improved memory suggesting cholesterol has a detrimental effect on learning and memory (Li et al., 2006).

It is unknown if a cholesterol diet has any effect on previously formed memories. To date, our observations about the effects of cholesterol on learning have been assessed during response acquisition i.e., the learning of a new memory (Schreurs et al., 2007b; Schreurs et al., 2003). Given that dietary cholesterol plays a significant role in learning new memories, we investigated whether a cholesterol diet might also have an effect on previously formed memories (memory recall) using our 2% cholesterol-fed rabbit model (Schreurs et al., 2007b; Schreurs et al., 2007a; Schreurs et al., 2003; Sparks & Schreurs, 2003).

To better understand the relationship between dietary cholesterol and learning and memory, it is important to know if dietary cholesterol has any direct effects on brain cholesterol. The relationship between high dietary cholesterol and brain cholesterol still remains unclear and the relationship between high dietary cholesterol and brain sulfatides has not been studied. Both cholesterol and sulfatides serve important roles in the CNS. Cholesterol plays an important role in synapse formation, receptor function, myelin formation and synaptic plasticity (Dufour et al., 2006; Goritz et al., 2002; Koudinov & Koudinova, 2001; Mauch et al., 2001; Sooksawate & Simmonds, 2001; Frank et al., 2008; Guo et al., 2008; Saher et al., 2005; Dietschy & Turley, 2004). Cholesterol levels in the CNS are tightly controlled and disturbances in cholesterol are correlated with disorders in humans such as Niemann-Pick disease Type C, Smith-Lemli-Opitz syndrome and Alzheimer's disease (Mori et al., 2001; Papassotiropoulos et al., 2002; Voikar et al., 2002; Xie et al., 2000; Whitney et al., 2002; Ikonen, 2006). Sulfatides are a class of sulfated galactocerebrosides that have an important role in myelin sheath and axon structure maintenance (Marcus et al., 2006; Ramakrishnan et al., 2007), modulation of potassium and sodium ion channels (Chi & Qi, 2006; Ishibashi et al., 2002), and learning and memory (D'Hooze et al., 2001). Furthermore, sulfatides have been shown to be altered in a number of neurological disorders including Alzheimer's disease (Han, 2007; Zeng & Han, 2008; Han et al., 2003; Han et al., 2002; Cheng et al., 2008).

Some studies have shown feeding animals high levels of cholesterol results in an increase in brain cholesterol levels (Sparks, 1997; Dufour et al., 2006). There is only one study, however, that reported a significant increase in the level of cholesterol in the brain of cholesterol-fed rabbits (Sparks, 1997). Another study found an increase in the cholesterol content of hippocampal neurons using immunofluorescent analysis but chemical analysis of total brain and hippocampus cholesterol levels showed no significant differences from control rabbits (Ghribi et al., 2006b). Finally, a study using cholesterol-fed rats detected differences in hippocampal cholesterol levels after a two-month feeding period but not after a five-month feeding period (Dufour et al., 2006). It is possible that some studies may have been insensitive to subtle changes in brain cholesterol levels of brain structures such as the frontal lobe and the hippocampus that contain smaller amounts of total cholesterol. To date,

there is no evidence that a high cholesterol diet has any effect on sulfatide levels in the brain.

The largest amounts of cholesterol and sulfatides in the brain are found in myelin, and recently it has been shown that cholesterol-fed rabbits suffer damage to the myelin sheath (Berkman et al., 2009). Therefore, in this experiment we used a technique for analyzing myelin and myelin-free fractions separately in order to reveal potentially subtle changes in cholesterol and sulfatide levels resulting from the diet. It is also possible that these levels are the same in a high-cholesterol and cholesterol-free diet but that the distribution of cholesterol and sulfatides among brain structures may be different. Given learning and memory are dependent on a number of brain structures (e.g., cerebellum, frontal lobe, hippocampus, and brainstem), we investigated the levels and distribution of cholesterol and sulfatides in each of these structures.

Methods

Subjects

Subjects were 24 two-month old male New Zealand white rabbits (*Oryctolagus cuniculus*) weighing approximately 2.0-2.5 kg at the beginning of the experiment. Animals were housed in individual cages, given free access to food and distilled water, and maintained on a 12-hour light/dark cycle. Rabbits were maintained in accordance with the National Institutes of Health guidelines. All procedures were approved by the West Virginia University ACUC.

All rabbits were initially given a standard Purina rabbit chow diet (contains 0% cholesterol) for the first ten days of training after which half were given a 2% cholesterol Purina rabbit chow diet (Dyets Inc.) and half were left on the standard Purina rabbit chow. All rabbits were maintained on their respective diet throughout the remainder of the experiment.

Apparatus

The apparatus and recording procedures for the rabbit NMR (nictitating membrane response) have been detailed by Schreurs and Alkon (1990) who modeled their apparatus after those described by Gormezano (Coleman & Gormezano, 1971; Gormezano, 1966). Each subject was restrained in a Plexiglas box and trained in a sound-attenuating, ventilated chamber (Coulbourn Instruments, Model E10-20). A stimulus panel containing a speaker and houselights (10-W, 120-V incandescent lamps) was mounted at a 45 degree angle, 15 cm anterior to and 15 cm above the subject's head. An ambient noise level of 65 dB in each chamber was provided by an exhaust fan.

Corneal air puff controlled by a programmable air pressure delivery system (Tescom Corp., Model ER-3000) was delivered through a tube (1 mm internal diameter) positioned 5 mm from and perpendicular to the center of the cornea. Air pressure intensity was measured at the orifice of the delivery tube in a closed system by a Smart Manometer (Meriam Instrument, 350 Series). Individual NM A/D outputs were stored on a trial-by-trial basis for subsequent analysis. Details of transducing nictitating membrane movements have been reported previously (Gormezano & Gibbs, 1988; Schreurs & Alkon, 1990). A 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 nictitating membrane (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). Data collection, analysis and stimulus delivery have been described (Buck et al., 2001) and were accomplished using a LabVIEW system (LabVIEW 5.1, National Instruments).

NMR classical conditioning

Rabbits received a day of adaptation, ten daily sessions of paired trace conditioned stimulus (CS)- unconditioned stimulus (US) presentations (n=12) or unpaired CS and US presentations (n=12), and then after a two-month period of dietary supplementation, ten days of CS-alone extinction followed by brain tissue analysis. On adaptation day, the rabbits were prepared for AP and recording of NM movement and then adapted to chambers for the same time as subsequent training sessions (60 min). Each of the paired trace conditioning sessions consists of 60 presentations of a 250-ms, 1-KHz, 82-dB, tone CS followed by a 250ms trace interval and then a 100-ms, 4-psi air puff US. Sessions for unpaired subjects consisted of 60 CS-alone and 60 US-alone presentations that occurred in an explicitly unpaired manner delivered, on average, every 30 s. Rabbits were then reassigned so that terminal levels of responding within paired and unpaired groups were comparable before beginning the two month feeding period. One group of rabbits from each training condition received a 2.0% cholesterol diet and the other received a 0.0% cholesterol diet [control]. For all rabbits, CS extinction sessions consisted of 60, 250-ms, 1-KHz, 82-dB, tone alone presentations with a trial delivered, on average, every 60 s. A conditioned response (CR) was defined as any extension of the NM exceeding 0.5 mm initiated after CS onset but before US onset for paired trials and the point at which the US would have occurred for CS-alone extinction trials. An unconditioned response (UR) was any extension of the NM exceeding 0.5 mm initiated after US onset.

Tissue collection

After the final day of CS-alone extinction, the animals were anesthetized with a solution containing Ketamine HCL (83.33 mg/ml) and Xylazine (16.67 mg/ml), followed by a lethal dose of Euthasol (sodium pentobarbital, 390 mg/ml). Following transcardial perfusion with heparinized saline, the brains were removed and the cerebellum, brainstem, hippocampi and a portion of rostral cortex were dissected. The tissue was weighed, immersed in 0.32M sucrose and frozen at -20 C.

Subcellular fractionation

Myelin was purified from each brain region by the flotation technique (Konat, 1981). Briefly, the tissue was homogenized in 0.88M sucrose, overlaid with 0.32M sucrose and centrifuged at 30,000 rpm for 1 hour at 4°C in a SW 40 Ti Beckman rotor. The myelin fraction located at the gradient interface and the non myelin fraction (pellet) were collected and washed free of sucrose. Protein content of subfractions was quantified using a bicinchoninic acid (BCA) kit from Pierce (Rockford, IL).

Lipid analysis

Total lipids were extracted from the subfractions with chloroform-methanol (Konat and Clausen, 1974). Cholesterol was quantified by the acetic anhydride (Eng & Noble, 1968). Briefly, lipid samples were solubilized in chloroform and after addition of acetic anhydride-sulfuric acid optical density was read at 620 nm. Sulfatides were determined by the Azure A method (Kean, 1968). Briefly, lipid samples were solubilized in chloroform-methanol mixed with acidified Azure A solution and optical density of the aqueous phase was read at 645 nm. The lipid content in the fractions was normalized to the amount of protein and expressed as nmols of lipid per mg of protein.

Statistical analysis

Preliminary analysis indicated non-homogeneity of variance for both sulfatide and cholesterol levels. As a result, analyses were conducted on natural log transformed data. Results in this paper are stated in the original units of measurement. Analysis of variance

(ANOVA) was performed and all post hoc analysis was done using statistical tests of contrasts with a Bonferroni correction between group means.

Results

Behavior

Cholesterol retards recall of previously acquired classical conditioning of the rabbit NMR. Figure 1 shows acquisition (Panel A) and the effects of feeding a 2%-cholesterol diet for eight weeks on CS-alone extinction (Panel B) of the rabbit NMR. An ANOVA on the acquisition data yielded a significant main effect of pairings, $F(1, 22) = 13.10$, $p < .005$, a significant interaction of days, $F(9, 198) = 31.12$, $p < .001$, and an interaction of pairings and days, $F(9, 198) = 19.08$, $p < .001$, confirming the clear differences in the levels of responding between the paired and unpaired rabbits. Panel B shows normal chow rabbits continued to respond at approximately 70% CRs over the first three days of extinction before decreasing over the remaining seven days. In contrast, cholesterol-fed rabbits responded at only 30% CRs on the first day of extinction, increased slightly to match normal chow levels of responding by the fourth day of extinction before declining with continued CS presentations. Responding to the tone in the unpaired groups was low and variable but there were no differences between groups. An ANOVA of the extinction data yielded a significant main effect of pairings, $F(1, 20) = 21.30$, $p < .001$, a significant interaction of pairings with days, $F(9, 180) = 2.84$, $p < .01$, and an interaction of pairings with days and cholesterol, $F(9, 180) = 2.67$, $p < .01$, confirming the clear differences in the levels of responding between the cholesterol-fed paired rabbits and controls. Post hoc comparisons confirmed a significant difference between paired normal and paired cholesterol groups on the first and second days of extinction (p 's $< .05$).

The behavioral data suggest cholesterol has an effect on a previously acquired memory. The nature of this effect is open to interpretation because deficits in responding appear to be overcome, at least temporarily, with continued CS-alone presentations. It is possible that cholesterol induced a state-dependent effect as a result of weight loss, liver dysfunction or heart disease (Finking & Hanke, 1997; Sparks, 1997). However, the recovery of responding as a function of repeated CS-alone presentations is similar to normal rabbits that show recovery of CRs as a function of repeated CS-alone presentations after much longer retention intervals – as long as six months after trace conditioning (Schreurs, 1998) and even nine months after delay conditioning (Schreurs, 1993). One might speculate that dietary cholesterol slowed retrieval of the memory so that after a two-month period the cholesterol fed rabbits had the retrieval issues of rabbits on a normal diet after six months. Importantly, we have shown that cholesterol-fed rabbits given CS-alone extinction immediately after tone-shock trace conditioning extinguish their CRs at the same rate and to the same level as normal diet controls (Schreurs et al., 2003).

Biochemical analysis

Although a comparison of brain sulfatide and cholesterol levels between the two diets did not reveal any significant differences (Figure 2), an ANOVA did reveal a significant difference in myelin, $F(1, 20) = 6.46$, $p < 0.05$, and highly significant difference in non myelin brain cholesterol levels as a main effect of pairings, $F(1, 20) = 21.55$, $p < 0.001$, and as an interaction of structure and pairings, $F(3, 60) = 6.86$, $p < 0.001$ and $F(3, 60) = 3.82$, $p < 0.05$, respectively. Figure 3 shows cholesterol levels for paired and unpaired groups in the frontal lobe, hippocampus, brainstem, and cerebellum in myelin (panel A) and non myelin (panel B) fractions. Post hoc analysis of the interactions of structure and pairings confirmed brain cholesterol levels between the paired (853.9 ± 74.0 nmol/mg) and unpaired groups (575.9 ± 28.3 nmol/mg) in the hippocampus myelin fraction were significantly different, $p <$

0.05. The analysis also confirmed a significant difference between the paired (154.6 ± 11.4 nmol/mg) and unpaired groups (98.9 ± 6.2 nmol/mg) in the frontal lobe non myelin fraction, $p < 0.05$.

A similar pattern of differences was found in the sulfatide levels as a function of pairing as shown in Figure 4. Statistical analysis revealed a significant difference in non myelin brain sulfatide levels as a main effect of pairing, $F(1, 20) = 28.64$, $p < 0.001$, and an interaction of structure and pairing, $F(3, 60) = 3.28$, $p < 0.05$. Post hoc analysis confirmed the difference between the paired (7.3 ± 0.52 nmol/mg) and unpaired group (4.8 ± 0.32 nmol/mg) in the frontal lobe was significant, $p < 0.05$. Statistical analysis failed to find a difference in myelin brain sulfatide levels as a main effect of pairing, $F(1, 20) = 2.32$, $p = 0.1434$, but there was as a significant interaction of structure and pairing, $F(3, 60) = 10.18$, $p < 0.001$. Post hoc analysis affirmed, as suggested in Figure 4, that there was a significant difference between the paired (100.9 ± 7.5 nmol/mg) and unpaired groups (68.8 ± 4.1 nmol/mg) in the hippocampus, $p < 0.05$.

Discussion

Our study revealed two major findings. First, we show that dietary cholesterol negatively affects recall of previously acquired classical conditioning of the rabbit NMR. This is the first time the effects of dietary cholesterol have been assessed on previously formed memories. Prior to this work, all our observations about the effects of cholesterol on learning had been assessed on response acquisition i.e., the learning of a new memory (Schreurs et al., 2007b; Schreurs et al., 2003). Second, we show associative learning-dependent changes in cholesterol and sulfatide levels in the hippocampus and frontal lobe. These are some of the first reported changes in cholesterol levels and, to our knowledge, the first reports of changes in sulfatide levels. It was interesting that we saw significant changes in both cholesterol and sulfatides specifically in the hippocampus and the frontal lobe since each is essential for some aspect of learning and memory, particularly the acquisition and extinction of trace conditioning (Bangasser et al., 2006; Frankland & Bontempi, 2005; Runyan et al., 2004; Cammarota et al., 2005; Izquierdo et al., 2007; Thompson & Kim, 1996).

Using our technique of separately analyzing myelin and myelin-free fractions, we did not find an effect of dietary cholesterol on brain cholesterol or sulfatide levels. There is research suggesting that dietary cholesterol affects brain cholesterol levels (Sparks, 1997; Dufour et al., 2006); however, other work has failed to support this finding (Ghribi et al., 2006b). It is unclear at present how increases in dietary cholesterol and the subsequent increase in plasma lipid levels are signaled to the CNS to bring about cholesterol-mediated changes in the brain (Dietschy & Turley, 2001). However, several hypotheses have been proposed. First, a number of researchers have demonstrated that although the blood brain barrier is usually impermeable to cholesterol, its integrity is compromised in the cholesterol-fed rabbit (Ghribi et al., 2006a; Sparks et al., 2000). Second, it has recently been demonstrated that there is a net flux of a cholesterol metabolite, 27-hydroxycholesterol, from the circulation into the brain in healthy human volunteers (Heverin et al., 2005). More importantly, it has been demonstrated that mice fed a diet rich in cholesterol increased this natural flux of 27-hydroxycholesterol into the brain (Heverin et al., 2005). Third, it has been demonstrated that dietary manipulations can alter cell membrane composition in the brain (Faulks et al., 2006). Therefore, it is possible that alterations in cholesterol homeostasis in the CNS may affect the composition of neuronal membranes by increasing or decreasing cholesterol and other lipids to maintain rigidity and function (Hayashi et al., 2002) including synaptic plasticity (Frank et al., 2008).

Interestingly, our data indicate clear evidence of associative learning-dependent changes in both cholesterol and sulfatide levels. Since brain cholesterol levels are increased in the paired animals that learn the hippocampally-dependent task, it is possible that these increases are the result of synaptic plasticity and axonal growth (Frank et al., 2008; Koudinov & Koudinova, 2001). Recently it was demonstrated that enzymes essential to cholesterol synthesis such as HMG-CoAR are present in adult neurons and are most prominently localized in cortical and hippocampal neurons (Korade et al., 2007). Cholesterol is essential for synaptic plasticity (Frank et al., 2008; Koudinov & Koudinova, 2001; Koudinov & Koudinova, 2003; Koudinov & Koudinova, 2005) and Dufour and colleagues (2006) have illustrated a correlation between an increase in hippocampal brain cholesterol levels and memory changes using rats fed a regular diet. Therefore it is possible that hippocampal neurons, as well as cortical neurons, utilize more cholesterol in response to associative learning (Inda et al., 2005) even if most neuronal cholesterol is shuttled to the neuron from glial cells (Pfrieger, 2002).

Given that cholesterol and sulfatide analysis only took place at the conclusion of CS extinction, it is not possible to parse out the relative contribution of acquisition and extinction to the cholesterol and sulfatide differences. Nevertheless, it is interesting to speculate about the relative contribution of these processes to the myelinated and unmyelinated fraction results. Increases in brain cholesterol and sulfatide levels in the hippocampal myelin fractions could be the result of trace conditioning, whereas the increases in the frontal lobe non myelin fraction could be the results of extinction. As discussed in the Introduction, both cholesterol and sulfatides have important roles in learning and memory. Brain maturation is not achieved until 13 to 26 weeks after birth in most mammals and not for many years in humans, during which time there is an excess amount of cholesterol in the CNS (Dietschy, 2009). Furthermore, studies have shown that increases in the levels of white matter are correlated with acquiring new skills (Bengtsson et al., 2005; Als et al., 2004). It is therefore possible that training young rabbits allows for incorporation of cholesterol and sulfatides into cellular membranes and myelin during learning.

The role of the hippocampus in learning a difficult behavioral task (such as trace conditioning) has been well documented (Bangasser et al., 2006; Frankland & Bontempi, 2005; Runyan et al., 2004). Using rabbits, one study illustrated how large bilateral lesions of the hippocampus made before training seriously impaired learning of a trace conditioning task. When the rabbits were trained first, lesions made immediately after training abolished the ability to remember the difficult task; however, lesions made one month after training had no effect on memory retention of the previously learned difficult task (Kim et al., 1995). The role of the frontal lobe, specifically the prefrontal lobe, in learning and memory has been well documented (Bangasser et al., 2006; Frankland & Bontempi, 2005; Runyan et al., 2004; Cammarota et al., 2005; Izquierdo et al., 2007; Thompson & Kim, 1996). The forebrain also plays a role in trace conditioning and extinction (Gruart et al., 2000; Weible et al., 2000). Given that myelin formation and change takes considerable time and trace conditioning occurred more than two months before tissue analysis, one could argue that increases in hippocampal brain cholesterol and sulfatide levels would be seen in the myelin fraction. On the other hand, since extinction occurred immediately before tissue analysis, increases in cholesterol and sulfatide levels may only have been seen in the non myelin fraction.

Taken together, our results show that dietary cholesterol appears to have a positive effect on learning but a negative effect on memory recall. How is this possible? As already noted, there are a large number of studies that have documented significant CNS as well as systemic consequences of a high-cholesterol diet. The CNS effects include, but are not

limited to, compromise of the blood brain barrier (Ghribi et al., 2006a; Sparks et al., 2000), increases in cholesterol metabolites such as 27-hydroxycholesterol (Heverin et al., 2005), an elevation in inflammatory markers (Yehuda et al., 2005; Rahman et al., 2005), the accumulation of beta amyloid (Sparks & Schreurs, 2003; Schreurs et al., 2007a), damage to the myelin sheath and axon (Berkman et al., 2009), and changes in membrane cholesterol (Ghribi et al., 2006b). Some of these processes take place within days of starting the diet whereas others may take weeks. It is therefore possible that when animals are fed cholesterol immediately after training, acquisition-specific changes in the hippocampus or long-term storage changes in the prefrontal cortex may be affected by the rapidly increasing CNS consequences of the diet. So when assessed for memory recall, animals perform poorly. On the other hand, when animals are placed on a cholesterol diet for two months before training, learning-specific changes are more likely to occur against a backdrop of steady-state cholesterol-induced CNS changes. We have shown that the training-specific changes remain intact and even benefit from the consequences of the diet when cholesterol-fed rabbits show better acquisition than controls (Schreurs et al., 2007a). These effects could be the result of the increased membrane cholesterol that is important for synaptic plasticity. If the cholesterol diet induces beta amyloid plaques, we have found that rabbits show significantly worse acquisition than controls (Sparks & Schreurs, 2003).

Acknowledgments

We thank Dr. Lauren B. Burhans, Jimena Gonzales-Joekes, and Carrie L. Smith-Bell for technical assistance and Dr. Gerry Hobbs for assistance in analyzing the data.

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Figure 1. Cholesterol retards memory for a previously acquired classically conditioned NMR Acquisition phase (A) of the twelve rabbits that received 60 daily pairings of a 250-ms, 82-dB, tone CS and 100-ms, 4-psi air puff US separated by a 250-ms trace (500-ms ISI, PD). Another twelve rabbits received 120 daily explicitly unpaired presentations of the tone and air puff (UP). Half the rabbits in each group were then placed on a 2% cholesterol diet (Cholesterol) and the other half remained on their normal diet (0% cholesterol, Normal). Extinction phase (B) of the twenty-four rabbits that received pairings ($n=12$) or unpaired presentations ($n=12$). Following an 8-week 2% cholesterol diet, rabbits previously conditioned to a tone CS show significantly lower levels of responding to the tone CS during tone-alone extinction. * $p < .05$ post hoc comparison of percent CRs between paired groups on day 1 and day 2 of extinction.

Figure 2. Dietary cholesterol has no effect on brain cholesterol and sulfatide levels

Cholesterol and sulfatides were extracted separately from myelin and non myelin fractions of the frontal lobe, hippocampus, brainstem, and cerebellum. These lipids were quantified as described in the Methods section and expressed as nmoles per mg of protein. Analysis of cholesterol levels in myelin and non myelin fractions (A) as a function of diet revealed no significant differences in any of the fractions ($n's = 12$). Similarly, no differences in sulfatide levels (B) as a function of diet were found in any of the myelin and non myelin fractions among the different structures ($n's = 12$).

Figure 3. Learning has a significant effect on brain cholesterol levels in (A) myelin fractions and (B) non myelin fractions

Cholesterol was extracted separately from myelin and non myelin fractions of the frontal lobe, hippocampus, brainstem, and cerebellum and quantified as described in the Methods section and expressed as nmoles per mg of protein. Analysis of cholesterol levels in the myelin fractions (A) as a function of pairing revealed a significant difference in the hippocampus between paired and unpaired groups ($n's = 12$). Analysis of cholesterol levels in the non myelin fractions (B) as a function of pairing revealed a significant difference in the frontal lobe ($n's = 12$). * $p < .05$.

Figure 4. Sulfatide levels change significantly as a function of pairing in (A) myelin fractions and (B) non myelin fractions

Sulfatides were extracted separately from myelin and non myelin fractions of the frontal lobe, hippocampus, brainstem, and cerebellum and quantified as described in the Methods section and expressed as nmoles per mg of protein. Analysis of sulfatide levels in the myelin fractions (A) as a function of pairing revealed a significant difference in the hippocampus between paired and unpaired groups ($n's = 12$). Analysis of sulfatide levels in the non myelin fractions (B) as a function of pairing revealed a significant difference in the frontal lobe ($n's = 12$). * $p < .05$.