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Dietary cholesterol degrades rabbit long term memory for discrimination learning but facilitates acquisition of discrimination reversal

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Abstract

We have shown previously that feeding dietary cholesterol before learning can improve acquisition whereas feeding cholesterol after learning can degrade long term memory. To examine these different findings within a single paradigm, we fed groups of rabbits 2% cholesterol or normal chow with or without 0.12 ppm copper added to the drinking water following two-tone discrimination learning of the nictitating membrane response in which a 8-kHz tone (conditioned stimulus, CS+) was followed by air puff and a 1-kHz tone (CS-) was not. After eight weeks on the diet, we assessed the rabbits' conditioned responding during testing and retraining. We then reversed the two-tone discrimination and assessed responding to the 1-kHz tone CS+ and the 8-kHz CS-. During testing, rabbits given cholesterol without copper had lower levels of responding to CS+ than rabbits in the other groups suggesting they did not retain the discrimination as well. However, during a brief discrimination retraining session, their response levels to the CS+ returned to the level of the other groups, demonstrating a return of the memory of the original discrimination. At the end of discrimination reversal, these same rabbits exhibited superior discrimination indexed by lower response levels to CS- but similar levels to CS+, suggesting they were better able to acquire the new relationship between the two tones by inhibiting CS- responses. These results add to our previous data by showing cholesterol diet-induced degradation of an old memory and facilitation of a new memory can both be demonstrated within a discrimination reversal paradigm. Given discrimination reversal is a hippocampally-dependent form of learning, the data support the role of cholesterol in modifying hippocampal function as we have shown previously with *in vitro* brain slice recordings.

Keywords

cholesterol; classical conditioning; discrimination; discrimination reversal; nictitating membrane response; memory; long term memory; rabbit

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1. Introduction

In rabbits fed 2% cholesterol for as little as eight weeks, there are as many as sixteen different indices of pathology that are similar to those seen in Alzheimer's Disease (AD). These indices include intracellular and extracellular beta amyloid accumulation, breaches of the blood brain barrier, activation of microglia, apoptosis, increased levels of Apolipoprotein E, phosphorylated tau protein, changes in the cerebrovasculature, and increases in ventricular volume (Deci, Lemieux, Smith-Bell, Sparks, & Schreurs, 2012; Ghribi, Golovko, Larsen, Schrag, & Murphy, 2006; Lemieux et al., 2010; Prasanthi et al., 2010; Prasanthi et al., 2008; Sparks et al., 1994; Sparks, 1997; Sparks, Kuo, Roher, Martin, & Lukas, 2000; Sparks & Schreurs, 2003; Woodruff-Pak, 2008; Woodruff-Pak, Agelan, & Del Valle, 2007; Zatta, Zambenedetti, Stella, & Licastro, 2002). Several of these indices have been shown to be exacerbated by the addition of very low concentrations of copper to the drinking water (Sparks, 2004; Sparks et al., 2006; Sparks, Martin, Stankovic, Waggoner, & Van Adel, 2007; Sparks & Schreurs, 2003; Woodruff-Pak et al., 2007).

Coinciding with cholesterol-induced changes in brain pathology, there have been some reports of detrimental effects of cholesterol on learning (Granholtm et al., 2008; Hooijmans et al., 2009; Lefterov et al., 2009; Micale et al., 2008; Sparks & Schreurs, 2003). Nevertheless, research with a number of animal models suggests that modifying dietary cholesterol may actually *improve* learning. Increasing cholesterol in mutant mice in which hippocampally-dependent spatial learning is normally impaired improves performance in the Morris water maze (Miller & Wehner, 1994; Upchurch & Wehner, 1988). Feeding cholesterol to young, normal rats also improves performance in the Morris water maze (Dufour, Liu, Gusev, Alkon, & Atzori, 2006; Ya et al., 2012). Feeding cholesterol to rats that are either deficient in cholesterol or have cholesterol synthesis blocked reverses problems with learning and memory (Endo, Nishimura, & Kimura, 1996; O'Brien et al., 2002; Voikar, Rauvala, & Ikonen, 2002; Xu et al., 1998). We have also shown that feeding rabbits cholesterol can facilitate trace classical conditioning of the nictitating membrane response (NMR) and of heart rate (Schreurs et al., 2007c; Schreurs, Smith-Bell, Darwish, Stankovic, & Sparks, 2007b; Schreurs, Smith-Bell, Lochhead, & Sparks, 2003).

In addition to the data on cholesterol's ability to facilitate learning, we have shown more recently that a cholesterol diet can have detrimental effects on the long term memory of classical conditioning of the rabbit NMR that was acquired immediately before the start of the cholesterol diet (Darwish, Wang, Konat, & Schreurs, 2010). We have since gone on to show that these effects are a function of the concentration and duration of the cholesterol diet (Schreurs et al., 2012). Importantly, these findings were in rabbits fed cholesterol without the addition of copper to their distilled drinking water. Although there is significant evidence that high cholesterol is correlated with cognitive impairment in humans (Goldstein et al., 2008; Solomon et al., 2007; Solomon et al., 2009; Zambon et al., 2010), there are just two studies showing that serum cholesterol has effects on long term memory but one is complicated by diabetic co-morbidity (Helkala, Niskanen, Viikari, Partanen, & Uusipata, 1995) and the other by atrial fibrillation (Tendolkar et al., 2012). The effects of copper on cognitive impairment is mixed (Kessler et al., 2008; Morris et al., 2006; Salustri et al., 2010) and significant controversy surrounds its role in dementia (Hung, Bush, & Cherny, 2010; Squitti, 2012).

Finally, there are a significant number of reports that cholesterol modifies the electrophysiological properties of neurons in the brain particularly the hippocampus (Dufour et al., 2006; Fester et al., 2009; Koudinov & Koudinova, 2001; Parkinson et al., 2009; Tanaka & Sokabe, 2012; Wang & Schreurs, 2010; Ya et al., 2012) and, when studied in the same animals, improves spatial maze learning (Dufour et al., 2006; Ya et al., 2012).

Although most of these reports have focused on synaptic plasticity (Koudinov & Koudinova, 2001; Ya et al., 2012), Wang and Schreurs (2010) have shown that a cholesterol diet also has significant effects on the membrane properties of hippocampal neurons including reductions in the size of the afterhyperpolarization – a property that has been shown to be important for learning and memory in rodents and rabbits (Bekisz et al., 2010; Disterhoft & Oh, 2006; Kaczorowski, Sametsky, Shah, Vassar, & Disterhoft, 2009; Kuiper et al., 2012; Matthews, Weible, Shah, & Disterhoft, 2008; Moyer, Jr., Thompson, & Disterhoft, 1996; Tombaugh, Rowe, & Rose, 2005).

The purpose of the present experiment was to combine the different effects of cholesterol on acquisition and retention into a single behavioral paradigm and also to examine whether the addition of copper could modify the effects of cholesterol and copper on memory for a previously acquired task or the acquisition of a new task. In this case, we used two-tone discrimination of the rabbit NMR followed by a 2% cholesterol diet with or without 0.12 ppm copper added to the drinking water to determine whether rabbits fed cholesterol could remember the original task followed by reversal of that discrimination to determine whether rabbits could acquire a difficult new task which has been shown to be dependent on the hippocampus (Berger & Orr, 1983; Churchill et al., 2001; Knuttinen, Power, Preston, & Disterhoft, 2001; Miller & Steinmetz, 1997). One prediction might be that cholesterol decreases the ability to remember the original discrimination but facilitates the acquisition of discrimination reversal and that the addition of copper might reverse those effects because it can retard learning (Schreurs, 2013; Sparks & Schreurs, 2003).

2. Methods and materials

2.1. Animals

A total of 55 male New Zealand White rabbits (*Oryctolagus cuniculus*) 3–4 months of age and weighing approximately 2 kg upon arrival were housed individually, with free access to Purina rabbit chow and water, maintained on a 12-h light/12-h dark cycle, weighed weekly, and treated following National Institutes of Health guidelines in experiments approved by the West Virginia University Animal Care and Use Committee.

The rabbits were assigned to groups that received one of four possible treatment conditions in which food (cholesterol vs. normal chow) and water (copper vs. distilled water) were manipulated after an initial 24 daily sessions of two-tone discrimination training. Rabbits received either Purina 5326 chow (high-fiber – containing 21% fiber and 0% cholesterol) and distilled water (Control, n=10), Purina 5326 chow and copper in distilled water (Copper, n=10), Purina 5326 chow plus 2% cholesterol (Test Diet, Richmond, IN) and distilled water (Cholesterol, n=19) or Purina 5326 chow plus 2% cholesterol and copper in distilled water (Cholesterol/Copper, n=16). Rabbits given copper received copper sulfate in their distilled drinking water with a final copper concentration of 0.12 ppm (0.12 mg/liter). Over the first four days, rabbits fed cholesterol were transitioned gradually so that 25%, 50%, 75% and then 100% of their food was the 2% cholesterol diet. All rabbits were kept on their respective diets for a total of 11 weeks with the last three weeks overlapping with behavioral training. Initial sample sizes for the cholesterol-fed groups were larger than for the normal chow-fed groups to accommodate both palatability issues that arose during the diet transition and hepatotoxicity that is known to develop with rabbits maintained on a 2% cholesterol diet for extended periods of time (de Wolf et al., 2003; Kainuma et al., 2006; Song, Min, Lee, & Cho, 2000; Sparks, 2007; Sparks et al., 2007). Rabbits that did not eat or drink for more than three consecutive days due to palatability or hepatotoxicity issues or that lost more than 15% of their body weight were withdrawn from the study. Final sample sizes after the institution of the different diets and removal of rabbits due to palatability,

hepatotoxicity or weight loss were Control, $n = 10$; Copper, $n = 10$; Cholesterol, $n = 10$; and Cholesterol/Copper, $n = 11$.

2.2. Apparatus

The apparatus has been detailed by Schreurs and Alkon (1990) who modeled their apparatus after those described by Gormezano (Coleman & Gormezano, 1971; Gormezano, 1966). 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, 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 & Gibbs, 1988; Schreurs & 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).

2.3. Procedures

All rabbits received one day of handling and brief restraint, one day of adaptation, 24 daily sessions of two-tone discrimination training, eight weeks of 2% cholesterol or normal chow with or without 0.12 ppm copper added to the drinking water, one brief session of tone testing immediately followed by one brief session of discrimination reminder training, and then 18 daily sessions of discrimination reversal. The cholesterol diet was maintained throughout discrimination testing, retraining, and reversal making the total time on the diet 11 weeks.

Brief handling and restraint was used to begin to acclimate rabbits to the behavioral procedures. This was continued the next day with the adaptation session which allowed rabbits to further habituate to restraint and become familiar with the training chambers. On the adaptation day, the rabbits were restrained, prepared for recording of NM movement and then adapted to the training chambers for the length of time of subsequent training sessions (60 min). Discrimination training was used to establish responding to one tone (8 kHz, CS+) that was followed by air puff unconditioned stimulus (US) and not to another tone (1 kHz, CS-) not followed by air puff. Each of the 24 discrimination sessions consisted of 60 presentations of a 400-ms, 8-kHz, 82-dB tone CS that coterminated with a 100-ms, 4-psi air puff US (i.e., 300-ms interstimulus interval,) and 60 presentations of a 400-ms, 1-kHz, 82-dB tone that was presented alone. To equate the level of discrimination in each treatment group, rabbits were assigned to dietary treatment conditions based on their levels of responding to CS+ and CS- at the end of discrimination training. Following eight weeks on their respective diets, rabbits were exposed to a brief session of discrimination testing during which both CS+ and CS- were presented alone. The testing session consisted of 30 presentations of the 400-ms, 8-kHz, 82-dB tone (CS+) and 30 presentations of the 400-ms 1-kHz, 82-dB tone (CS-) with no presentations of air puff. We used only 30 presentations of each stimulus to test memory and immediately followed testing with a brief session of

discrimination reminder training in order to minimize any extinction effects from CS alone presentations. The reminder training session consisted of 30 presentations of the 400-ms, 8-kHz, 82-dB tone CS+ that coterminated with a 100-ms, 4-psi air puff US and 30 presentations of the 400-ms 1-kHz, 82-dB tone CS– that was presented alone. Beginning the next day, all rabbits received 18 daily sessions of discrimination reversal training during which the tone that previously served as CS+ (8 kHz) became the CS– and was no longer followed by air puff and the tone that previously served as CS– (1 kHz) became the CS+ and was always followed by air puff. Thus, each of the discrimination reversal sessions consisted of 60 presentations of the 400-ms, 1-kHz, 82-dB tone CS that coterminated with a 100-ms, 4-psi air puff US (CS+) and 60 presentations of a 400-ms 8-kHz tone CS that was presented alone (CS–). For all sessions, stimulus presentations were delivered, on average, every 30 s (25–35 s range) with the restriction that no more than three of the same stimulus type (CS+ or CS–) could occur consecutively. The tones were not counterbalanced within groups because pilot testing revealed that although having identical intensities (82 dB), the 1-kHz tone was more salient than the 8-kHz tone. As a result, both cholesterol-fed and normal chow control rabbits were unable to learn the discrimination reversal when the CS+ was initially the 1-kHz tone and CS– was the 8-kHz tone within the limited time available (Nokia & Wikgren, 2010). The time allotted for reversal training was limited as a result of the cholesterol diet causing hepatotoxicity that compromises the health of the rabbits as noted above (Kainuma et al., 2006; Song et al., 2000; Sparks et al., 2007). This occurred despite a high-fiber diet used to slow the absorption of cholesterol (Kritchevsky & Story, 1978). Therefore, we needed to use a reversal paradigm that allowed a significant level of discrimination to occur within the available time for the reversal training. The high levels of discrimination and discrimination reversal observed across all groups to the specific tones used as CS+ and CS– suggest that any sensory or performance effects of the independent variables (cholesterol, copper) were not confounded with associative effects.

2.4. Behavioral data

A nictitating membrane conditioned response was defined as any extension of the nictitating membrane exceeding 0.5 mm that was initiated after CS onset but prior to US onset on CS+ trials and at the point at which the US would have occurred on CS– trials.

2.5. Statistical analyses

Behavioral data were analyzed using a mixed model Analysis of Variance (ANOVA) with the significance level set at $p < .05$ (SYSTAT, Version 8, Chicago, IL). As a result of previous work suggesting that cholesterol and distilled water could improve acquisition of a new memory but degrade recall of an old memory (Darwish et al., 2010; Schreurs et al., 2007b) an orthogonal contrast was used to compare the Cholesterol group with the other dietary treatment groups (SYSTAT, Version 8, Chicago, IL).

3. Results

The four panels of Figure 1 show mean responding to CS+ and CS– for all 44 sessions of the experiment for rabbits fed normal chow and given distilled water (Control, top left panel), rabbits fed normal chow and given copper in distilled water (Copper, top right panel), rabbits fed 2% cholesterol and given distilled water (Cholesterol, bottom left panel), and rabbits fed cholesterol and given copper in distilled water (Cholesterol/Copper, bottom right panel). The break in the x-axis designates the eight weeks during which rabbits were fed their respective diets and remained in their home cages.

3.1. Two-tone discrimination

The left side of Figure 1 shows that all animals were eventually able to discriminate between CS+ and CS− by the end of the 24 sessions of discrimination training. The long period of overlap between levels of responding to CS+ and CS− suggests the discrimination was difficult to learn. An analysis of variance with the dummy factor of dietary treatment and factors of discrimination (CS+ vs. CS−) and sessions yielded significant effects of discrimination ($F(1, 51) = 18.11, p < .001$) and sessions ($F(23, 1173) = 87.78, p < .001$), and an interaction of discrimination and sessions ($F(23, 1173) = 19.84, p < .001$). There were no effects of the dummy variable of dietary treatments ($F's < 1$).

3.2. Discrimination testing

Eight weeks following the beginning of the four different dietary treatments, rabbits were returned to the behavioral chambers to assess the levels of responding to CS+ and CS−. The panels of Figure 1 show that all groups showed a substantial drop in responding to CS+ and CS− from levels at the end of discrimination training to testing and a reduction in the discrimination between CS+ and CS− suggesting a substantial level of forgetting (Schreurs, 1993; Schreurs, 1998). An ANOVA comparing responding to CS+ and CS− on the final day of discrimination training (before the dietary treatments) with tone testing 8 weeks after diet initiation resulted in a significant effect of the 8-week diet interval ($F(1, 44) = 146.97, p < .001$), the discrimination ($F(1, 44) = 39.00, p < .001$), and an interaction of the diet interval and discrimination ($F(1, 44) = 7.98, p < .005$) confirming that responding decreased over the eight weeks on the diet and that the extent of the discrimination between CS+ and CS− was reduced. An analysis of responding to CS+ and CS− during discrimination testing yielded a significant effect of discrimination ($F(1, 44) = 19.12, p < .001$) suggesting that, even though the overall level of responding may have dropped, the majority of rabbits had retained some level of discrimination over the two months in their home cage.

Further inspection of the panels of Figure 1 also suggests that the level of responding to CS+ and CS− during tone testing, shown more clearly as bar graphs in Figure 2, was lower in the Cholesterol group than in the other dietary treatment groups. An orthogonal contrast of responding to CS+ and CS− comparing the Cholesterol group to the other three dietary treatment groups yielded a significantly lower level of responding to CS+ ($F(1, 44) = 6.24, p < .02$) but not to CS− ($F(1, 44) = 3.27, p < .08$) suggesting that, consistent with our previous data on the effects of cholesterol and distilled water on long term memory (Darwish et al., 2010; Schreurs et al., 2012), rabbits in the Cholesterol group did not remember the discrimination as well as rabbits in the other dietary treatment groups. This impression was substantiated by the insets in Figure 2 which show responding to CS+ and CS− for each of the dietary treatment groups as a function of 10-trial blocks during testing. Examination of responding across the 10-trial blocks during testing shows there is a general increase in responding to CS+ and CS− in all groups except the Cholesterol group suggesting that the other dietary treatment groups appeared to “remember” the stimuli, particularly CS+, with repeated presentations. In contrast, the low, steady level of responding in the Cholesterol group was an effect of cholesterol on memory that did not improve or extinguish with repeated presentations. An ANOVA of responding to CS+ and CS− as a function of 10-trial blocks yielded a significant effect of discrimination ($F(1, 44) = 19.22, p < .001$) and 10-trial blocks ($F(2, 88) = 14.37, p < .001$) confirming differences in the levels of responding to CS+ and CS−, and an overall increase in responding. There was also an interaction of dietary treatment groups with 10-trial blocks ($F(6, 88) = 2.19, p = .05$), and orthogonal contrasts comparing the Cholesterol group with the other three dietary treatment groups across the three 10-trial blocks yielded significantly lower levels of responding to CS+ on the second and third blocks ($p's < .02$) and lower responding to CS− on the third block ($p < .05$).

3.3. Discrimination reminder training

The panels of Figure 1 also show that rabbits in each of the dietary treatment groups remembered the initial discrimination during a session of discrimination reminder training. An ANOVA of responding to CS+ and CS– yielded a significant effect of discrimination ($F(1, 44) = 31.21, p < .001$) but no effects of dietary treatments (F 's < 1.2). An orthogonal contrast comparing the Cholesterol group with the other three groups revealed no significant effects for responding to CS+ or CS–. An ANOVA of responding to CS+ and CS– as a function of 10-trial blocks also yielded a significant effect of discrimination ($F(1, 44) = 31.44, p < .001$) and 10-trial blocks ($F(2, 88) = 13.00, p < .001$) suggesting responding increased as a function of reminder training but there were no effects of dietary treatment (F 's < 1.1). An orthogonal contrast comparing the Cholesterol group with the other three dietary treatment groups across the three 10-trial blocks yielded no significant effects among dietary treatments.

3.4. Discrimination reversal

The right side of the panels in Figure 1 shows responding during the 18 sessions of discrimination reversal training and shows that discrimination gradually emerged after several days of overlap in high response levels to both CS+ and CS–. Rabbits in all dietary treatment groups were able to discriminate between the new CS+ and CS– but responding to CS– by rabbits in the Cholesterol group appears to be lower than responding by rabbits in the other treatment groups by the end of training. An ANOVA of responding to CS+ and CS– across the 18 sessions of discrimination reversal for the four dietary treatment groups resulted in a significant effect of discrimination ($F(1, 44) = 125.73, p < .001$), sessions ($F(17, 629) = 146.97, p < .001$), and an interaction between discrimination and sessions ($F(17, 44) = 50.96, p < .001$) confirming that the discrimination reversal was acquired. Although there were no overall effects of dietary treatment (F 's < 1.2), the same orthogonal contrast comparing the Cholesterol group to the other three dietary treatment groups used in analyzing testing and reminder training yielded a significantly lower level of responding to CS– on the final day of discrimination reversal ($F(1, 37) = 9.57, p < .03$) but not CS+ ($F(1, 37) = 1.77, p < .20$) suggesting that, consistent with our previous data on the effects of cholesterol and distilled water on acquisition of a new association (Schreurs et al., 2007c; Schreurs, Smith-Bell, Darwish, Stankovic, & Sparks, 2007a; Schreurs et al., 2007b; Schreurs et al., 2003), rabbits in the Cholesterol group acquired the new discrimination better than rabbits in the other treatment groups. In this case, rabbits in the Cholesterol group responded less to the new CS– suggesting they were better able to inhibit responding to a tone previously followed by air puff. This difference became even clearer with analyses of percent discrimination between CS+ and CS– where the Cholesterol group showed better discrimination than the other dietary treatment groups on Sessions 6 ($p < .02$), 15 ($p < .006$) and 18 ($p < .03$).

4. Discussion

The two principal findings of the present experiment were that dietary cholesterol degraded long term memory for a two-tone discrimination that was acquired before the cholesterol diet and facilitated acquisition of a subsequent reversal of that discrimination after the diet. These findings add two-tone discrimination of the rabbit NMR to our previous data showing cholesterol can degrade retention of an old NMR trace conditioning memory (Darwish et al., 2010; Schreurs et al., 2012) and facilitate acquisition of a new trace conditioning memory for the NMR and for heart rate (Schreurs et al., 2007c; Schreurs et al., 2007b; Schreurs et al., 2003). The former finding seems to be unique in the animal literature because studies have examined learning and memory immediately after the cholesterol diet rather than the long-term effects of cholesterol on a previously acquired task. The latter finding is supported by

studies showing that cholesterol can facilitate learning and recent memory in rodents – in this case spatial navigation in a water maze (Dufour et al., 2006; Li et al., 2012; Micale et al., 2008; Ya et al., 2012). Indirect support also comes from studies showing that experimental or genetic perturbations of cholesterol metabolism that impair spatial learning or eyelid conditioning in rodents can be overcome by treating the perturbation (Miller & Wehner, 1994; O'Brien, Xu, Tint, Salen, & Servatius, 2000; Xu et al., 1998).

Due to the very low levels of responding to CS+ and CS– by the Cholesterol group during discrimination testing that were indicative of degraded long term memory (Schreurs, 1993), it could be argued that the subsequent lower responding to CS– during discrimination reversal by rabbits in the Cholesterol group was a direct result of the low response levels during testing carrying over to discrimination reversal. However, closer examination of initial response levels to both CS+ and CS– during discrimination reversal show they were no lower for the Cholesterol group than response levels for the other dietary treatment groups. In fact, mean responding to CS+ and CS– during the first session of discrimination reversal by the Cholesterol group was well within the range of the other three treatment groups, and there were no significant differences between the treatment groups ($F_s < 1.0$). In other words, by the beginning of discrimination reversal, responding by rabbits in the Cholesterol group had recovered from the low levels of responding seen during testing. The recovery of responding during both reacquisition and reversal in the Cholesterol group also argues against any potential nonassociative performance deficits caused by the cholesterol diet or the choice of CS+ and CS–. An alternative interpretation of the initial low level of responding seen during testing is that it represents a failure of retrieval of the original memory in response to non-reinforced CS presentations. During reacquisition, the addition of the shock US may have provided a more powerful cue to aid in retrieval, allowing the cholesterol rabbits to overcome their initial impairment and exhibit significant savings.

There is evidence from research in rabbits that acquisition of discrimination is not dependent on the hippocampal complex (Burhans & Gabriel, 2007; Freeman, Jr., Weible, Rossi, & Gabriel, 1997; Nokia & Wikgren, 2010), but an intact, normally functioning hippocampus is necessary for discrimination reversal (Berger & Orr, 1983; Churchill et al., 2001; Miller & Steinmetz, 1997). For example, Berger and Orr (1983) showed that a hippocampal lesion selectively disrupted two-tone discrimination reversal conditioning of the rabbit NMR but did not affect acquisition of the discrimination or extinction of that discrimination. Miller and Steinmetz (1987) showed that hippocampal neural activity during discrimination reversal learning was significantly different from activity during discrimination learning. Importantly, lesions of the hippocampus prevent rabbits from inhibiting responding to the new CS– – the stimulus that is no longer paired with air puff (Berger & Orr, 1983) – and this is also true for the administration of drugs that affect hippocampal activity (Churchill et al., 2001).

Another body of literature provides strong evidence that cholesterol affects both synaptic plasticity and membrane excitability of neurons in the hippocampus (Dufour et al., 2006; Fester et al., 2009; Koudinov & Koudinova, 2001; Parkinson et al., 2009; Tanaka & Sokabe, 2012; Wang & Schreurs, 2010; Ya et al., 2012). For example, Koudinov and Koudinov (2001) showed that a cholesterol diet significantly enhanced hippocampal long term potentiation, a finding that was also noted by Dufour et al. (2006). Wang and Schreurs (2010) have shown that a cholesterol diet significantly reduces the amplitude of the CA1 pyramidal cell afterhyperpolarization – a measure of membrane excitability that has been shown to be important for learning and memory in rodents and rabbits (Bekisz et al., 2010; Disterhoft & Oh, 2006; Kaczorowski et al., 2009; Kuiper et al., 2012; Matthews et al., 2008; Moyer, Jr. et al., 1996; Tombaugh et al., 2005). It is therefore possible that feeding rabbits a cholesterol diet leads to changes in hippocampal properties that may account for the

improved discrimination reversal seen in the current experiment. In other words, if the hippocampus is important for inhibiting responding to the former CS+ during discrimination reversal, the stronger inhibition of responding to that CS shown by rabbits fed cholesterol in the present experiment may have resulted from changes in hippocampal synaptic plasticity and/or membrane excitability.

The relevance of the cholesterol-fed rabbit model to the cognitive deficits in Alzheimer's Disease depends upon how cholesterol affects learning *and* memory. One of the most devastating hallmarks of Alzheimer's Disease is the loss of recent memory (Albert, 1997; Albert, 2002; Fleischman et al., 2005; Gabrieli, 1996; Grady & Craik, 2000; Solomon, Beal, & Pendelbury, 1988). The present results show that a cholesterol diet can indeed affect memory retention as we have shown previously (Darwish et al., 2010; Schreurs et al., 2012). What remains unclear is the difference in the effects of cholesterol on learning and memory in rabbits that receive copper in their drinking water and those that do not. We have noted elsewhere that the most significant index of rabbit pathology induced by a diet of cholesterol that is worsened by the addition of copper to the drinking water is the deposition and accumulation of beta amyloid plaques (Sparks & Schreurs, 2003; Woodruff-Pak, 2008). In a number of other studies where copper has been added to the drinking water in concentrations and durations similar to the present experiment, other indices of pathology including increased ventricular volume and narrowing of the cerebrovasculature were similar in the presence and absence of copper in the drinking water and may have reached asymptote (Deci et al., 2012; Lemieux et al., 2010). Our efforts to extend the time available for discrimination reversal learning beginning eight weeks after the start of the cholesterol diet included the use of a high-fiber diet that reduces the rate of cholesterol absorption and the consequent accumulation of beta amyloid in the brain (Schreurs, 2013). In addition, heightened awareness of the potential for hepatotoxicity may have caused rabbits to be removed from the study than in our initial studies (Schreurs, 2013). This may have actually biased against the deposition of plaques and the resultant degradation of learning (Sparks & Schreurs, 2003).

Although the beta amyloid hypothesis has held considerable sway in explaining the pathology and memory losses found in Alzheimer's Disease (Ondrejcek et al., 2010; Shankar & Walsh, 2009; Walsh & Selkoe, 2007), the failure of beta amyloid-reduction strategies to ameliorate cognitive impairment (Teich & Arancio, 2012) has caused some to reconsider the primacy of the accumulation of beta amyloid as a causal factor in the disease (Benilova, Karran, & De Strooper, 2012; Bishop & Robinson, 2002; Roseblum, 2002). The accumulation of beta amyloid in our cholesterol-fed rabbits in the absence of plaques has not prevented significant learning (Schreurs et al., 2007a; Schreurs et al., 2007b; Schreurs et al., 2003) just as an elevated beta amyloid burden in humans may still result in normal cognitive performance (Aizenstein et al., 2008; Rodrigue et al., 2012).

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Reference List

- Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. *Archives of Neurology*. 2008; 65:1509–1517. [PubMed: 19001171]
- Albert MS. The ageing brain: normal and abnormal memory. *Philosophical Transactions of the Royal Society London B*. 1997; 352:1703–1709.

- Albert MS. Memory decline: the boundary between aging and age-related disease. *Annals of Neurology*. 2002; 51:282–284. [PubMed: 11891821]
- Bekisz M, Garkun Y, Wabno J, Hess G, Wrobel A, Kossut M. Increased excitability of cortical neurons induced by associative learning: an ex vivo study. *European Journal of Neuroscience*. 2010; 32:1715–1725. [PubMed: 20964731]
- Benilova I, Karran E, De Strooper B. The toxic A β oligomer and Alzheimer's disease: an emperor in need of clothes. *Nature Neuroscience*. 2012; 15:1–9.
- Berger TW, Orr WB. Hippocampectomy selectively disrupts discrimination reversal conditioning of the rabbit nictitating membrane response. *Behavioural Brain Research*. 1983; 8:49–68. [PubMed: 6849679]
- Bishop GA, Robinson SR. The amyloid hypothesis: let sleeping dogmas lie? *Neurobiology of Aging*. 2002; 23:1101–1105. [PubMed: 12470810]
- Burhans LB, Gabriel M. Contextual modulation of conditioned responses: Role of the ventral subiculum and nucleus accumbens. *Behavioral Neuroscience*. 2007; 121:1243–2157. [PubMed: 18085878]
- Churchill JD, Green JT, Voss SE, Manley E, Steinmetz JE, Garraghty PE. Discrimination reversal conditioning of an eyeblink response is impaired by NMDA receptor blockade. *Integrative Physiological and Behavioral Science*. 2001; 36:62–74. [PubMed: 11484997]
- Coleman SR, Gormezano I. Classical conditioning of the rabbit's (*Oryctolagus cuniculus*) nictitating membrane response under symmetrical CS-US interval shifts. *Journal of Comparative and Physiological Psychology*. 1971; 77:447–455. [PubMed: 5118253]
- Darwish DS, Wang D, Konat GW, Schreurs BG. Dietary cholesterol impairs memory and memory increases brain cholesterol and sulfatide levels. *Behavioral Neuroscience*. 2010; 124:115–123. [PubMed: 20141286]
- de Wolf ID, Fielmich-Bouman XM, Lankhorst A, den Bieman M, van Oost BA, Beynen AC, et al. Cholesterol and copper in the liver of rabbit inbred strains with differences in dietary cholesterol response. *Journal of Nutritional Biochemistry*. 2003; 14:459–465. [PubMed: 12948876]
- Deci S, Lemieux SK, Smith-Bell CA, Sparks DL, Schreurs BG. Cholesterol increases ventricular volume in a rabbit model of Alzheimer's Disease. *Journal of Alzheimer's Disease*. 2012; 29:283–292.
- Disterhoft JF, Oh MM. Learning, aging and intrinsic neuronal plasticity. *Trends in Neurosciences*. 2006; 29:587–599. [PubMed: 16942805]
- Dufour F, Liu Q-Y, Gusev P, Alkon DL, Atzori M. Cholesterol-enriched diet affects spatial learning and synaptic function in hippocampal synapses. *Brain Research*. 2006; 1103:88–98. [PubMed: 16814755]
- Endo Y, Nishimura J-I, Kimura F. Impairment of maze learning in rats following long-term glucocorticoid treatments. *Neuroscience Letters*. 1996; 203:199–202. [PubMed: 8742027]
- Fester L, Zhou L, Butow A, Huber C, von Lossow R, Prange-Kiel J, et al. Cholesterol-promoted synaptogenesis requires the conversion of cholesterol to estradiol in the hippocampus. *Hippocampus*. 2009; 19:692–705. [PubMed: 19156851]
- Fleischman DA, Wilson RS, Gabrieli JDE, Schneider JA, Bienias JL, Bennett DA. Implicit memory and Alzheimer's disease neuropathology. *Brain*. 2005; 128:2006–2015. [PubMed: 15975947]
- Freeman JH Jr. Weible AP, Rossi J, Gabriel M. Lesions of the entorhinal cortex disrupt behavioral and neuronal responses to context change during extinction of discriminative avoidance behavior. *Experimental Brain Research*. 1997; 115:445–457.
- Gabrieli JDE. Memory systems analyses of mnemonic disorders in aging and age-related diseases. *Proceedings of the National Academy of Sciences, USA*. 1996; 93:13534–13540.
- Ghribi O, Golovko MY, Larsen B, Schrag M, Murphy EJ. Deposition of iron and β -amyloid plaques is associated with cortical cellular damage in rabbits fed with long-term cholesterol-enriched diets. *Journal of Neurochemistry*. 2006; 99:438–449. [PubMed: 17029598]
- Goldstein FC, Ashley AV, Endeshaw Y, Hanfelt J, Lah JJ, Levey AI. Effects of hypertension and hypercholesterolemia on cognitive functioning in patients with Alzheimer's disease. *Alzheimer's Disease and Associated Disorders*. 2008; 22:336–342.

- Gormezano, I. Classical conditioning. In: Sidowski, JB., editor. *Experimental methods and instrumentation in psychology*. New York: McGraw-Hill; 1966. p. 385-420.
- Gormezano I, Gibbs CM. Transduction of the rabbit's nictitating membrane response. *Behavior Research Methods, Instruments, & Computers*. 1988; 20:18–21.
- Grady CL, Craik FIM. Changes in memory processing with age. *Current Opinion in Neurobiology*. 2000; 10:224–231. [PubMed: 10753795]
- Granhölm A-C, Bimonte-Nelson HA, Moore AB, Nelson ME, Freeman LR, Sambamurti K. Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in middle-aged rats. *Journal of Alzheimer's Disease*. 2008; 14:133–145.
- Helkala E-L, Niskanen L, Viikari H, Partanen J, Uusipata M. Short-term and long-term memory in elderly patients with NIDDM. *Diabetes Care*. 1995; 18:681–685. [PubMed: 8586007]
- Hooijmans CR, Van Der Zee CEEM, Deeg DJH, Brouwer KM, Reijmer YD, van Groen T, et al. DHA and cholesterol containing diets influence Alzheimerlike pathology, cognition and cerebral vasculature in APP^{SW}/PS1^{ΔE9} mice. *Neurobiology of Disease*. 2009; 33:482–498. [PubMed: 19130883]
- Hung YH, Bush AI, Cherny RA. Copper in the brain and Alzheimer's disease. *Journal of Biological Inorganic Chemistry*. 2010; 15:61–76. [PubMed: 19862561]
- Kaczorowski CC, Sametsky E, Shah S, Vassar R, Disterhoft JF. Mechanisms underlying basal and learning-related intrinsic excitability in a mouse model of Alzheimer's disease. *Neurobiology of Aging*. 2009; 32:1452–1465. [PubMed: 19833411]
- Kainuma M, Fujimoto M, Sekiya N, Tsuneyama K, Cheng C, Takano Y, et al. Cholesterol-fed rabbit as a unique model of nonalcoholic, nonobese, non-insulin-resistant fatty liver disease with characteristic fibrosis. *Journal of Gastroenterology*. 2006; 41:971–980. [PubMed: 17096066]
- Kessler H, Bayer TA, Bach D, Schneider-Axmann T, Supprian T, Herrmann W, et al. Intake of copper has no effect on cognition in patients with mild Alzheimer's disease: a pilot phase 2 clinical trial. *Journal of Neural Transmission*. 2008; 115:1181–1187. [PubMed: 18587525]
- Knuttnen M-G, Power JM, Preston AR, Disterhoft JF. Awareness in classical differential eyeblink conditioning in young and aging humans. *Behavioral Neuroscience*. 2001; 115:747–757. [PubMed: 11508714]
- Koudinov AR, Koudinova NV. Essential role of cholesterol in synaptic plasticity and neuronal degeneration. *FASEB Journal*. 2001; 15:1858–1860. [PubMed: 11481254]
- Kritchevsky D, Story JA. Fiber, hypercholesterolemia, and atherosclerosis. *Lipids*. 1978; 13:336–339.
- Kuiper EF, Nelemans A, Luiten P, Nijholt I, Dolga A, Eisel U. K_{Ca}2 and K_{Ca}3 channels in learning and memory processes, and neurodegeneration. *Frontiers in Pharmacology*. 2012; 3:107. [PubMed: 22701424]
- Lefterov I, Fitz NF, Cronican A, Lefterov P, Staufenbiel M, Koldamova R. Memory deficits in APP23/Abca1^{+/-} mice correlate with the level of Aβ oligomers. *ASN Neuro*. 2009; 1(2) art:e00006.
- Lemieux SK, Smith-Bell CA, Wells JR, Ezerioha NM, Carpenter JS, Sparks DL, et al. Neurovascular changes measured by TOF-MRA in cholesterol-fed rabbits with cortical amyloid beta-peptide accumulation. *Journal of Magnetic Resonance Imaging*. 2010; 32:306–314. [PubMed: 20677255]
- Li L, Xiao N, Yang X, Gao J, Ding J, Wang T, et al. A high cholesterol diet ameliorates hippocampus-related cognitive and pathological deficits in ovariectomized mice. *Behavioural Brain Research*. 2012; 230:251–258. [PubMed: 22366266]
- Matthews EA, Weible AP, Shah S, Disterhoft JF. The BK-mediated fAHP is modulated by learning a hippocampus-dependent task. *Proceedings of the National Academy of Sciences*. 2008; 105:15154–15159.
- Micale V, Scapagnini G, Colombrita C, Mazzola C, Alkon DL, Drago F. Behavioral effects of dietary cholesterol in rats tested in experimental models of mild stress and cognition tasks. *European Journal of Neuropsychopharmacology*. 2008; 18:462–471.
- Miller DP, Steinmetz JE. Hippocampal activity during classical discrimination-reversal eyeblink conditioning in rabbits. *Behavioral Neuroscience*. 1997; 111:70–79. [PubMed: 9109625]
- Miller S, Wehner JM. Cholesterol treatment facilitates spatial learning performance in DBA/2Jb mice. *Pharmacology Biochemistry and Behavior*. 1994; 49:257–261.

- Morris MC, Evans DA, Tangney CC, Bienias JL, Schneider JA, Wilson RS, et al. Dietary copper and high saturated and *trans* fat intakes associated with cognitive decline. *Archives of Neurology*. 2006; 63:1085–1088. [PubMed: 16908733]
- Moyer JR Jr, Thompson LT, Disterhoft JF. Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. *Journal of Neuroscience*. 1996; 16:5536–5546. [PubMed: 8757265]
- Nokia MS, Wikgren J. Hippocampal theta activity is selectively associated with contingency detection but not discrimination in rabbit discrimination-reversal eyeblink conditioning. *Hippocampus*. 2010; 20:457–460. [PubMed: 19693781]
- O'Brien WT, Xu G, Batta A, Tint GS, Salen G, Dyer CA, et al. Developmental sensitivity of associative learning to cholesterol synthesis inhibitors. *Behavioural Brain Research*. 2002; 129:141–152. [PubMed: 11809505]
- O'Brien WT, Xu G, Tint GS, Salen G, Servatius RJ. Blocking cholesterol synthesis impairs acquisition of the classically conditioned eyeblink response. *Integrative Physiological and Behavioral Science*. 2000; 35:120–131. [PubMed: 11021337]
- Ondrejcek T, Klyubin I, Hu N-W, Barry AE, Cullen WK, Rowan MJ. Alzheimer's disease amyloid β -protein and synaptic function. *NeuroMolecular Medicine*. 2010; 12:13–26. [PubMed: 19757208]
- Parkinson PF, Kannangara TS, Eadie BD, Burgess BL, Wellington CL, Christie BR. Cognition, learning behaviour and hippocampal synaptic plasticity are not disrupted in mice over-expressing the cholesterol transporter ABCG1. *Lipids in Health and Disease*. 2009; 8:5. [PubMed: 19239689]
- Prasanthi JR, Dasari B, Marwarha G, Larson T, Chen X, Geiger JD, et al. Caffeine protects against oxidative stress and Alzheimer's disease-like pathology in rabbit hippocampus induced by cholesterol-enriched diet. *Free Radical Biology & Medicine*. 2010; 49:1212–1220. [PubMed: 20638472]
- Prasanthi JRP, Schommer E, Thomasson S, Thompson A, Feist G, Ghribi O. Regulation of β -amyloid levels in the brain of cholesterol-fed rabbit, a model system for sporadic Alzheimer's disease. *Mechanisms of Ageing and Development*. 2008; 29:649–655.
- Rodrigue KM, Kennedy KM, Devous MD, Rieck JR, Hebrank AC, Diaz-Arrastia R, et al. β -amyloid burden in healthy aging. *Neurology*. 2012; 778:387–395. [PubMed: 22302550]
- Roseblum WI. Structure and location of amyloid beta peptide chains and arrays in Alzheimer's disease: new findings require reevaluation of the amyloid hypothesis and of tests of the hypothesis. *Neurobiology of Aging*. 2002; 23:225–230. [PubMed: 11804706]
- Salustri C, Barbati G, Ghidoni R, Quintiliani L, Ciappina S, Binetti G, et al. Is cognitive function linked to serum free copper levels? A cohort study in a normal population. *Clinical Neurophysiology*. 2010; 121:502–507. [PubMed: 20097602]
- Schreurs BG. Long-term memory and extinction of the classically conditioned rabbit nictitating membrane response. *Learning & Motivation*. 1993; 24:293–302.
- Schreurs BG. Long-term memory and extinction of rabbit nictitating membrane trace conditioning. *Learning & Motivation*. 1998; 29:68–82.
- Schreurs BG. Cholesterol and copper affect learning and memory in the rabbit. *International Journal of Alzheimer's Disease*. 2013 in press.
- Schreurs BG, Alkon DL. US-US conditioning of the rabbit's nictitating membrane response: emergence of a conditioned response without alpha conditioning. *Psychobiology*. 1990; 18:312–320.
- Schreurs BG, Smith-Bell CA, Darwish DS, Stankovic G, Sparks DL. Classical conditioning of the rabbit's nictitating membrane response is a function of the duration of dietary cholesterol. *Nutritional Neuroscience*. 2007a; 10:159–168. [PubMed: 18019398]
- Schreurs BG, Smith-Bell CA, Darwish DS, Stankovic G, Sparks DL. High dietary cholesterol facilitates classical conditioning of the rabbit's nictitating membrane response. *Nutritional Neuroscience*. 2007b; 10:31–43. [PubMed: 17539481]
- Schreurs BG, Smith-Bell CA, Darwish DS, Wang D, Burhans L, Gonzalez-Joekes J, et al. Cholesterol enhances classical conditioning of the rabbit heart rate response. *Behavioural Brain Research*. 2007c; 181:52–63. [PubMed: 17466388]

- Schreurs BG, Smith-Bell CA, Lochhead J, Sparks DL. Cholesterol modifies classical conditioning of the rabbit (*Oryctolagus cuniculus*) nictitating membrane response. *Behavioral Neuroscience*. 2003; 117:1220–1232. [PubMed: 14674842]
- Schreurs BG, Wang D, Smith-Bell CA, Burhans LB, Bell R, Gonzales-Joekes J. Dietary cholesterol concentration and duration degrade long-term memory of classical conditioning of the rabbit's nictitating membrane response. *International Journal of Alzheimer's Disease*. 2012; 2012
- Shankar GM, Walsh DM. Alzheimer's disease: synaptic dysfunction and A β . *Molecular Neurodegeneration*. 2009; 4:48. [PubMed: 19930651]
- Solomon A, Kareholt I, Ngandu T, Winblad B, Nissinen A, Tuomilehto J, et al. Serum cholesterol changes after midlife and late-life cognition. *Neurology*. 2007; 68:751–756. [PubMed: 17339582]
- Solomon A, Kareholt I, Ngandu T, Wolozin B, MacDonald SWS, Winblad B, et al. Serum cholesterol, statins and cognition in non-demented elderly. *Neurobiology of Aging*. 2009; 30:1006–1009. [PubMed: 18022292]
- Solomon PR, Beal MF, Pendelbury WW. Age-related disruption of classical conditioning: a model systems approach to memory disorders. *Neurobiology of Aging*. 1988; 9:535–546. [PubMed: 3062464]
- Song S-H, Min B-I, Lee J-H, Cho KS. Chronological effects of atherogenic diets on the aorta, liver and spleen of rabbits. *Journal of Korean Medical Science*. 2000; 15:413–419. [PubMed: 10983689]
- Sparks DL. Dietary cholesterol induces Alzheimer-like β -amyloid immunoreactivity in rabbit brain. *Nutrition, Metabolism and Cardiovascular Diseases*. 1997; 7:255–266.
- Sparks DL. Cholesterol, copper, and accumulation of thioflavine s-reactive Alzheimer's-like amyloid β in rabbit brain. *Journal of Molecular Neuroscience*. 2004; 24:97–104. [PubMed: 15314257]
- Sparks DL. Cholesterol metabolism and brain amyloidosis: evidence for a role of copper in the clearance of A β through the liver. *Current Alzheimer Research*. 2007; 4:165–169. [PubMed: 17430242]
- Sparks DL, Friedland R, Petanceska S, Schreurs BG, Shi J, Perry G, et al. Trace copper levels in the drinking water, but not zinc or aluminum influence CNS Alzheimerlike pathology. *Journal of Nutrition, Health and Aging*. 2006; 10:247–254.
- Sparks DL, Kuo Y-M, Roher AE, Martin TA, Lukas RJ. Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Preliminary observations. *Annals New York Academy of Sciences*. 2000; 903:335–344.
- Sparks DL, Martin T, Stankovic G, Waggoner T, Van Adel R. Influence of water quality on cholesterol induced systemic pathology. *The Journal of Nutrition, Health & Aging*. 2007; 11:189–193.
- Sparks DL, Scheff SW, Hunsaker III JC, Liu H, Landers T, Gross DR. Induction of Alzheimer-like β -amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Experimental Neurology*. 1994; 126:88–94. [PubMed: 8157129]
- Sparks DL, Schreurs BG. Trace amounts of copper in water induce β -amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proceedings of the National Academy of Sciences, USA*. 2003; 100:11065–11069.
- Squitti R. Copper dysfunction in Alzheimer's disease: from meta-analysis of biochemical studies to new insights into genetics. *Journal of Trace Elements in Medicine and Biology*. 2012; 26:93–96. [PubMed: 22565015]
- Tanaka M, Sokabe M. Continuous de novo synthesis of neurosteroids is required for normal synaptic transmission and plasticity in the dentate gyrus of the rat hippocampus. *Neuropharmacology*. 2012; 62:2372–2386.
- Teich AF, Arancio O. Is the amyloid hypothesis of Alzheimer's disease therapeutically relevant? *Biochemical Journal*. 2012; 446:165–177. [PubMed: 22891628]
- Tendolkar I, Enajat M, Zwiers MP, van WG, de Leeuw FE, van KJ, et al. One-year cholesterol lowering treatment reduces medial temporal lobe atrophy and memory decline in stroke-free elderly with atrial fibrillation: evidence from a parallel group randomized trial. *International Journal of Geriatric Psychiatry*. 2012; 27:49–58. [PubMed: 21308791]

- Tombaugh GC, Rowe WB, Rose GM. The slow afterhyperpolarization in hippocampal CA1 neurons covaries with spatial learning ability in aged fisher 344 rats. *The Journal of Neuroscience*. 2005; 25:2609–2616. [PubMed: 15758171]
- Upchurch M, Wehner JM. DBA/2J mice are incapable of cholinergically-based learning in the Morris water maze. *Pharmacology Biochemistry & Behavior*. 1988; 29:325–329.
- Voikar V, Rauvala H, Ikonen E. Cognitive deficit and development of motor impairment in a mouse model of Niemann-Pick type C disease. *Behavioural Brain Research*. 2002; 132:1–10. [PubMed: 11853852]
- Walsh DM, Selkoe DJ. A β oligomers - a decade of discovery. *Journal of Neurochemistry*. 2007; 101:1172–1184. [PubMed: 17286590]
- Wang D, Schreurs BG. Dietary cholesterol modulates the excitability of rabbit hippocampal CA1 pyramidal neurons. *Neuroscience Letters*. 2010; 479:327–331.
- Woodruff-Pak DS. Animal models of Alzheimer's disease: therapeutic implications. *Journal of Alzheimer's Disease*. 2008; 17:507–521.
- Woodruff-Pak DS, Agelan A, Del Valle L. A rabbit model of Alzheimer's disease: valid at neuropathological, cognitive, and therapeutic levels. *Journal of Alzheimer's Disease*. 2007; 11:371–383.
- Xu G, Servatius RJ, Shefer S, Tint GS, O'Brien WT, Batta AK, et al. Relationship between abnormal cholesterol synthesis and retarded learning in rats. *Metabolism*. 1998; 47:878–882. [PubMed: 9667239]
- Ya BL, Liu WY, Ge F, Zhang YX, Zhu BL, Bai B. Dietary cholesterol alters memory and synaptic structural plasticity in young rat brain. *Neurological Science*. 2012
- Zambon D, Quintana M, Mata P, Alonso R, Benavent J, Cruz-Sanchez F, et al. Higher incidence of mild cognitive impairment in familial hypercholesterolemia. *American Journal of Medicine*. 2010; 123:267–274. [PubMed: 20193836]
- Zatta P, Zambenedetti P, Stella MP, Licastro F. Astrocytosis, microgliosis, metallothionein-I-II and amyloid expression in high cholesterol-fed rabbits. *Journal of Alzheimer's Disease*. 2002; 4:1–9.

HIGHLIGHTS

- Feeding cholesterol to rabbits affects both learning and memory
- Cholesterol degrades long term memory and facilitates new learning
- Discrimination reversal can be used to test old memories and new learning
- Cholesterol may have behavioral effects by modifying hippocampal function

Figure 1.

Mean ($\pm$ SEM) percent conditioned responding (CRs) to CS+ and CS- for all 44 sessions of the experiment for rabbits fed normal chow and given distilled water (Control, top left panel), rabbits fed normal chow and given copper in distilled water (Copper, top right panel), rabbits fed 2% cholesterol and given distilled water (Cholesterol, bottom left panel), and rabbits fed cholesterol and given copper in distilled water (Cholesterol/Copper, bottom right panel). Rabbits received twenty four sessions of two-tone discrimination training (Discrimination), one session of discrimination testing (Test), one session of discrimination reminder training (Train) and eighteen sessions of discrimination reversal training (Discrimination Reversal). The break in the x-axis designates the eight weeks during which rabbits were fed their respective diets and remained in their home cages.

Figure 2.

Mean ($\pm$ SEM) percent conditioned responding (CRs) to CS+ and CS- during discrimination testing for rabbits fed normal chow and given distilled water (Control), rabbits fed normal chow and given copper in distilled water (Copper), rabbits fed 2% cholesterol and given distilled water (Cholesterol), and rabbits fed cholesterol and given copper in distilled water (Cholesterol/Copper). The insets show responding to CS+ and CS- in ten-trial blocks during discrimination testing for the four dietary treatment groups. * $p < .05$.