Behavioral Neuroscience
2003, Vol. 117, No. 6, 1220–1232

Copyright 2003 by the American Psychological Association, Inc.
0735-7044/03/$12.00 DOI: 10.1037/0735-7044.117.6.1220

Cholesterol Modifies Classical Conditioning of the Rabbit (*Oryctolagus cuniculus*) Nictitating Membrane Response

Bernard G. Schreurs and Carrie A. Smith-Bell
Blanchette Rockefeller Neurosciences Institute and
West Virginia University

Jeff Lochhead and D. Larry Sparks
Sun Health Research Institute

Cholesterol plays an important role in synapse formation, receptor function, and synaptic plasticity, and animal studies show that modifying cholesterol may improve learning and memory. Other data show that feeding animals cholesterol can induce beta amyloid accumulation. Rabbits (*Oryctolagus cuniculus*) fed 2% cholesterol for 8 weeks were given trace conditioning of the nictitating membrane response using a 100-ms tone, a 700-ms trace, and periorbital electrical stimulation or airpuff. Rabbits fed cholesterol showed significant facilitation of trace conditioning to airpuff and conditioning-specific reflex modification to periorbital electrical stimulation and airpuff. The cholesterol-fed rabbits had beta amyloid accumulation in the cortex, but little in the hippocampus. The data suggest cholesterol had facilitative effects that outweighed potential amnesic effects of cortical beta amyloid.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

There is a considerable body of data suggesting that modifying cholesterol may improve learning and memory. Elevating cholesterol in young DBA/2 mutant mice improves performance on the Morris water maze, a task that is normally impaired in this mutant (Miller & Wehner, 1994; Upchurch & Wehner, 1988). Feeding rats cholesterol 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). Many of these positive effects of cholesterol may be due to the crucial role cholesterol plays in the formation of new synapses, normal receptor function, and synaptic plasticity (Goritz, Mauch, Nagler, & Pfrieger, 2002; Koudinov & Koudinova, 2001; Mauch et al., 2001; Sooksawate & Simmonds, 2001a, 2001b). Indeed, cholesterol is so crucial to normal neural function that it is synthesized de novo in the central nervous system, and 25% of the body's total unestrified cholesterol is found in the brain (Dietschy & Turley, 2001).

However, there are studies that show high cholesterol levels may be detrimental to learning and memory. Human studies show that elevated serum cholesterol is a significant risk factor for mild cognitive impairment (Kivipelto et al., 2001; Näslund et al., 2000; Yaffe, Barret-Connor, Lin, & Grady, 2002) and that cholesterol levels may be negatively correlated with measures of intelligence (Atzmon et al., 2002; Muldoon, Ryan, Matthews, & Manuck, 1997; Reitan & Shipley, 1963; van Exel et al., 2002; Yaffe et al., 2002). A recent study of cholesterol synthesis shows that high levels of the cholesterol precursors lanosterol and lathosterol are correlated with lower memory performance as subjects age (Teunissen et al., 2003). Epidemiological evidence also suggests a strong relationship between cholesterol levels and Alzheimer's disease, a disease noted for its severe decline in learning and memory (Evans et al., 2000; Hartmann, 2001; Jarvik et al., 1995; Notkola et al., 1998; Simons, Keller, Dichgans, & Schulz, 2001; Stewart et al., 2001).

In addition to human studies, animal studies in which there is a manipulation of cholesterol show an inverse relationship between cholesterol and memory. For example, decreasing cholesterol in aged animals improves learning and memory for tasks such as the Morris water maze (Kessler, Kessler, & Yehuda, 1986; Yehuda & Carasso, 1993; Yehuda, Rabinovitz, & Motofsky, 1998). In addition, rats, mice, and rabbits given calcium channel blockers that, among other things, decrease the level of cholesterol by reducing the estrification of cholesterol and increasing hydrolysis of existing cholesterol esters (Nayler, 1999; Schachter, 1997), demonstrate improvements in learning and memory (Deyo, Straube, & Disterhoft, 1989; Kane & Robinson, 1999; Quartermain, 2000; Woodruff-Pak, Chi, Li, Pak, & Fanelli, 1997).

Given that high cholesterol may play a significant role in learning and memory and contribute to beta amyloid deposition and Alzheimer's disease, a cholesterol-fed animal model would be useful for studying the role of elevated cholesterol in learning and memory. We have adopted the hippocampally dependent task of trace conditioning (Kim, Clark, & Thompson, 1995; McEchron & Disterhoft, 1999; Moyer, Deyo, & Disterhoft, 1990; Moyer, Thompson, & Disterhoft, 1996; Port, Mikhail, & Patterson, 1985; Port, Romano, Steinmetz, Mikhail, & Patterson, 1986; Seager, Asaka, & Berry, 1999; Solomon, Vander Schaaf, Thompson, & Weisz, 1986) to study the effects of cholesterol on classical conditioning of the rabbit nictitating membrane response (NMR). Although the cholesterol-fed rabbit has been used as a model for atherosclerosis since 1913 (Finking & Hanke, 1997) and continues

Bernard G. Schreurs and Carrie A. Smith-Bell, Blanchette Rockefeller Neurosciences Institute, Morgantown, West Virginia, and Department of Physiology and Pharmacology, West Virginia University; Jeff Lochhead and D. Larry Sparks, Sun Health Research Institute, Sun City, Arizona.

This research was supported by funds from the National Institutes of Health (Grant MH64715 to Bernard G. Schreurs), the Blanchette Rockefeller Neurosciences Institute, and the Arizona Disease Control Research Commission (to D. Larry Sparks).

Correspondence concerning this article should be addressed to Bernard G. Schreurs, Blanchette Rockefeller Neurosciences Institute and Department of Physiology and Pharmacology, West Virginia University, P.O. Box 9302, Morgantown, West Virginia. E-mail: bschreurs@hsc.wvu.edu

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

to be the model of choice for examining atherogenesis (Bocan, 1998; Brousseau & Hoeg, 1999; Fan & Watanabe, 2000; Nayler, 1999; Schachter, 1997), there have been no previous reports of the cholesterol-fed rabbit being used to study the effects of cholesterol on learning and memory.

Experiment 1

The only previous studies of explicit cholesterol manipulation and classical conditioning have focused on the effects of cholesterol deficits on eyelid conditioning in rats (O'Brien et al., 2002; O'Brien, Xu, Tint, Salen, & Servatius, 2000; Xu et al., 1998). These experiments were designed to examine the developmental effects of genetic disorders of cholesterol synthesis such as Smith-Lemli-Optiz syndrome. Servatius and colleagues found that if just-weaned rats had their cholesterol synthesis inhibited, they did not learn as well as controls and that these learning deficits could be overcome by removing the synthesis inhibitor or beginning the experiment 30 days after weaning (O'Brien et al., 2002). In a number of rabbit classical conditioning studies in which aged animals were used, researchers showed that the calcium channel blocker nimodipine facilitated trace and long delay conditioning (Deyo et al., 1989; Solomon et al., 1995; Woodruff-Pak et al., 1997). Although nimodipine is primarily a calcium channel blocker and its effects on learning are argued to involve improved hippocampal neuronal activity (Moyer, Thompson, Black, & Disterhoft, 1992; Power, Wu, Sametsky, Oh, & Disterhoft, 2002), at sufficiently high doses, nimodipine, like other dihydropyridines, may also reduce cholesterol-induced atherosclerosis (Nayler, 1999).

The purpose of the present experiment was to assess the effects of adding 2% cholesterol to a normal diet in young adult rabbits on the acquisition and extinction of trace conditioning of the NMR. In designing this experiment, we noted that a large number of substances may affect classical conditioning of the rabbit NMR (Du & Harvey, 1996; Du, Weiss, & Harvey, 2000; Harvey & Romano, 1993; Kronforst-Collins et al., 1997; Moore, Goodall, & Solomon, 1976; Oh, Power, Thompson, Moriearty, & Disterhoft, 1999; Solomon et al., 1995; Weiss et al., 2000; Welsh, Romano, & Harvey, 1998; Woodruff-Pak, 1997; Woodruff-Pak & Santos, 2000). However, not all compounds that affect responding during classical conditioning affect associative processes (P. Chen, Ghoneim, & Gormezano, 1992; Schindler, Gormezano, & Harvey, 1984). Assessing the effects of cholesterol on classical conditioning of the rabbit NMR also requires assessing and controlling for potential sensory, motor, and nonassociative effects of the substance (P. Chen et al., 1992; Gormezano, 1994; Moon, Ghoneim, & Gormezano, 1994; Schindler et al., 1984). For example, cholesterol may decrease responding to a tone conditioned stimulus (CS) that was paired with an airpuff or periorbital electrical stimulation unconditioned stimulus (US) because it affects sensory perception (e.g., the animal may become hard of hearing) or motor performance (e.g., the animal may develop muscle weakness) rather than associative processes. Explicitly unpaired presentations of the CS and US have been used to assess the various nonassociative contributors to responding, including sensitization and pseudoconditioning, as well as providing a simple assessment of sensory perception and motor performance (P. Chen et al., 1992; Gormezano, 1984; Gormezano & Kehoe, 1981; Gormezano, Kehoe, & Marshall, 1983; Moon et al., 1994; Schindler, Gormezano, & Harvey, 1983; Schindler et al., 1984). Additional CS and US intensity manipulations have been used to provide more thorough and extensive assessments of sensory processing and motor performance (P. Chen et al., 1992; Gormezano, 1984; Gormezano & Kehoe, 1981; Gormezano et al., 1983; Moon et al., 1994; Schindler et al., 1983, 1984). The manipulation of US intensity provides a means of assessing the sensory processing of the US and motor performance of the unconditioned response (UR) system being studied. The ability to manipulate US intensity also allows us to assess the effects of classical conditioning on the UR and potential interactions of cholesterol with the UR and its modification by classical conditioning (Gruart & Yeo, 1995; Schreurs, Oh, Hirashima, & Alkon, 1995; Schreurs, Shi, Pineda, & Buck, 2000; Wikgren, Ruusuvirta, & Korhonen, 2002). Manipulating CS intensity after conditioning allows the conditioned response (CR) to act as an objective test of whether the subject can detect the CS (P. Chen et al., 1992; Moon et al., 1994; Schindler et al., 1984). Various associative aspects of classical conditioning can also be tested by the selection of specific behavioral paradigms. For example, the ability to bridge a temporal gap can be assessed using trace conditioning in which there is a "trace" interval between the offset of the CS and the onset of the US. Retention and forgetting can be assessed by presenting the CS by itself in an extinction procedure. The retarding effects of prior experience can be assessed by presenting CS–US pairings after explicitly unpaired presentations of the CS and US (Napier, Macrae, & Kehoe, 1992). Consequently, in the present experiment, an explicitly unpaired control group was used to assess nonassociative contributors to responding and manipulations of CS, and US intensities were used to assess potential effects of cholesterol on sensory processing, motor performance, and conditioning-specific reflex modification (Buck, Seager, & Schreurs, 2001; Schreurs et al., 1995; Schreurs et al., 2000; Seager, Smith-Bell, & Schreurs, 2003). It should be remembered that these behavioral manipulations take place against the backdrop of a cholesterol diet that was originally designed to create an atherosclerotic rabbit (Finking & Hanke, 1997) that also develops beta amyloid accumulation in the brain (Sparks, 1997; Sparks et al., 1994).

Method

Subjects. Twenty one male New Zealand white rabbits (*Oryctolagus cuniculus*) weighing ~2.2 kg were housed individually, with free access to food and water, and maintained on a 12-hr light–dark cycle, all following American Psychological Association and National Institutes of Health guidelines. Cholesterol-fed rabbits received 2% cholesterol in Purina rabbit chow (Dyets., Bethlehem, PA) for 8 weeks prior to behavioral experiments, and normal diet control rabbits receive standard rabbit chow (0% cholesterol). Rabbits were placed on the 8-week diet because, in addition to producing atherosclerosis (Finking & Hanke, 1997), it takes 8 weeks for there to be a significant accumulation of beta amyloid in the brain (Sparks, 1997; Sparks, Kuo, Roher, & Martin, 2000; Sparks et al., 1994). Analysis of variance indicated that all rabbits increased in weight, $F(1, 7) = 429.55$, $p < .001$, with cholesterol rabbits gaining less weight (2.83 kg) than controls (3.24 kg), $F(1, 21) = 20.54$, $p < .001$. Rabbits were maintained on their respective diets throughout the course of the behavioral experiments and, because of the atherogenic nature of the cholesterol diet, their weight and general health were closely monitored.

Apparatus. The apparatus and recording procedures for the rabbit NMR, first described by Gormezano (1966; Gormezano, Schneiderman, Deaux, & Fuentes, 1962), have been detailed previously (Schreurs & Alkon, 1990; Schreurs et al., 2000). Each rabbit was restrained in a

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

Plexiglas box and trained in a sound-attenuating, ventilated chamber. A stimulus panel containing a speaker and 10-W houselights was mounted above the subject's head. Ambient noise (65 dB) was provided by an exhaust fan. Periorbital electrical stimulation (ES) was delivered by means of a programmable two-pole shocker to stainless steel Autoclip wound clips positioned 10 mm below and 10 mm posterior to the right eye. Transducing nictitating membrane (NM) movements involved a hook and L-shaped lever attached to a 6-0 nylon loop sutured into but not through the NM. The other end of the lever was attached to a rotary encoder connected to a 12-bit A/D converter (5-ms sampling rate; 0.05-mm resolution). Individual NM A/D outputs were stored on a trial-by-trial basis for subsequent analysis. Data collection, analysis, and stimulus delivery were accomplished using a LabVIEW software system (Schreurs et al., 2000).

Procedure. Following 8 weeks on their respective diets, 11 cholesterol-fed rabbits and 10 control rabbits fed the normal diet were assigned to two groups that received 1 day of adaptation, one 80-trial session of US ES pretesting, then either six daily sessions of paired CS–US presentations (cholesterol diet paired [CP], normal diet control paired [NP], *n*s = 7) or six daily sessions of unpaired CS and US presentations (cholesterol diet unpaired [CU], $n = 4$; normal diet control unpaired [NU], $n = 3$), followed by an 80-trial session of US posttesting. After US posttesting, paired subjects were given one session of CS intensity testing followed by six daily sessions of CS extinction, whereas unpaired rabbits were given six daily sessions of paired CS–US presentations.

On adaptation day, the rabbits were prepared for ES and recording of NM movement and then adapted to the training chambers for the length of time of subsequent training sessions (80 min). On both the pretest and the posttest day, all of the subjects received a total of 80 trials of ES presented at an average intertrial interval (ITI) of 60 s (50–70 s range). Each trial involved the presentation of 1 of 20 possible combinations of stimulus intensity (0.1, 0.25, 0.5, 1.0, 2.0 mA) and stimulus duration (10, 25, 50, or 100 ms). Four separately randomized sequences of the 20 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 six paired-conditioning sessions consisted of 80 presentations of a 100-ms, 1-KHz, 82-dB tone CS that was followed by a 700-ms trace interval and then a 100-ms, 60-Hz, 2-mA ES US (i.e., 800-ms interstimulus interval [ISI]). Paired stimulus presentations were delivered, on average, every 60 s (50–70 s range). Sessions for unpaired subjects consisted of 80 CS-alone and 80 US-alone presentations that occurred in an explicitly unpaired manner delivered, on average, every 30 s (20–40 s range). CS intensity testing consisted of seven 100-ms tone intensities (60, 65, 70, 75, 80, 85, 90 dB) plus a zero intensity (0 dB) paired with the ES US (800-ms ISI) and presented 10 times as a randomized sequence with each trial delivered, on average, every 60 s (50–70 s range). CS extinction sessions consisted of 80 CS-alone presentations with each trial delivered, on average, every 60 s (50–70 s range).

A 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 UR was defined as any extension of the NM exceeding 0.5 mm that was initiated within 800 ms of US onset. The UR criterion was based on the observation that, following CS–US pairings, URs at the lower US intensities (e.g., 0.25 and 0.5 mA) had onset latencies that fell into the range of latencies for CRs (Buck et al., 2001; Schreurs et al., 2000). Amplitude of a response was scored in millimeters as the maximum extension of the NM. Onset latency of a response was identified as the latency in milliseconds from stimulus onset at which a response rose 0.1 mm above the baseline. Peak latency of a response was determined as the latency in milliseconds from stimulus onset for maximum extension of the NM. Area of a response was calculated as the total area under the response curve from US onset to the end of the trial.

Histology. Rabbits were anesthetized deeply with a cocktail of Ketamine (35 mg/kg)/Xylazine (5 mg/kg) and perfused transcardially with 0.5% paraformaldehyde to determine the effects of the cholesterol diet on the brain. Brains were extracted and postfixed for 14 days in 4% paraformaldehyde. Fifty-micron vibratome sections of the hippocampus and temporal cortex of the brain were immunostained with an antibody to beta amyloid (10D5; provided by Dale Schenk of Elan Pharmaceuticals) using published peroxidase–antiperoxidase immunohistochemical methods (Sparks et al., 1994). The cells stained for the10D5 antibody within a 0.5 × 0.5 mm square grid were counted in at least three randomly selected areas and averaged for the hippocampus and for the temporal cortex by an experimenter (Jeff Lochhead) blind to the rabbit's diet.

Results

Behavior. The four panels of Figure 1 show responding to the tone CS during paired and unpaired stimulus presentations (Acquisition), tone intensity testing (Tone Intensity), and extinction (Extinction) for paired rabbits and CS–US acquisition (Unpaired Acquisition) for unpaired rabbits. Examination of the Acquisition panel shows that all rabbits given paired CS–US presentations reached a level of 90% CRs by the last session of training and that there were no differences in the rate or level of CR acquisition between cholesterol-fed and normal diet control rabbits. The panel also shows that responding by unpaired rabbits was at baseline levels and did not differ between cholesterol-fed and normal diet control rabbits. Statistical analysis confirmed significant differences in the level of responding to the CS between paired and unpaired rabbits as a main effect of groups, $F(1, 18) = 34.10$, $p < .001$, and an interaction of Group × Sessions, $F(1, 90) = 17.30$, $p < .001$. There were no significant differences between cholesterol-fed and normal diet control rabbits detected as a main effect of diet, $F(1, 18) = 0.25$, $p > .62$, or as an interaction of Diet × Group, $F(1, 18) = 0.72$, $p > .40$, nor were there any interactions of either of these effects with sessions, $F(5, 90) = 0.41$, $p > .84$, and $F(5, 90) = 0.25$, $p > .93$, respectively.

The Tone Intensity panel illustrates that both cholesterol-fed and normal diet control rabbits given paired training gave an increasing frequency of CRs as the tone intensity increased but that there were no differences between the two groups. These observations were confirmed by a main effect of tone intensity, $F(7, 84) = 121.92$, $p < .001$, and no main effect of diet, $F(1, 12) = 0.01$, $p > .93$, nor interaction of Diet × Tone Intensity, $F(7, 84) = 0.54$, $p > .80$. The Extinction panel shows that both the paired cholesterol-fed and paired normal diet control rabbits extinguished CRs to the tone as reflected by a main effect of sessions, $F(5, 60) = 4.44$, $p < .005$; the groups did not differ as indicated by no main effect of diet, $F(1, 12) = 0.24$, $p > .63$; and the rate of extinction did not differ between groups as indicated by a lack of Diet × Sessions interaction, $F(5, 60) = 0.56$, $p > .73$. The Unpaired Acquisition panel shows that both cholesterol-fed and normal diet control rabbits previously given unpaired stimulus presentations acquired CRs as a function of CS–US pairings indicated by a significant main effect of session, $F(5, 25) = 12.52$, $p < .001$, but that the two groups did not differ, $F(1, 5) = 0.11$, $p > .75$, nor did their rate of acquisition differ, $F(5, 25) = 0.14$, $p > .99$. As noted in other studies (Napier et al., 1992), the rate of acquisition was noticeably slower during CS–US pairings for rabbits previously given unpaired stimulus presentations than for rabbits originally given paired stimulus presentations. This observation was confirmed by a main effect of group, $F(1, 16) = 8.73$, $p < .005$, and an interaction of Group × Session, $F(5, 80) = 5.65$, $p < .001$.

In sum, the panels show and statistical analyses confirm there were no significant differences between cholesterol-fed and normal diet control rabbits in terms of CR acquisition, responsiveness to the CS or US during unpaired stimulus presentations, respon-

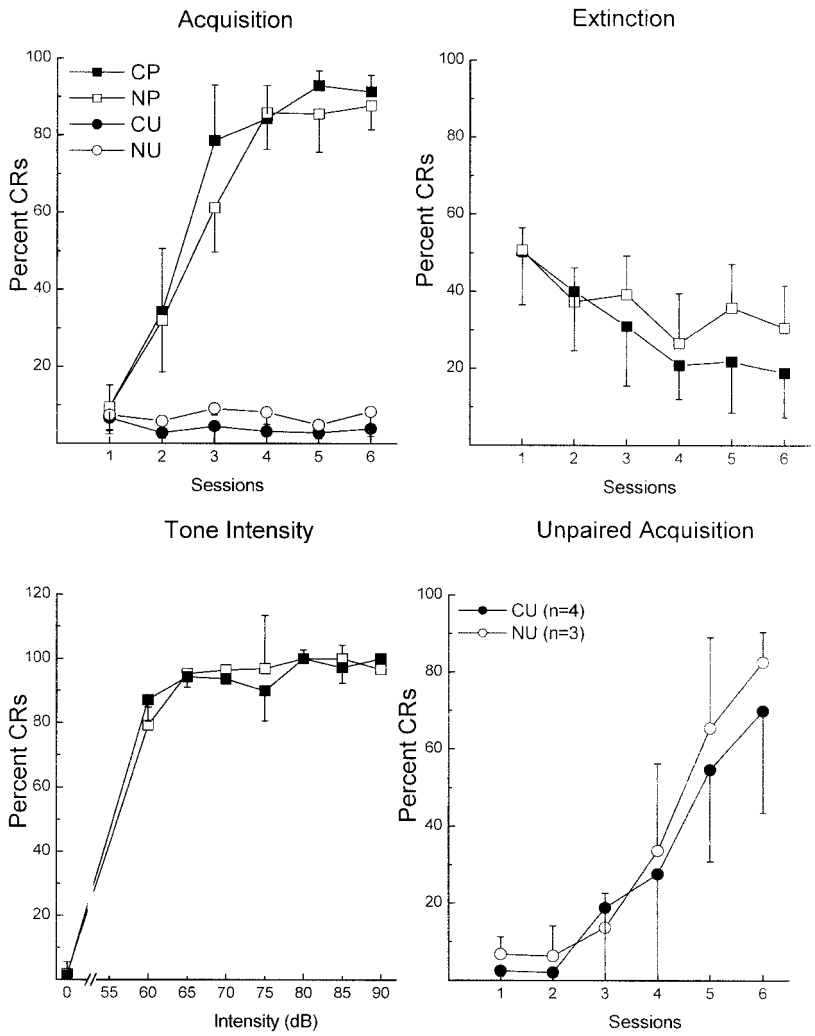

Figure 1. Effects of cholesterol on responding during paired and unpaired tone and periorbital electrical stimulation (ES) presentations (Acquisition), tone intensity testing (Tone Intensity), and extinction (Extinction) for paired rabbits; and conditioned stimulus (CS)–unconditioned stimulus (US) acquisition for rabbits initially given unpaired tone and ES presentations (Unpaired Acquisition). Paired rabbits reached a level of 90% conditioned responses (CRs) over the course of six daily sessions of 80 CS–US pairings of a 100-ms, 1000-Hz, 82-dB tone CS followed by a 700-ms trace interval and a 100-ms, 60-Hz, 2-mA electrical pulse US. Extinction occurred over six daily sessions of 80 CS-alone presentations. For tone intensity, some *SEM*s are too small for error bars to be visible. CP = cholesterol diet paired; NP = normal diet control paired; CU = cholesterol diet unpaired; NU = normal diet control unpaired.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

siveness to different tone intensities following CR acquisition, rate of extinction following CR acquisition, or rate and level of CR acquisition following unpaired stimulus presentation.

An analysis of responding to the US during the US pretest session found no significant differences in response frequency, onset latency, amplitude, peak latency, or area between the URs of cholesterol-fed rabbits and normal diet control rabbits, indicating that cholesterol did not have any effect on sensitivity to the US nor performance of the UR. Similarly, an analysis of responding to the US during the US posttest session for rabbits that received unpaired CS and US presentations found no significant differences in response frequency, onset latency, amplitude, peak latency, or area between the URs of unpaired cholesterol-fed rabbits and unpaired normal diet controls. Finally, with the exception of a significant decrease in peak latency from pretest to posttest at 0.5 mA (157 ± 5.4 vs. 108 ± 22.5 ms, $M \pm SEM$), for normal controls, $F(1, 5) = 19.10$, $p < .05$, there were no significant pretest–posttest differences in response frequency, onset latency, amplitude, peak latency, or area of the URs of unpaired cholesterol-fed rabbits and unpaired normal diet controls.

In strong contrast to the pretest data for all rabbits and the posttest data for the unpaired rabbits, an analysis of the posttest data for paired rabbits found substantial increases from their responses during the pretest session and significant differences between cholesterol-fed rabbits and normal diet controls. In previous examinations of US responding after classical conditioning of the

rabbit NMR (Gruart & Yeo, 1995; Schreurs et al., 1995; Schreurs et al., 2000), rabbits showed an increase in the size and shape of the UR as a result of classical conditioning, a phenomenon we call *conditioning-specific reflex modification* (CRM). This was also true in the present experiment. Figure 2 shows average response topographies to five US intensities during the first 20 pretest trials (dotted lines) and the first 20 posttest trials (solid lines) for the cholesterol-fed rabbits and normal diet controls given paired CS–US presentations. The figure shows that responses were larger and peaked later on posttest (solid lines) than on pretest (dotted lines) for both groups, a finding that replicates our previously observed CRM effect when periorbital electrical stimulation was used as the US (Schreurs et al., 1995). Statistical analysis confirmed that there was a significant increase in peak latency at 0.5 mA from pretest to posttest (106 ± 6.3 vs. 223 ± 30.1 ms) for paired normal diet control rabbits, $F(1, 11) = 12.47$, $p < .01$. More interestingly, analysis of the cholesterol-fed rabbit posttest data revealed a significant increase from pretest in response frequency at 0.25 mA (0% ± 0% vs. 42.9% ± 17.4%), $F(1, 13) = 6.07$, $p < .05$, in peak latency (107 ± 16.1 vs. 481 ± 99.9 ms), $F(1, 13) =$

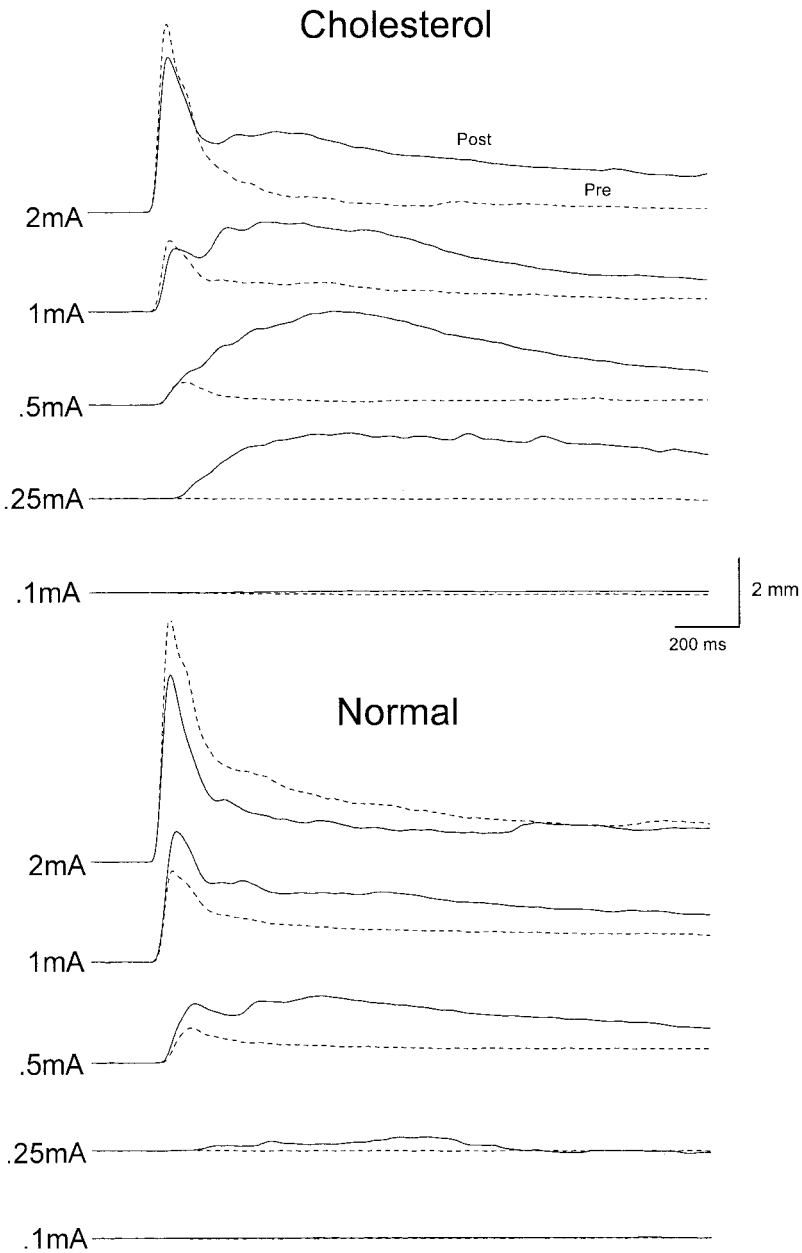

Figure 2. Effects of cholesterol on response topographies to five unconditioned stimulus intensities on the first 20 pretest trials (Pre; dotted lines) and first 20 posttest trials (Post; solid lines) for rabbits given paired conditioned stimulus–unconditioned stimulus presentations. The top graphs show a clear increase in response size after conditioning for cholesterol-fed rabbits, and the bottom graphs show very modest changes in response size for normal diet control rabbits.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

49.46, $p < .001$, and area (12,567 ± 4,981 vs. 59,673 ± 8,506 arbitrary units), $F(1, 13) = 19.57$, $p < .001$, at 0.5 mA; and in peak latency (73 ± 6.7 vs. 234 ± 63.0 ms), $F(1, 13) = 6.49$, $p < .05$, and area (16,184 ± 7,017 vs. 54,373 ± 14,040 arbitrary units), $F(1, 13) = 5.92$, $p < .05$, at 1.0 mA.

A comparison of the posttest response topographies for paired cholesterol-fed and paired normal diet control rabbits shown in Figure 2 revealed that cholesterol-fed rabbits had URs that were larger and peaked later than those of the normal diet control rabbits. This is particularly true at 0.25 mA for UR amplitude (4.8 ± 0.6 vs.1.2 ± 0.2 mm), $F(1, 6) = 15.92$, $p < .05$, and area (91,248 ± 13,991 vs.13,032 ± 3,105 units), $F(1, 6) = 12.35$, $p < .05$, and at 0.5 mA for UR peak latency (481 ± 47.0 vs. 223 ± 30.1 ms), $F(1, 11) = 18.55$, $p < .005$. Thus, the data suggest that although CRM was observed in both groups following classical conditioning, cholesterol-fed rabbits had a significantly higher level of conditioning-specific reflex modification than paired normal diet control rabbits.

Histology. The panels of Figure 3 show 10D5 immunoreactivity in the cortex and hippocampus of a normal diet control rabbit (left) and a cholesterol-fed rabbit (right). Examination of the middle panels shows 10D5 immunoreactivity in the cortex of cholesterol-fed rabbits (right) with little labeling in the cortex of normal diet controls (left), confirming that a 2% cholesterol diet can induce intracellular beta amyloid accumulation. The 10D5 labeling of the cortex shown in the right middle panel appears to be uniformly dense, and labeled cells are distributed evenly throughout the cortex as previously reported (Sparks et al., 1994). An analysis of the number of immunoreactive cells in the temporal cortex revealed a significantly higher level in cholesterol-fed rabbits than in the normal diet controls (25.98 ± 6.39 vs.10.6 ± 0.79 cells, $p < .05$). The lower panels of Figure 3 illustrate the level of beta amyloid accumulation in the hippocampus of cholesterol-fed rabbits (right) compared with normal diet controls (left). Although a number of cells are 10D5 immunoreactive, labeling is sporadic and there are no extracellular plaques. Analysis of the hippocampal tissue indicated that the level of labeling was quite variable between rabbits, and although suggestive, there was no significant difference in the level of immunoreactive cells in hippocampus of cholesterol-fed rabbits compared with the normal diet controls (36.91 ± 8.63 vs. 14.67 ± 9.31 cells, $p > .09$).

Discussion

The results of the dietary manipulation in Experiment 1 show that an 8-week diet of 2% cholesterol induces a reduction in weight gain, atherosclerosis, and significant accumulation of beta amyloid in neurons of the cortex with less consistent accumulation in the hippocampus (Sparks, 1997; Sparks et al., 1994; Sparks et al., 2000). The behavioral data show that rabbits fed the cholesterol diet did as well as rabbits given the normal control diet on all standard learning and memory measures, including acquisition of CRs, extinction of those CRs, responsiveness to the US before CS–US presentations, and responsiveness to the tone CS after CS–US pairings. It is important to note that there were no differences between cholesterol-fed rabbits and controls in responding during unpaired presentations of the CS and US, suggesting that cholesterol did not alter the normally low level of nonassociative responding. At the same time, however, rabbits fed the cholesterol diet and given paired training showed a significant increase in the level of conditioning-specific reflex modification compared with paired rabbits on the normal control diet. Taken together, the data suggest that a cholesterol diet was able to induce modest intracellular beta amyloid accumulation in the cortex and facilitate conditioning-specific reflex modification without significant effects on the rabbit's ability to detect the CS or US or to acquire or extinguish CRs when periorbital electrical stimulation was used as the US.

Perhaps the most interesting result of Experiment 1 was that cholesterol had a facilitative effect on CRM without affecting CR acquisition. This is clearly an associative phenomenon because, as in previous experiments (Schreurs et al., 1995), the present data show that CRM was only detected in rabbits that received CS–US pairings resulting in high levels of CR acquisition and not detected in rabbits receiving unpaired presentations of the CS and US. CRM is also a clear index of the strength of conditioning because we have shown previously that the level of CRM is a function of the level of CR acquisition (Schreurs et al., 1995).

As noted earlier, there is evidence that feeding cholesterol can improve learning and memory in both rats and mice. However, these effects have only been shown in mutant mice (Miller & Wehner, 1994; Upchurch & Wehner, 1988) and in cholesterol-deficient rats (O'Brien et al., 2000; O'Brien et al., 2002; Servatius, 2000; Tapp, Servatius, Hunt, & Powell, 1997; Xu et al., 1998). Here we show a facilitative effect of dietary cholesterol on CRM, an associative process (Schreurs et al., 1995), in normal adult rabbits.

It is possible that the cholesterol diet did not produce changes in acquisition, extinction, or acquisition after unpaired stimulus presentations because the task was too easy and rabbits quickly reached a response ceiling and, thus, all our behavioral measures except CRM were insensitive to the effects of cholesterol. The relatively rapid rate of CR acquisition for a brief tone followed by a 700-ms trace and periorbital electrical stimulation US suggests that the task was not as difficult as a 500-ms trace with an airpuff US (Moyer et al., 1990; Thompson, Moyer, & Disterhoft, 1996). We and others have noted that a periorbital US affects sensory inputs and neural pathways that are different from those affected by airpuff (Buck et al., 2001; McEchron, McCabe, Green, Llabre, & Schneiderman, 1991). Indeed, trace conditioning using an electrical US almost certainly taps into structures other than the hippocampus, such as the amygdala, that mediate conditioned fear (McEchron et al., 1991). On the other hand, there is considerable evidence that the hippocampus is also involved in fear conditioning (C. Chen, Kim, Thompson, & Tonegawa, 1996; Crestani et al., 2002; Gewirtz, McNish, & Davis, 2000; Maren & Holt, 2000; McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; McEchron, Tseng, & Disterhoft, 2000). McEchron et al. (1991) have shown that when heart rate conditioning is used as a measure of conditioned fear, heart rate conditioning is supported by an electrical US but not by an airpuff US. We have shown elsewhere that a relatively intense periorbital electrical stimulation US (e.g., 2 and 4 mA) can support substantial levels of CRM, whereas a weaker periorbital electrical US (e.g., 1 mA) or an airpuff US (e.g., 4 psi) does not, even though both can support levels but not rates of CR acquisition comparable with those supported by a 2-mA US (Buck et al., 2001; Seager et al., 2003). This point is important in the present experiment because the relatively intense US that supported CRM may have prevented detection of cholesterol-

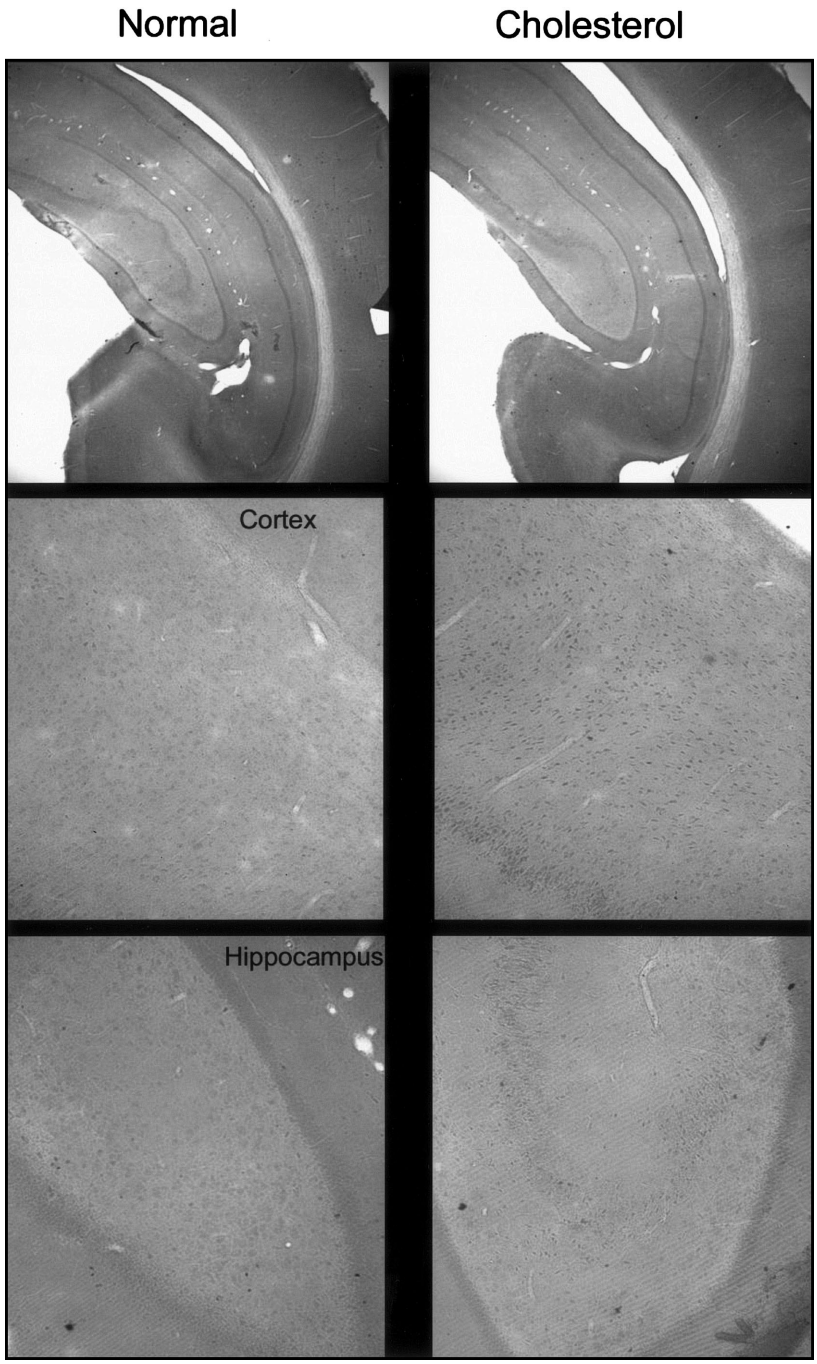

Figure 3. Beta amyloid labeling in the rabbit cortex and hippocampus. The left panels show low- and high-magnification representative sections of the rabbit cortex and hippocampus from a rabbit fed a normal diet (0% cholesterol), and the right panels show sections from a rabbit fed a diet containing 2% cholesterol.

induced differences by more commonly used measures of classical conditioning such as CR acquisition and extinction.

Experiment 2

Given the likelihood that the electrical US used in Experiment 1 was too intense to be sensitive to the effects of cholesterol on CR acquisition, we next used an airpuff US to repeat the essential features of Experiment 1. The use of airpuff avoided the potential involvement of fear-related structures such as the amygdala and ensured that the task was hippocampally dependent (Alkon et al., 1991; McEchron & Disterhoft, 1999; Moyer et al., 1990; Moyer et al., 1996). In addition, trace conditioning with airpuff as the US has been shown to be acquired more slowly than the acquisition rate shown by rabbits in Experiment 1 (Moyer et al., 1990) and so would allow any potential cholesterol-mediated differences in acquisition rate or level to emerge before an asymptotic level of

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

conditioning was acquired. However, we have found that the use of a weaker US may preclude detection of CRM (Buck et al., 2001; Seager et al., 2003).

Method

Unless otherwise noted, the methods were the same as those used in Experiment 1. The US in the present experiment was a 4-psi puff of air to the cornea. Following 8 weeks on their respective diets, 7 cholesterol-fed rabbits and 6 rabbits fed the normal control diet received a 1-day session of adaptation, one 60-trial session of airpuff US pretesting, 18 daily sessions of tone and airpuff trace conditioning, a 60-trial session of US posttesting, and 4 daily sessions of tone and airpuff delay conditioning.

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 posttest day, all of the subjects received a total of 60 trials of airpuff presented at an average ITI of 60 s (50–70 s range). Each trial involved the presentation of 1 of 15 possible combinations of stimulus intensity (0.5, 1.0, 2.0, 4.0, or 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 3 consecutive trials. Each of the 18 paired trace conditioning sessions consisted of 60 presentations of a 100-ms, 1-KHz, 82-dB tone CS that was followed by a 700-ms trace interval and then a 100-ms, 4-psi airpuff US (i.e., 800-ms ISI). Each of the 4 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 airpuff US (i.e., 300-ms ISI). Stimulus presentations were delivered, on average, every 60 s (50–70 s range). Corneal airpuff was computer controlled and delivered to the cornea through a 1-mm diameter tube. To facilitate the delivery of the airpuff US, we held the eyelids loosely with tailors hooks attached to an adjustable elastic strap (Buck et al., 2001).

Results

Figure 4 shows responding for rabbits in the cholesterol and normal diet control groups across 18 days of trace conditioning. The figure shows that CRs emerged slowly across the 18 days of pairings and that cholesterol-fed rabbits conditioned to higher average levels (50% CRs) than the normal diet control rabbits

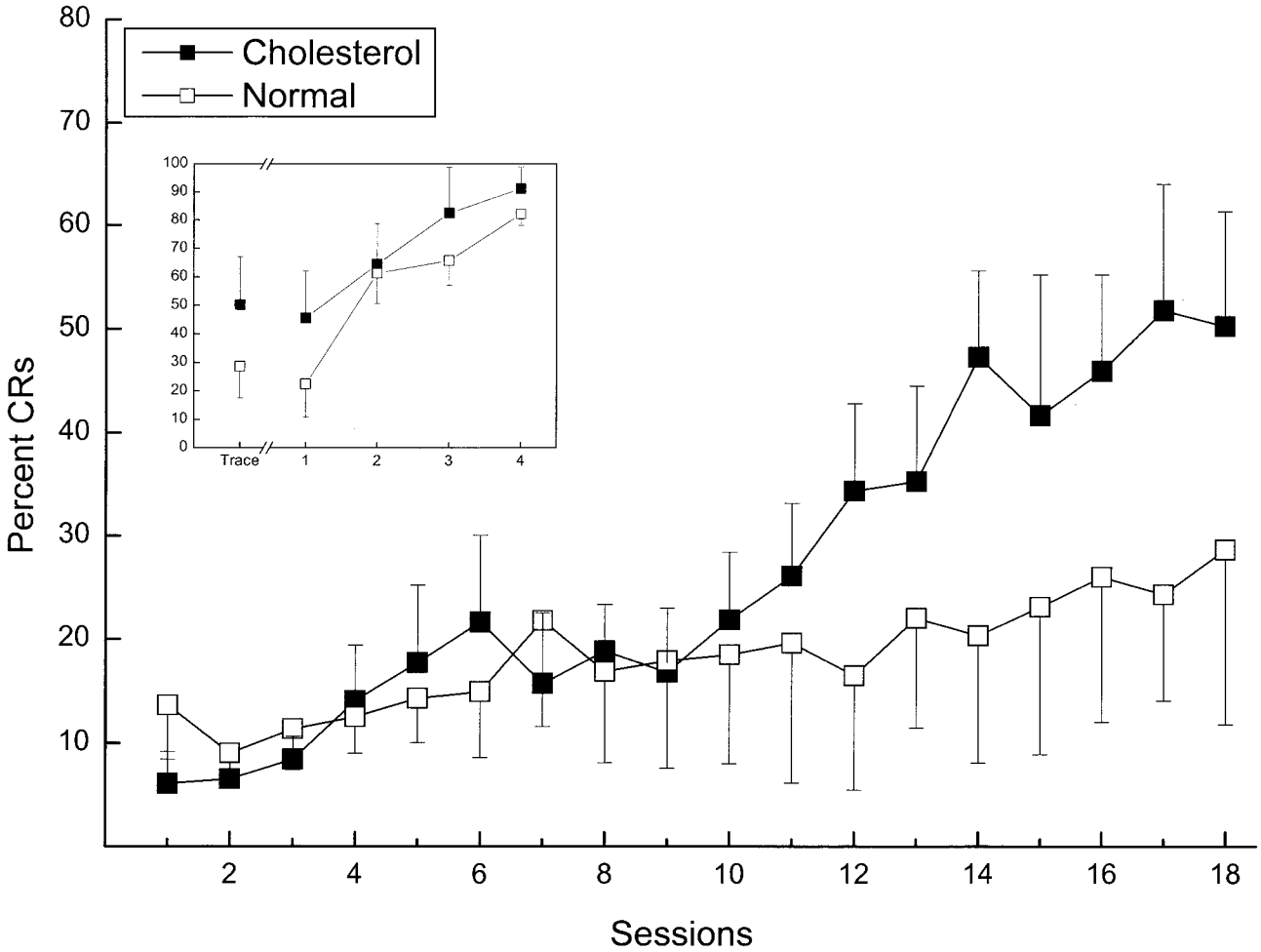

Figure 4. Effects of cholesterol on percent conditioned responses (CRs) to 700-ms trace conditioning across 18 daily sessions of 60 conditioned stimulus (CS)–unconditioned stimulus (US) pairings with a 100-ms, 1000-Hz, 82-dB tone followed by a 700-ms trace and a 100-ms, 4-psi airpuff. The inset shows percent CRs on the final day of trace conditioning and across four subsequent daily sessions of pairings with a 400-ms, 1000-Hz, 82-dB tone CS that coterminated with a 4-psi airpuff US.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

(25% CRs). Analysis of variance of percentage CRs across the sessions of trace conditioning revealed a significant main effect of sessions, $F(17, 187) = 7.33$, $p < .001$, and significant interaction of Diet × Sessions, $F(17, 187) = 2.31$, $p < .005$. Examination of responding by individual rabbits showed that only 2 of the 6 normal diet animals (33.3%) reached a criterion of 60% CRs (Thompson et al., 1996), whereas 5 of 7 cholesterol-fed rabbits (71.4%) reached a criterion of 60% CRs. The inset in Figure 4 shows averaged CRs on the final day of trace conditioning and across the 4 days of delay conditioning. The inset clearly indicates that all of the rabbits were able to acquire levels of responding approaching 90% CRs by the 4th day of delay conditioning, providing evidence that all of the rabbits were capable of learning the easier delay conditioning task. Statistical analysis of percentage CRs across delay conditioning yielded a highly significant main effect of sessions, $F(3, 30) = 26.4$, $p < .001$, but no significant main effect of diet, $F(1, 10) = 0.94$, $p > .35$, or Diet × Session interaction, $F(3, 30) = 1.00$, $p > .40$.

Statistical analysis of responses to airpuff on pretest showed that, with the exception of a difference in response area at 1.0 psi, $F(1, 11) = 11.54$, $p < .01$, there were no significant differences in response frequency, onset latency, amplitude, peak latency, or area between the URs of cholesterol-fed rabbits and those of normal diet control rabbits. Consequently, as noted in Experiment 1, the cholesterol diet did not have any overall effect on sensitivity to the US or performance of the UR. An analysis of responding to the airpuff US during US pretest by the subset of rabbits that showed significant levels of classical conditioning, that is, with CRs greater than 60% (Moyer et al., 1990), also did not yield any significant differences in response frequency, onset latency, amplitude, peak latency, or area between URs of cholesterol-fed rabbits and normal diet controls, corroborating that cholesterol did not have any effect on sensitivity to the US or performance of the UR on rabbits that subsequently conditioned. Consistent with our previous efforts to detect CRM using airpuff (Buck et al., 2001), a comparison of pretest and posttest responding for rabbits that did condition found a small number of differences that were indicative of CRM. Specifically, there was a significant increase in the frequency (66.7 ± 10.3% vs. 95.2 ± 4.8%), $F(1, 9) = 6.30$, $p < .05$, and area (19,692 ± 6,833 vs. 49,017 ± 8,063 arbitrary units), $F(1, 9) = 13.71$, $p < .01$, of URs for cholesterol-fed rabbits at 4.0 psi. There were no significant pretest–posttest differences in URs for the normal diet control rabbits that conditioned (Buck et al., 2001).

Discussion

Our data from the present experiment confirm that cholesterol has significant effects on learning and memory. Cholesterol-fed rabbits performed significantly better than normal diet controls during acquisition of a trace-conditioning paradigm using airpuff as the US—a task that has been shown to be dependent on the hippocampus (Alkon et al., 1991; McEchron & Disterhoft, 1999; Moyer et al., 1990; Moyer et al., 1996). In addition, the cholesterol-fed rabbits showed a modest level of CRM compared with normal diet controls (Buck et al., 2001), providing further support for the suggestion that cholesterol facilitated learning. Finally, as in Experiment 1 in which a ceiling effect occurred, the facilitative effects of cholesterol disappeared during delay conditioning when all the rabbits were able to learn the simpler delay task at comparable rates and to identical asymptotic levels.

General Discussion

Taken together, the present results indicate that a diet of 2% cholesterol can modify trace conditioning of the rabbit NMR. The evidence for this modification comes from a significant increase in the level of conditioning as a result of tone–airpuff trace conditioning and significantly enhanced conditioning-specific modification as a result of tone–electrical stimulation trace conditioning and more modest enhancement as a result of tone–airpuff trace conditioning.

We have already noted that cholesterol is crucial for the formation of new synapses, normal receptor function, and synaptic plasticity, and the present facilitative effects of a cholesterol diet are consistent with these findings (Goritz et al., 2002; Koudinov & Koudinova, 2001; Mauch et al., 2001; Sooksawate & Simmonds, 2001a, 2001b). New synapses are formed by neurons through glial-derived cholesterol that also increases the frequency of spontaneous synaptic activity by enhancing quantal size and efficiency of transmitter release (Goritz et al., 2002; Mauch et al., 2001). Cholesterol is critical to the function of membrane receptor proteins, including the nicotinic acetylcholine receptor, cholecystokinin receptor, and the gamma-aminobutryic acid ($GABA_A$) receptor (Sooksawate & Simmonds, 2001a), and can modulate the function of these receptors by changing membrane fluidity or binding directly with the protein to change its conformation (Burger, Gimpl, & Fahrenholz, 2000; Mauch et al., 2001). Cholesterol also seems to be important for synaptic plasticity because depletion of cholesterol in rat hippocampal slices impairs long-term potentiation (Koudinov & Koudinova, 2001). Thus, one implication of the present experiments is that the addition of cholesterol to the diet appears to facilitate learning through the effects of cholesterol on synaptic function and plasticity. Nevertheless, it should be noted that a long-term, high-cholesterol diet is extremely unhealthy, and rabbits on cholesterol for 8 weeks do become atherosclerotic and eventually die of heart disease (Finking & Hanke, 1997).

The present results, although consistent with evidence that elevating cholesterol in cholesterol-deficient rats and mice can improve learning (Miller & Wehner, 1994; O'Brien et al., 2000; O'Brien et al., 2002; Xu et al., 1998), seem to be at odds with results from rabbit experiments in which the calcium channel blocker nimodipine, that may reduce cholesterol, improved learning (Deyo et al., 1989; Kowalska & Disterhoft, 1994; Solomon et al., 1995; Woodruff-Pak et al., 1997). There are several potential reasons for this apparent discrepancy. Perhaps the most obvious is the fact that doses of nimodipine used in the rabbit classical conditioning experiments were lower than doses that act directly on cholesterol (Nayler, 1999). Second, the nimodipine studies used predominantly aged rabbits that show a considerable deficit in the rate and level of trace conditioning compared with the young adult rabbits used in the present study (Deyo et al., 1989; Kowalska & Disterhoft, 1994; Solomon et al., 1995; Woodruff-Pak et al., 1997). Third, nimodipine's site of activity at the doses used in the rabbit experiments appears to be centered at the hippocampal pyramidal cells in which excitability was increased (Moyer et al., 1992; Power et al., 2002). Taken together, the evidence suggests that in the rabbit classical conditioning experiments using aged rabbits, a low dose of nimodipine was acting centrally as a calcium

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

channel blocker in the hippocampus rather than more peripherally to reduce cholesterol levels.

The fact that the present experiments show a facilitative effect of cholesterol on learning does not seem to be consistent with a body of evidence documenting the significant accumulation of beta amyloid in the brain of cholesterol-fed rabbits (Sparks, 1997; Sparks et al., 1994; Sparks et al., 2000; Zatta, Zambenedetti, Stella, & Licastro, 2002). Moreover, experiments show that beta amyloid may act as an *N*-methyl-D-aspartate (NMDA) receptor agonist (Cowburn, Wiehager, Trief, Li-Li, & Sundstrom, 1997; Parks, Smith, Trimmer, Bennett, & Parker, 2001), and rabbit classical conditioning experiments suggest that an NMDA receptor agonist may facilitate learning (Thompson & Disterhoft, 1997). Despite the fact that we noted uniform, significant beta amyloid accumulation in the temporal cortex, there was more sporadic and variable accumulation of beta amyloid in the hippocampus—the structure required for trace conditioning using an airpuff US (Moyer et al., 1990; Thompson et al., 1996). In addition, although beta amyloid NMDA receptor activation may have mediated the present effects, the accumulation of beta amyloid was intracellular (Sparks, 1996, 1997), and there may not have been sufficient extracellular beta amyloid to activate the NMDA receptor directly.

In addition, recent evidence suggests that the level of beta amyloid accumulation in the rabbit brain may be influenced by dietary factors in addition to cholesterol and, in particular, the constituents of the drinking water (Sparks, Lochhead, Horstman, Wagoner, & Martin, 2002). Specifically, Sparks and colleagues have found that distilled water significantly reduces the level of beta amyloid deposition and plaque formation in the brain of cholesterol-fed rabbits. In contrast, tap water from certain regions, including Arizona and Kentucky, where the original cholesterol-beta amyloid connections were observed, increased the intensity and extent of beta amyloid accumulation (Sparks et al., 2002). An analysis of tap water in Morgantown, West Virginia (USFilter, Northbrook, IL), where the present experiments were conducted, indicated a large number of significant differences in tap water contents from those in Arizona and Kentucky, including concentrations of bicarbonate, chloride, copper, nitrate, silica, sulfate, and zinc. A number of factors including silica, nitrates, and sulfates (Basun, Forssell, Wetterberg, & Winblad, 1991; Bonay & Avila, 2001; Jacqmin-Gadda, Commenges, Letenneur, & Dartigues, 1996; Tohgi et al., 1998) as well as trace metals such as zinc, iron, and copper may play a significant role in Alzheimer's disease (Atwood et al., 2000; Bush & Tanzi, 2002; Curtain et al., 2001; Gonzalez et al., 1999; Lovell, Robertson, Teesdale, Campbell, & Markesbery, 1998; Perry et al., 2002). A comparison of the histological sections from rabbits given tap water or distilled water in Sun City, Arizona (Sparks et al., 2002) and those in the present experiment (Figure 5) suggests that the number and density of 10D5 immunoreactive neurons in rabbits given local tap water are comparable with the rabbits given distilled water in the study by Sparks et al. (2002). As a result, the facilitative effects on classical conditioning and conditioning-specific reflex modification may have had more to do with the cholesterol diet than with the accumulation of beta amyloid. Consequently, it may be the case that rabbits in the present experiments, although receiving a diet of 2% cholesterol, did not have the level of beta amyloid accumulation normally associated with such a cholesterol burden.

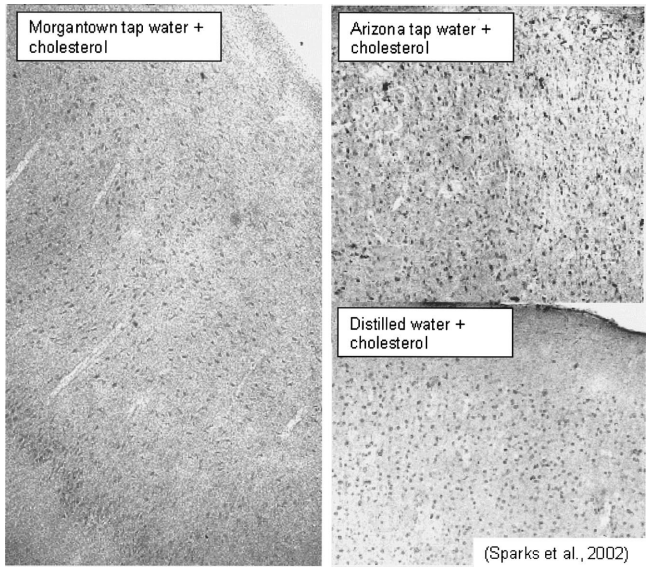

Figure 5. Comparison of histological sections from a cholesterol-fed rabbit given tap water from Morgantown, WV (left) and from Sun City, AZ (top right) and distilled water (bottom right). Note the comparable number and density of 10D5 immunoreactive neurons in sections from the cholesterol-fed rabbit given Morgantown tap water (left) and distilled water (bottom right). Bottom right panel adapted from "Water Quality Has a Pronounced Effect on Cholesterol-Induced Accumulation of Alzheimer amyloid (aβ) in Rabbit Brain," by D. L. Sparks, J. Lochead, D. Horstman, T. Wagoner, and T. Martin, 2002, *Journal of Alzheimer's Disease, 4,* p. 520. Copyright 2002 by IOS Press. Adapted with permission.

References

Alkon, D. L., Amaral, D. G., Bear, M. F., Black, J., Carew, T. J., Cohen, N. J., et al. (1991). Learning and memory. *Brain Research Reviews, 16,* 193–220.

Atwood, C. S., Scarpa, R. C., Huang, X., Moir, R. D., Jones, W. D., Fairlie, D. P., et al. (2000). Characterization of copper interactions with Alzheimer amyloid β peptides: Identification of an attomolar-affinity copper binding site on amyloid β1-42. *Journal of Neurochemistry, 75,* 1219–1233.

Atzmon, G., Gabriely, I., Griener, W., Davidson, D., Schechter, C., & Brazilai, N. (2002). Plasma HDL levels highly correlate with cognitive function in exceptional longevity. *Journal of Gerontology Series A, Biological Sciences and Medical Sciences, 57A,* M712–M715.

Basun, H., Forssell, L. G., Wetterberg, L., & Winblad, B. (1991). Metals and trace elements in plasma and cerebrospinal fluid in normal aging and Alzheimer's disease. *Journal of Neural Transmission: Parkinson's and Dementia Section, 3,* 231–258.

Bocan, T. M. A. (1998). Animal models of atherosclerosis and interpretation of drug intervention. *Current Pharmaceutical Design, 4,* 37–52.

Bonay, P., & Avila, J. (2001). Apolipoprotein E4 stimulates sulfation of glycosaminoglycans in neural cells. *Biochimica et Biophysica Acta, 1535,* 217–220.

Brousseau, M. E., & Hoeg, J. M. (1999). Transgenic rabbits as a model for atherosclerosis research. *Journal of Lipid Research, 40,* 365–375.

Buck, D. L., Seager, M. A., & Schreurs, B. G. (2001). Conditioning-specific reflex modification of the rabbit (*Oryctolagus cuniculus*) nictitating membrane response: Generality and nature of the phenomenon. *Behavioral Neuroscience, 115,* 1039–1047.

Burger, K., Gimpl, G., & Fahrenholz, F. (2000). Regulation of receptor function by cholesterol. *Cellular and Molecular Life Sciences, 57,* 1577–1592.

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

Bush, A. I., & Tanzi, R. E. (2002). The galvanization of β-amyloid in Alzheimer's disease. *Proceedings of the National Academy of Sciences, USA, 99,* 7317–7319.

Chen, C., Kim, J. J., Thompson, R. F., & Tonegawa, S. (1996). Hippocampal lesions impair contextual fear conditioning in two strains of mice. *Behavioral Neuroscience, 110,* 1177–1180.

Chen, P., Ghoneim, M. M., & Gormezano, I. (1992). Sodium pentobarbital: Sensory and associative effects in classical conditioning of the rabbit nictitating membrane response. *Psychopharmacology, 107,* 365–372.

Cowburn, R. F., Wiehager, B., Trief, E., Li-Li, M., & Sundstrom, E. (1997). Effects of β-amyloid-(25–35) peptides on radioligand binding to excitatory amino acid receptors and voltage-dependent calcium channels: Evidence for a selective affinity for the glutamate and glycine recognition sites of the NMDA receptor. *Neurochemical Research, 22,* 1437–1442.

Crestani, F., Keist, R., Fritschy, J.-M., Benke, D., Vogt, K., Prut, L., et al. (2002). Trace fear conditioning involves hippocampal α_5 $GABA_A$ receptors. *Proceedings of the National Academy of Sciences, USA, 99,* 8980–8985.

Curtain, C. C., Ali, F., Volitakis, I., Cherny, R. A., Norton, R. S., Beyreuther, K., et al. (2001). Alzheimer's disease amyloid-β binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. *Journal of Biological Chemistry, 276,* 20466–20473.

Deyo, R. A., Straube, K. T., & Disterhoft, J. F. (1989, February 10). Nimodipine facilitates associative learning in aging rabbits. *Science, 243,* 809–811.

Dietschy, J. M., & Turley, S. D. (2001). Cholesterol metabolism in the brain. *Current Opinion in Lipidology, 12,* 105–112.

Du, W., & Harvey, J. A. (1996). The nitric oxide synthesis inhibitor L-name facilitates associative learning. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 20,* 1183–1195.

Du, W., Weiss, H., & Harvey, J. A. (2000). Associative learning is enhanced by selective neuronal nitric oxide synthase inhibitors and retarded by a nitric oxide donor in the rabbit. *Psychopharmacology, 150,* 264–271.

Endo, Y., Nishimura, J.-I., & Kimura, F. (1996). Impairment of maze learning in rats following long-term glucocorticoid treatments. *Neuroscience Letters, 203,* 199–202.

Evans, R. M., Emsley, C. L., Gao, S., Sahota, A., Hall, K. S., Farlow, M. R., & Hendrie, H. (2000). Serum cholesterol, *APOE* genotype, and the risk of Alzheimer's disease: A population-based study of African Americans. *Neurology, 54,* 240–242.

Fan, J., & Watanabe, T. (2000). Cholesterol-fed and transgenic rabbit models for the study of atherosclerosis. *Journal of Atherosclerosis and Thrombosis, 7,* 26–32.

Finking, G., & Hanke, H. (1997). Nikolaj Nicolajewisch Anitschkow (1885–1964) established the cholesterol-fed rabbit as a model for atherosclerosis research. *Atherosclerosis, 135,* 1–7.

Gewirtz, J. C., McNish, K. A., & Davis, M. (2000). Is the hippocampus necessary for contextual fear conditioning? *Behavioural Brain Research, 110,* 83–95.

Gonzalez, C., Martin, T., Cacho, J., Brenas, M. T., Arroyo, T., Garcia-Berrocal, B., et al. (1999). Serum zinc, copper, insulin and lipids in Alzheimer's disease epsilon 4 apolipoprotein E allele carriers. *European Journal of Clinical Investigation, 29,* 637–642.

Goritz, C., Mauch, D. H., Nagler, K., & Pfrieger, F. W. (2002). Role of glial-derived cholesterol in synaptogenesis: New revelations in the synapse-glia affair. *Journal of Physiology (Paris), 96,* 257–263.

Gormezano, I. (1966). Classical conditioning. In J. B. Sidowski (Ed.), *Experimental methods and instrumentation in psychology* (pp. 385–420). New York: McGraw-Hill.

Gormezano, I. (1984). The study of associative learning with CS–CR paradigms. In D. L. Alkon (Ed.), *Primary neural substrates of learning and behavioral change* (pp. 5–24). New York: Cambridge University Press.

Gormezano, I. (1994). MDA effects on classical appetitive conditioning of the rabbit jaw movement response. *Brain Research Bulletin, 35,* 183–187.

Gormezano, I., & Kehoe, E. J. (1981). Classical conditioning and the law of contiguity. In P. Harzem (Ed.), *Predictability, correlation, and contiguity* (2nd ed., pp. 1–45). New York: Wiley.

Gormezano, I., Kehoe, E. J., & Marshall, B. S. (1983). Twenty years of classical conditioning research with the rabbit. In J. M. Sprague (Ed.), *Progress in psychobiology and physiological psychology* (10th ed., pp. 197–275). New York: Academic Press.

Gormezano, I., Schneiderman, N., Deaux, E. G., & Fuentes, I. (1962, October 5). Nictitating membrane: Classical conditioning and extinction in the albino rabbit. *Science, 138,* 33–34.

Gruart, A., & Yeo, C. H. (1995). Cerebellar cortex and eyeblink conditioning: Bilateral regulation of conditioned responses. *Experimental Brain Research, 104,* 431–448.

Hartmann, T. (2001). Cholesterol, Aβ and Alzheimer's disease. *Trends in Neurosciences, 11,* S45–S48.

Harvey, J. A., & Romano, A. G. (1993). Harmaline-induced impairment of Pavlovian conditioning in the rabbit. *Journal of Neuroscience, 13,* 1616–1623.

Jacqmin-Gadda, H., Commenges, D., Letenneur, L., & Dartigues, J.-F. (1996). Silica and aluminum in drinking water and cognitive impairment in the elderly. *Epidemiology, 7,* 281–285.

Jarvik, G. P., Wijsman, E. M., Kukull, W. A., Schellenberg, G. D., Yu, C., & Larson, E. B. (1995). Interactions of apolipoprotein E genotype, total cholesterol level, age, and sex in prediction of Alzheimer's disease. *Neurology, 45,* 1092–1096.

Kane, K. A., & Robinson, G. B. (1999). Effect of chronic nimodipine on spatial learning and on long-term potentiation. *Behavioural Brain Research, 98,* 95–101.

Kessler, A. R., Kessler, B., & Yehuda, S. (1986). In vivo modulation of brain cholesterol level and learning performance by a novel plant lipid: Indication of interactions between hippocampal-cortical cholesterol and learning. *Life Sciences, 38,* 1185–1192.

Kim, J. J., Clark, R. E., & Thompson, R. F. (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuroscience, 109,* 195–203.

Kivipelto, M., Helkala, E.-L., Hanninen, T., Laakso, M. P., Hallikainen, M., Alhainen, K., et al. (2001). Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study. *Neurology, 56,* 1683–1689.

Koudinov, A. R., & Koudinova, N. V. (2001). Essential role of cholesterol in synaptic plasticity and neuronal degeneration. *FASEB Journal, 15,* 1858–1860.

Kowalska, M., & Disterhoft, J. F. (1994). Relation of nimodipine dose and serum concentration to learning enhancement in aging rabbits. *Experimental Neurology, 127,* 159–166.

Kronforst-Collins, M. A., Moriearty, P. P. L., Ralph, M., Becker, R. E., Schmidt, B., Thompson, L. T., & Disterhoft, J. F. (1997). Metrifonate treatment enhances acquisition of eyeblink conditioning in aging rabbits. *Pharmacology Biochemistry and Behavior, 56,* 103–110.

Lovell, M. A., Robertson, J. D., Teesdale, W. J., Campbell, J. J., & Markesbery, W. R. (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. *Journal of Neurological Sciences, 158,* 47–52.

Maren, S., & Holt, W. (2000). The hippocampus and contextual memory retrieval in Pavlovian conditioning. *Behavioural Brain Research, 110,* 97–108.

Mauch, D. H., Nagler, K., Schumacher, S., Goritz, C., Muller, E.-C., Otto, A., & Pfrieger, F. W. (2001). CNS synaptogenesis promoted by glial-derived cholesterol. *Science, 294,* 1354–1357.

McEchron, M. D., Bouwmeester, H., Tseng, W., Weiss, C., & Disterhoft,

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

J. F. (1998). Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. *Hippocampus, 8,* 638–646.

McEchron, M. D., & Disterhoft, J. F. (1999). Hippocampal encoding of non-spatial trace conditioning. *Hippocampus, 9,* 385–396.

McEchron, M. D., McCabe, P. M., Green, E. J., Llabre, M. M., & Schneiderman, N. (1991). Air puff versus shock unconditioned stimuli in rabbit heart rate conditioning. *Physiology & Behavior, 51,* 195–199.

McEchron, M. D., Tseng, W., & Disterhoft, J. F. (2000). Neurotoxic lesions of the dorsal hippocampus disrupt auditory-cued trace heart rate (fear) conditioning in rabbits. *Hippocampus, 10,* 739–751.

Miller, S., & Wehner, J. M. (1994). Cholesterol treatment facilitates spatial learning performance in DBA/2Ibg mice. *Pharmacology Biochemistry and Behavior, 49,* 257–261.

Moon, Y., Ghoneim, M. M., & Gormezano, I. (1994). Nitrous oxide: Sensory, motor, associative, and behavioral tolerance effects in classical conditioning of the rabbit nictitating membrane response. *Pharmacology Biochemistry and Behavior, 47,* 523–529.

Moore, J. W., Goodall, N. A., & Solomon, P. R. (1976). Central cholinergic blockade by scopolamine and habituation, classical conditioning, and latent inhibition of the rabbit's nictitating membrane response. *Physiological Psychology, 4,* 395–399.

Moyer, J. R., Jr., Deyo, R. A., & Disterhoft, J. F. (1990). Hippocampectomy disrupts trace eye-blink conditioning in rabbits. *Behavioral Neuroscience, 104,* 243–252.

Moyer, J. R., Jr., Thompson, L. T., Black, J. P., & Disterhoft, J. F. (1992). Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age- and concentration-dependent manner. *Journal of Neurophysiology, 68,* 2100–2109.

Moyer, J. R., Jr., Thompson, L. T., & Disterhoft, J. F. (1996). Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. *Journal of Neuroscience, 16,* 5536–5546.

Muldoon, M. F., Ryan, C. M., Matthews, K. A., & Manuck, S. B. (1997). Serum cholesterol and intellectual performance. *Psychosomatic Medicine, 59,* 382–387.

Napier, R. M., Macrae, M., & Kehoe, E. J. (1992). Rapid reacquisition in conditioning of the rabbit's nictitating membrane response. *Journal of Experimental Psychology: Animal Behavior Processes, 18,* 182–192.

Näslund, J., Haroutunian, V., Mohs, R., Davis, K. L., Davies, P., Greengard, P., & Buxbaum, J. D. (2000). Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. *Journal of the American Medical Association, 283,* 1571–1577.

Nayler, W. G. (1999). Review of preclinical data of calcium channel blockers and atherosclerosis. *Journal of Cardiovascular Pharmacology, 33,* S7–S11.

Notkola, I.-L., Sulkava, R., Pekkanen, J., Erkinjunti, T., Ehnholm, C., Kivinen, P., et al. (1998). Serum total cholesterol, apolipoprotein Eε4 allele, and Alzheimer's disease. *Neuroepidemiology, 17,* 14–20.

O'Brien, W. T., Xu, G., Batta, A., Tint, G. S., Salen, G., Dyer, C. A., et al. (2002). Developmental sensitivity of associative learning to cholesterol synthesis inhibitors. *Behavioural Brain Research, 129,* 141–152.

O'Brien, W. T., Xu, G., Tint, G. S., Salen, G., & Servatius, R. J. (2000). Blocking cholesterol synthesis impairs acquisition of the classically conditioned eyeblink response. *Integrative Physiological and Behavioral Science, 35,* 120–131.

Oh, M. M., Power, J. M., Thompson, L. T., Moriearty, P. L., & Disterhoft, J. F. (1999). Metrifonate increases neuronal excitability in CA1 pyramidal neurons from both young and aging rabbit hippocampus. *Journal of Neuroscience, 19,* 1814–1823.

Parks, J. K., Smith, T. S., Trimmer, P. A., Bennett, J. P., Jr., & Parker, W. D., Jr. (2001). Neurotoxic Aβ peptides increase oxidative stress in vivo through NMDA-receptor and nitric-oxide-synthase mechanisms, and inhibit complex IV activity and induce a mitochondrial permeability transition in vitro. *Journal of Neurochemistry, 76,* 1050–1056.

Perry, G., Sayre, L. M., Atwood, C. S., Castellani, R. J., Cash, A. D., Rottkamp, C. A., & Smith, M. A. (2002). The role of iron and copper in the aetiology of neurodegenerative disorders. *CNS Drugs, 16,* 339–352.

Port, R. L., Mikhail, A. A., & Patterson, M. M. (1985). Differential effects of hippocampectomy on classically conditioned rabbit nictitating membrane response related to interstimulus interval. *Behavioral Neuroscience, 99,* 200–208.

Port, R. L., Romano, A. G., Steinmetz, J. E., Mikhail, A. A., & Patterson, M. M. (1986). Retention and acquisition of classical trace conditioned responses by rabbits with hippocampal lesions. *Behavioral Neuroscience, 100,* 745–752.

Power, J. M., Wu, W. W., Sametsky, E., Oh, M. M., & Disterhoft, J. F. (2002). Age-related enhancement of the slow outward calcium-activated potassium current in hippocampal CA1 pyramidal neurons in vitro. *Journal of Neuroscience, 22,* 7234–7243.

Quartermain, D. (2000). Chronic administration of the Ca^{2+} channel blocker amlodipine facilitates learning and memory in mice. *European Journal of Pharmacology, 399,* 57–63.

Reitan, R. M., & Shipley, R. E. (1963). The relationship of serum cholesterol changes to psychological abilities. *Journal of Gerontology, 18,* 350–357.

Schachter, M. (1997). Calcium antagonists and atherosclerosis. *International Journal of Cardiology, 62,* S9–S15.

Schindler, C. W., Gormezano, I., & Harvey, J. A. (1983). Effect of morphine on acquisition of the classically conditioned nictitating membrane response of the rabbit. *Journal of Pharmacology and Experimental Therapeutics, 227,* 639–643.

Schindler, C. W., Gormezano, I., & Harvey, J. A. (1984). Sensory and associative effects of morphine and naloxone in classical conditioning of the rabbit nictitating membrane response. *Psychopharmacology, 83,* 114–121.

Schreurs, B. G., & Alkon, D. L. (1990). US–US conditioning of the rabbit's nictitating membrane response: Emergence of a conditioned response without alpha conditioning. *Psychobiology, 18,* 312–320.

Schreurs, B. G., Oh, M. M., Hirashima, C., & Alkon, D. L. (1995). Conditioning-specific modification of the rabbit's unconditioned nictitating membrane response. *Behavioral Neuroscience, 109,* 24–33.

Schreurs, B. G., Shi, T., Pineda, S. I., & Buck, D. L. (2000). Conditioning the unconditioned response: Modification of the rabbit's (*Oryctolagus cuniculus*) unconditioned nictitating membrane response. *Journal of Experimental Psychology: Animal Behavior Processes, 26,* 144–156.

Seager, M. A., Asaka, Y., & Berry, S. D. (1999). Scopolamine disruption of behavioral and hippocampal responses in appetitive trace classical conditioning. *Behavioural Brain Research, 100,* 143–151.

Seager, M. A., Smith-Bell, C. A., & Schreurs, B. G. (2003). Conditioning-specific reflex modification of the rabbit (*Oryctolagus cuniculus*) nictitating membrane response: US intensity effects. *Learning & Behavior, 31,* 292–298.

Servatius, R. J. (2000). Eyeblink conditioning in the freely moving rat: Square-wave stimulation as the unconditioned stimulus. *Journal of Neuroscience Methods, 102,* 35–42.

Simons, M., Keller, P., Dichgans, J., & Schulz, J. B. (2001). Cholesterol and Alzheimer's disease: Is there a link? *Neurology, 57,* 1089–1093.

Solomon, P. R., Vander Schaaf, E. R., Thompson, R. F., & Weisz, D. J. (1986). Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. *Behavioral Neuroscience, 100,* 729–744.

Solomon, P. R., Wood, M. S., Groccia-Ellison, M. E., Yang, B. Y., Fanelli, R., & Mervis, R. F. (1995). Nimodipine facilitates retention of the classically conditioned nictitating membrane response in aged rabbits over long retention intervals. *Neurobiology of Aging, 16,* 791–796.

Sooksawate, T., & Simmonds, M. A. (2001a). Effects of membrane cholesterol on the sensitivity of the $GABA_A$ receptor to GABA in acutely dissociated rat hippocampal neurones. *Neuropharmacology, 40,* 178–184.

Sooksawate, T., & Simmonds, M. A. (2001b). Influence of membrane

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.

cholesterol on modulation of the $GABA_A$ receptor by neuroactive steroids and other potentiators. *British Journal of Pharmacology, 134,* 1303–1311.

Sparks, D. L. (1996). Intraneuronal β-amyloid immunoreactivity in the CNS. *Neurobiology of Aging, 17,* 291–299.

Sparks, D. L. (1997). Dietary cholesterol induces Alzheimer-like β-amyloid immunoreactivity in rabbit brain. *Nutrition, Metabolism and Cardiovascular Diseases, 7,* 255–266.

Sparks, D. L., Kuo, Y.-M., Roher, A. E., & Martin, T. A. (2000). Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation: Preliminary observations. In R. N. Kalaria & P. Ince (Eds.), *Annals of the New York Academy of Sciences: Vol. 903. Vascular factors in Alzheimer's disease* (pp. 335–344). New York: New York Academy of Sciences.

Sparks, D. L., Lochhead, J., Horstman, D., Wagoner, T., & Martin, T. (2002). Water quality has a pronounced effect on cholesterol-induced accumulation of Alzheimer amyloid (aβ) in rabbit brain. *Journal of Alzheimer's Disease, 4,* 519–525.

Sparks, D. L., Scheff, S. W., Hunsaker, J. C., III, Liu, H., Landers, T., & Gross, D. R. (1994). Induction of Alzheimer-like β-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Experimental Neurology, 126,* 88–94.

Stewart, R., Russ, C., Richards, M., Brayne, C., Lovestone, S., & Mann, A. (2001). Apolipoprotein E genotype, vascular risk and early cognitive impairment in an African Caribbean population. *Dementia Geriatric Cognitive Disorders, 12,* 251–256.

Tapp, W., Servatius, R., Hunt, J., & Powell, D. A. (1997). Vagal activity predicts eyeblink conditioning in human subjects. *NeuroReport, 8,* 1203–1207.

Teunissen, C. E., de Vente, J., von Bergmann, K., Bosma, H., Van Boxtel, M. P. J., De Bruijn, C., et al. (2003). Serum cholesterol, precursors and metabolites and cognitive performance in an aging population. *Neurobiology of Aging, 24,* 147–155.

Thompson, L. T., & Disterhoft, J. F. (1997). Age- and dose-dependent facilitation of associative eyeblink conditioning by D-cycloserine in rabbits. *Behavioral Neuroscience, 111,* 1303–1312.

Thompson, L. T., Moyer, J. R. J., & Disterhoft, J. F. (1996). Trace eyeblink conditioning in rabbits demonstrates heterogeneity of learning ability both between and within age groups. *Neurobiology of Aging, 17,* 619–629.

Tohgi, H., Abe, T., Yamazaki, K., Murata, T., Isobe, C., & Ishizaki, E. (1998). The cerebrospinal fluid oxidized NO metabolites, nitrite and nitrate, in Alzheimer's disease and vascular dementia of Binswanger type and multiple small infarct type. *Journal of Neural Transmission, 105,* 1283–1291.

Upchurch, M., & Wehner, J. M. (1988). DBA/2Ibg mice are incapable of cholinergically-based learning in the Morris water maze. *Pharmacology Biochemistry and Behavior, 29,* 325–329.

van Exel, E., de Craen, A. J. M., Gussekloo, J., Houx, P., Bootsma-van der Weil, A., Macfarlane, P. W., et al. (2002). Association between high-density lipoprotein and cognitive impairment in the oldest old. *Annals of Neurology, 51,* 716–721.

Voikar, V., Rauvala, H., & Ikonen, E. (2002). Cognitive deficit and development of motor impairment in a mouse model of Niemann-Pick Type C disease. *Behavioural Brain Research, 132,* 1–10.

Weiss, C., Preston, A. R., Oh, M. M., Schwarz, R. D., Welty, D., & Disterhoft, J. F. (2000). The M1 muscarinic agonist Cl-1017 facilitates trace eyeblink conditioning in aging rabbits and increases the excitability of CA1 pyramidal cells. *Journal of Neuroscience, 20,* 783–790.

Welsh, S. E., Romano, A. G., & Harvey, J. A. (1998). Effects of serotonin 5-HT2A/2C antagonists on associative learning in the rabbit. *Psychopharmacology, 137,* 157–163.

Wikgren, J., Ruusuvirta, T., & Korhonen, T. (2002). Reflex facilitation during eyeblink conditioning and subsequent interpositus nucleus inactivation in the rabbit (*Oryctolagus cuniculus*). *Behavioral Neuroscience, 116,* 1052–1058.

Woodruff-Pak, D. S. (1997). Nefiracetam ameliorates learning deficits in older rabbits and may act via the hippocampus. *Behavioural Brain Research, 83,* 179–184.

Woodruff-Pak, D. S., Chi, J., Li, Y.-T., Pak, M. H., & Fanelli, R. J. (1997). Nimodipine ameliorates impaired eyeblink classical conditioning in order rabbits in the long-delay paradigm. *Neurobiology of Aging, 18,* 641–649.

Woodruff-Pak, D. S., & Santos, I. S. (2000). Nicotinic modulation in an animal model of a form of associative learning impaired in Alzheimer's disease. *Behavioural Brain Research, 113,* 11–19.

Xu, G., Servatius, R. J., Shefer, S., Tint, G. S., O'Brien, W. T., Batta, A. K., & Salen, G. (1998). Relationship between abnormal cholesterol synthesis and retarded learning in rats. *Metabolism, 47,* 878–882.

Yaffe, K., Barret-Connor, E., Lin, F., & Grady, D. (2002). Serum lipoprotein levels, statin use, and cognitive function in older women. *Archives of Neurology, 59,* 378–384.

Yehuda, S., & Carasso, R. L. (1993). Modulation of learning, pain thresholds, and thermoregulation in the rat by preparations of free purified α-linolenic and linoleic acids: Determination of the optimal ω3-to-ω6 ratio. *Proceedings of the National Academy of Sciences, USA, 90,* 10345–10349.

Yehuda, S., Rabinovitz, S., & Motofsky, D. I. (1998). Modulation of learning and neuronal membrane composition in the rat by essential fatty acid preparation: Time-course analysis. *Neurochemical Research, 23,* 627–634.

Zatta, P., Zambenedetti, P., Stella, M. P., & Licastro, F. (2002). Astrocytosis, microgliosis, metallothionein-I-II and amyloid expression in high cholesterol-fed rabbits. *Journal of Alzheimer's Disease, 4,* 1–9.

Received April 8, 2003
Revision received June 17, 2003
Accepted June 25, 2003 ■

This document is copyrighted by the American Psychological Association or one of its allied publishers.
This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.