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Research Report

Cholesterol-enriched diet affects spatial learning and synaptic function in hippocampal synapses

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Abbreviations:
AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
CA1, cornu ammonis 1
CD, cholesterol-enriched diet
DNQX, 6,7-dinitroquinoxaline-2, 3-dione
EPSC, excitatory postsynaptic current
GABA, gamma aminobutyric acid
I_{AMPA}, AMPA-receptor mediated current
I_{NMDA}, NMDA-receptor mediated current
IPSC, inhibitory postsynaptic current
LDL, low-density lipoproteins
NMDA, *N*-methyl-D-aspartate
PPD, paired-pulse depression
PPF, paired-pulse facilitation
RD, regular diet

ABSTRACT

The aim of the present study was to determine the effect of a cholesterol-rich diet on learning performance and monitor possible related changes in synaptic function. To this purpose, we compared controls with rats fed with a cholesterol-enriched diet (CD). By using a Morris water-maze paradigm, we found that CD rats learned a water-maze task more quickly than rats fed with a regular diet (RD). A longer period of this diet tended to alter the retention of memory without affecting the improvement in the acquisition of the task. Because of the importance of the hippocampus in spatial learning, we hypothesized that these behavioral effects of cholesterol would involve synaptic changes at the hippocampal level. We used whole-cell patch-clamp recording in the CA1 area of a hippocampal rat slice preparation to test the influence of the CD on pre- and postsynaptic function. CD rats displayed an increase in paired-pulse ratio in both glutamatergic synapses (+48 ± 9%) and GABAergic synapses (+41 ± 8%), suggesting that the CD induces long-lasting changes in presynaptic function. Furthermore, by recording NMDA-receptor-mediated currents (I_{NMDA}) and AMPA-receptor-mediated currents (I_{AMPA}) in the same set of cells we found that CD rats display a lower I_{NMDA}/I_{AMPA} ratio (I_{NMDA}/I_{AMPA} = 0.75 ± 0.32 in RD versus 0.10 ± 0.03 in CD), demonstrating that cholesterol regulates also postsynaptic function. We conclude that a cholesterol-rich diet affects learning speed and performance, and that these behavioral changes occur together with robust, long-lasting, synaptic changes at both the pre- and postsynaptic level.

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1. Introduction

The physiological role of cholesterol in the brain is still unclear. Recent studies have implicated cholesterol in synaptic plasticity and learning, but its physiological relevance in this context is still enigmatic. Both local synthesis and external transport contribute to enrich the brain pool of cholesterol: astrocytes synthesize cholesterol (Dietschy and Turley, 2001) and deliver it to neurons via apoE-dependent transporters, such as the low-density lipoproteins (LDL) (Poirier et al., 1995; Herz and Beffert, 2000), whereas peripheral cholesterol may also be transferred to astrocytes via LDL transport across the blood–brain barrier (Dietschy and Turley, 2001; Dehouck et al., 1997). Cholesterol is involved in multiple functions in the central nervous system: neurons use exogenous cholesterol to assemble the exocytosis apparatus (Pfrieger, 2003), while they use cholesterol provided by astrocytes as glial factor promoting synaptogenesis (Mauch et al., 2001). These and other studies suggest that a cholesterol-enriched diet may elicit a number of changes in synaptic plasticity (Boleman et al., 1998; Koudinov and Koudinova, 2001a; Malavolti et al., 1991; Schoknecht et al., 1994; Sparks et al., 2000) and memory encoding and storage (Martin et al., 2000).

In spite of this wealth of data, investigations on the role of cholesterol in learning and memory did not focus on the association between behavioral and cellular changes induced by a cholesterol-rich diet (Teunissen et al., 2003; Yaffe et al., 2002; Voikar et al., 2002; Kessler et al., 1986; Wagstaff et al., 2003; Miller and Wehner, 1994; O'Brien et al., 2002). In the present study, we wanted to determine whether an

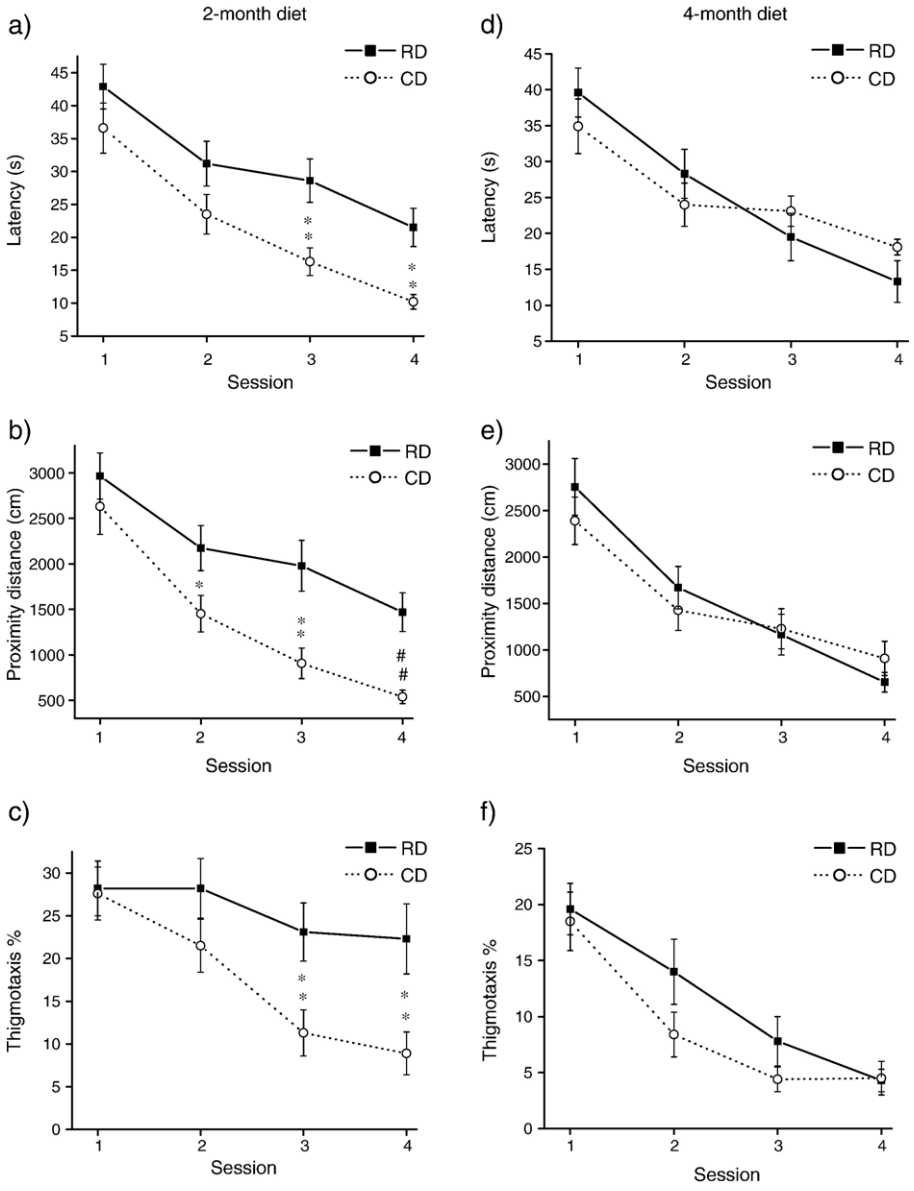


Fig. 1 – Effect of cholesterol diet on water-maze training performances. Rats were fed with a regular (RD) or high-cholesterol (CD) diet for 2 (a – c) and 4 months (d – f), and then trained to solve a water-maze task. Latency (a, d), proximity distance (b, e) and thigmotaxis (c, f) were compared between RD and CD animals for each training session ($n = 8$, $P < 0.01$).

administration of exogenous cholesterol affecting learning could also affect synaptic function. We found that a cholesterol-rich diet induced large changes in basic physiological synaptic properties as well as in the capacity to solve a learning paradigm using the Morris water-maze (D'hooge and De Deyn, 2001; Morris, 1984).

2. Results

2.1. *Influence of the diet on the animal weight and cholesterol concentration in blood and brain*

No difference in weight was noted between RD rats and CD rats after 2 or 4 months of diet (456.2 ± 10.3 g versus 459.4 ± 13.2 g, and 572.5 ± 18.5 g versus 572.5 ± 16.1 g, respectively, average ± standard error). In the mean time, the blood concentration of cholesterol was almost doubled by a 2-month or 4-month diet enriched with cholesterol, compared to a regular diet: 1.11 ± 0.14 g/L and 1.14 ± 0.06 g/L, compared to and 0.66 ± 0.04 g/L and 0.59 ± 0.03 g/L, respectively ($P < 0.01$). After 2 months of diet, the hippocampal cholesterol concentration was higher ($P < 0.01$) in CD (109.4 ± 0.1 ng/μg proteins) than in RD (85.8 ± 0.1 ng/μg proteins). Hippocampal cholesterol concentrations were comparable in both RD and CD rats (116.7 ± 0.1 ng/μg versus 115.6 ± 0.1 ng/μg) fed for 4 months.

2.2. *Effect of a 2-month cholesterol diet on water-maze learning*

2.2.1. *Training*

A 2 (groups) × 4 (sessions) analysis of escape latencies variance (ANOVA) with repeated measures of the second factor revealed a significant effect of groups ($F_{(1,14)} = 6.175$, $P = 0.026$), days ($F_{(3,42)} = 24.39$, $P < 0.0001$) and trials ($F_{(3,42)} = 10.74$, $P < 0.0001$). RD latencies were significantly higher than CD at session 3 and 4 (respectively, $P = 0.010$, $P = 0.001$, Fisher's post hoc test; Fig. 1a).

Similar analysis of proximity distance showed a significant effect of groups ($F_{(1,14)} = 5.78$, $P = 0.031$), days ($F_{(3,42)} = 29.41$, $P < 0.0001$) and trials ($F_{(3,42)} = 13.37$, $P < 0.0001$). An additional Fisher's post hoc test showed a significant lower CD proximity distance for sessions 2, 3 and 4, respectively ($P = 0.027$, $P = 0.002$, $P < 0.0001$; Fig. 1b).

A thigmotaxis nonsignificant effect of groups ($F_{(1,14)} = 3.138$, $P > 0.09$), significant effect of days ($F_{(3,42)} = 7.48$, $P = 0.0004$) and trials ($F_{(3,42)} = 4.772$, $P = 0.006$) was measured. RD thigmotaxis was significantly higher on days 3 and 4 (respectively, $P = 0.009$, $P = 0.006$, Fisher's post hoc test; Fig. 1c). In CD rats, escape latency and thigmotaxis were significantly correlated ($R = 0.97$, $P = 0.028$). This correlation was not observed with RD rat performances ($R = 0.80$, $P = 0.19$).

2.2.2. *Probe test*

On day 3, CD animals spent more time in the target area than the RD rats (Table 1). Also, CD rats crossed the platform location more often than the RD rats (Table 1). No significant difference was found between groups with speed (17.0 ± 2.2 versus 18.0 ± 2.1 cm/s) nor proximity distance (Table 1). Rats did not show any preference for the target quadrant on day 3 (Fig. 2a).

On day 5, a clear quadrant effect was measured for swimming distance in both RD ($F_{(3,24)} = 4.83$, $P = 0.009$) and CD ($F_{(3,24)} = 10.80$, $P = 0.0001$) rats. Fisher's post hoc comparison revealed that in both groups, rats had a preference for the target quadrant (Fig. 2b, $P < 0.02$ for all quadrants except quadrant 3, RD, where $P = 0.057$). A more stringent analysis of the rats' bias showed that CD animals spent more time in the target area than RD rats (Table 1). The proximity distance was lower with CD than RD rats (Table 1), and no significant difference was found between groups concerning speed (18.7 ± 1.7 versus 19.7 ± 1.5 cm/s) or target crossings (Table 1).

2.3. *Effect of a 4-month cholesterol diet on water-maze learning*

2.3.1. *Training*

A 2 (groups) × 4 (sessions) analysis of escape latencies variance (ANOVA) with repeated measures of the second factor revealed a significant effect of days ($F_{(3,42)} = 16.77$, $P < 0.0001$) and trials ($F_{(3,42)} = 11.34$, $P < 0.0001$). There was no difference between RD and CD (Fig. 1d).

Similarly, analysis of proximity distance showed an effect of days ($F_{(3,42)} = 33.92$, $P < 0.0001$) and trials ($F_{(3,42)} = 15.72$, $P < 0.0001$), but no effect of groups (Fig. 1e).

A similar effect of days ($F_{(3,42)} = 24.72$, $P < 0.0001$) and trials ($F_{(3,42)} = 26.30$, $P < 0.0001$) was measured on thigmotaxis, but no difference was noted between RD and CD rats (Fig. 1f).

Finally, escape latency and thigmotaxis were significantly correlated in RD and CD rats ($R = 0.99$ $P = 0.003$, $R = 0.95$ $P = 0.045$, respectively).

Table 1 – Effect of a 2-month cholesterol diet on water-maze spatial bias

	2-month diet			
	2 sessions		4 sessions	
	RD	CD	RD	CD
% Target area	4.0 ± 1.7	14.3 ± 3.0**	8.2 ± 2.9	22.6 ± 2.7**
Target crossings	1.0 ± 0.3	2.9 ± 0.5**	2.0 ± 0.6	3.0 ± 0.4
Proximity distance (cm)	2220.6 ± 392.1	1798.7 ± 390.5	2013.6 ± 125.9	1440.0 ± 193.3**

Rats were fed for 2 months with a regular diet (RD) or a cholesterol-enriched diet (CD). They were train to solve a water-maze task, then challenged in a probe test after 2 and 4 training sessions. Their spatial preference was compared by measuring the % of time spent in a designed target area, the number of target crossings and the proximity to the target ($n = 8$, **$P < 0.01$).

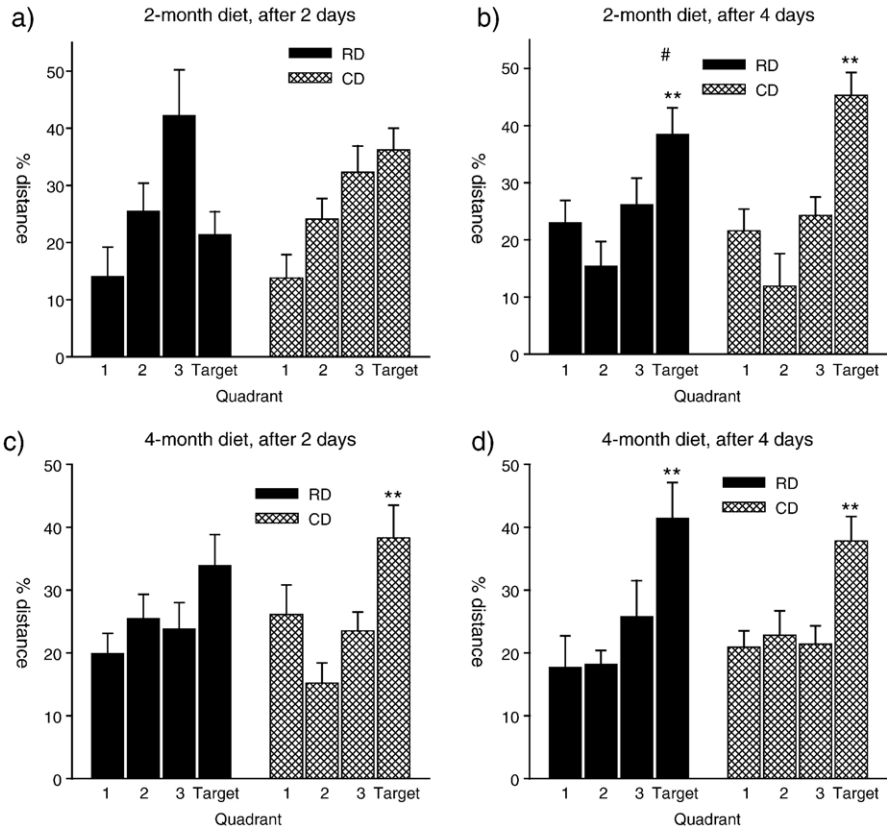


Fig. 2 – Effect of cholesterol diet on quadrant test performances. Rats were fed with a regular (RD) or high-cholesterol (CD) diet for 2 (a, b) and 4 months (c, d) and then trained to solve a water-maze task. The two asterisks (**) indicate that the target quadrant differs from the others with $P < 0.02$. (b) # indicates that the target quadrant differs from quadrant 3 with $P = 0.058$. The spatial bias was assessed by a quadrant test after 2 (a, c) or 4 (b, d) days of training ($n = 8$).

2.3.2. *Probe test*

On day 3, CD rats showed a quadrant effect ($F_{(3,28)} = 5.34$, $P = 0.005$) with a significant preference for the target quadrant ($P = 0.045$, $P = 0.001$, $P = 0.017$) (Fig. 2c). RD rats did not show any quadrant preference ($F_{(3,28)} = 2.076$, $P = 0.126$; Fig. 2c. The percentage of time spent by CD rats in the target area was significantly higher than the RD animals (Table 2). Also, CD rats crossed the target position more often than RD rats did (Table 2). No significant difference was found between groups with speed (21.6 ± 2.9 versus 23.9 ± 2.1 cm/s). Proximity distance tended to be lower than within RD rats, but the difference was not statistically significant (Table 2).

On day 5, a clear quadrant effect for swimming distance was shown in both RD ($F_{(3,28)} = 5.12$, $P = 0.006$) and CD ($F_{(3,28)} = 5.75$, $P = 0.003$) rats. In both groups, Fisher's post hoc comparison revealed a clear bias for the target quadrant (Fig. 2d). No difference between groups was found with speed (21.0 ± 1.8 versus 24.4 ± 1.9 cm/s), target crossings and proximity distance (Table 2). A high tendency to spend more time in the target area has been noticed with RD compared to CD diet, but this was not statistically significant (Table 2).

Table 2 – Effect of a 4-month cholesterol diet on water-maze spatial bias

	4-month diet			
	2 sessions		4 sessions	
	RD	CD	RD	CD
% Target area	6.9 ± 2.6	15.5 ± 1.4*	20.5 ± 4.4	14.9 ± 3.6
Target crossings	1.2 ± 0.2	3.1 ± 0.5**	2.6 ± 0.5	2.9 ± 0.4
Proximity distance (cm)	1877.4 ± 105.3	1572.0 ± 104.3	1482.6 ± 118.9	1652.5 ± 126.9

Rats were fed for 4 months with a regular diet (RD) or a cholesterol-enriched diet (CD). They were train to solve a water-maze task, then challenged in a probe test after 2 and 4 training sessions. Their spatial preference was compared by measuring the % of time spent in a designed target area, the number of target crossings, and the proximity to the target ($n = 8$, *$P < 0.05$, **$P < 0.01$).

2.4. *Effect of age on cholesterol concentrations and learning skills*

The concentration of cholesterol in the hippocampus was significantly higher in 5-month-old RD with respect to 3-month-old rats (116.7 ± 0.1 versus 85.8 ± 0.1 ng/μg proteins, respectively, $P < 0.01$). The blood concentration of cholesterol in CD rats did not change with the age of the animals.

We compared learning patterns in relation to the age of the animals. Applying repeated measures of ANOVA, we found that the proximity distance was significantly lower for RD 5-month-old rats than RD 3-month-old ones on sessions 3 ($P = 0.025$) and 4 ($P = 0.001$). No age-dependent difference was revealed between CD groups.

Performances during probe tests were also compared at different ages. No difference was found in the day 3 test. On the other hand, on the day 5 test, the proximity distance was significantly reduced in 5-month-old RD rats compared to 3-month-old ones ($P = 0.008$). Moreover, RD 5-month-olds spent more time in the target area than the younger animals ($P = 0.035$). On the contrary, CD rats showed a tendency to decrease their dwell time in the target area with age ($P = 0.061$). No other difference related to age was observed. The results from this and the previous section indicate that the presence of cholesterol induced a change in the behavioral responses of the animals, suggesting the possibility that a cholesterol-rich diet alters neuronal excitability. We used patch-clamp recording to investigate the presence of differences in synaptic function in RD versus CD animals.

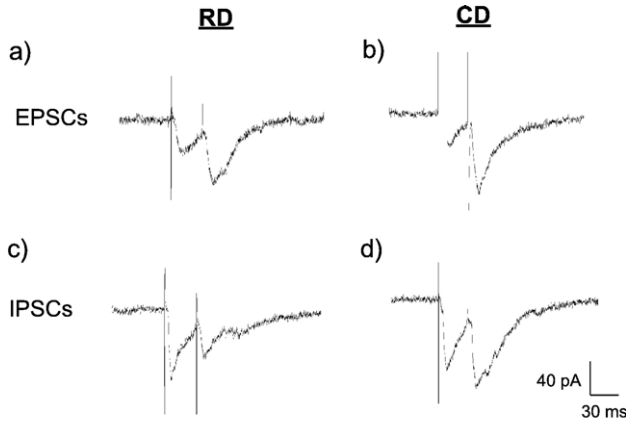
2.5. *Modulation of short-term synaptic plasticity by a cholesterol diet*

Because a prominent effect of cholesterol is to alter mobility and physicochemical properties of lipid bilayer of neurotransmitter vesicles, we first investigated the possibility that cholesterol affected presynaptic function.

EPSCs and IPSCs induced with a paired-pulse protocol were recorded in hippocampus slices from 3-month-old naive (i.e., without training) RD and CD rats (6 each). Fig. 3 shows representatives traces of EPSCs (a and b) and IPSCs (c and d), in RD (a and c) and CD (b and d) rat slices. This protocol induced facilitation of EPSCs in both RD and CD rats (Figs. 3a and b). The average of the first pulse response was similar in the two groups, but the amplitude of the EPSCs second response was larger in CD ($n = 8$) than in RD animals ($n = 7$; Fig. 3e). Paired-pulse depression of IPSCs was detected in the RD hippocampus ($n = 6$), whereas the cholesterol diet induced IPSCs facilitation ($n = 7$, Figs. 3c and d). Similar to the previous experiment, although the first pulse response was similar between both groups, the amplitude of the second response was larger in CD animals with respect to RD animals (Fig. 3e). These data confirmed our hypothesis suggesting an involvement of cholesterol in the regulation of presynaptic function.

2.6. *Modulation of the glutamatergic currents in CA1 pyramidal cells by a cholesterol diet*

A change in the mobility of the lipid bilayer has the potential to affect postsynaptic receptor trafficking and assembly.



e)

		A 1 (pA)	A 2 (pA)	Ratio
EPSCs	RD	32.3 ± 2.9	56.6 ± 4.7	1.86 ± 0.09
	CD	34.9 ± 4.1	72.6 ± 7.4*	2.32 ± 0.09*
IPSCs	RD	86.9 ± 9.1	58.7 ± 5.8	0.83 ± 0.09
	CD	81.1 ± 7.4	91.9 ± 8.8*	1.24 ± 0.07*

Fig. 3 – Effect of cholesterol diet on short-term synaptic plasticity in CA1 neurons. A paired-pulse stimulation of the Schaffer collateral pathway induced a higher facilitation of EPSCs in cholesterol-fed animals (CD) (b) compared with rats fed with a regular rodent diet (RD) (a). The same stimulation of the stratum pyramidale layer induced a depression of the IPSCs in RD rats (c) and a facilitation of IPSCs in CD animals (d). (e) The average of the ratio between the second and the first peak of the response, and each one of the components in the control-fed rats or in the cholesterol-rich diet fed rats.

Excitatory currents are determined by the combined activation of I_{NMDA} and I_{AMPA}. The ratio I_{NMDA}/I_{AMPA} determines the immediate synaptic efficacy and the capability of developing long-term changes at an excitatory synapse. For these reasons, we measured I_{NMDA} and I_{AMPA} in the presence of Mg^{2+} ions.

Fast currents were reliably detected in all recorded cells were they displayed the typical kinetic profile (rise time 1–3 ms, tau < 10 ms, Figs. 4a and c). They were blocked by application of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM, $n = 3$), indicative of their nature of AMPA-receptor-mediated glutamatergic currents. Outward currents were measured at a membrane potential V_h = + 40 mV. They were blocked by aminophosphovaleric acid (100 μM) and had slower kinetic (peak at ~10 ms; Figs. 4b and d) typical of I_{NMDA}. I_{NMDA} displayed a large variability from cell to cell. The ratio I_{NMDA}/I_{AMPA} was much larger in RD versus CD rats (5 each, Fig. 4e, control $n = 9$, cholesterol diet $n = 10$). In CD rats, I_{NMDA} were so small that in most recordings it was impossible to measure their kinetics (rise and decay times).

3. Discussion

To assess the effect of cholesterol on learning, we trained young adult rats to learn the location of a hidden platform in a

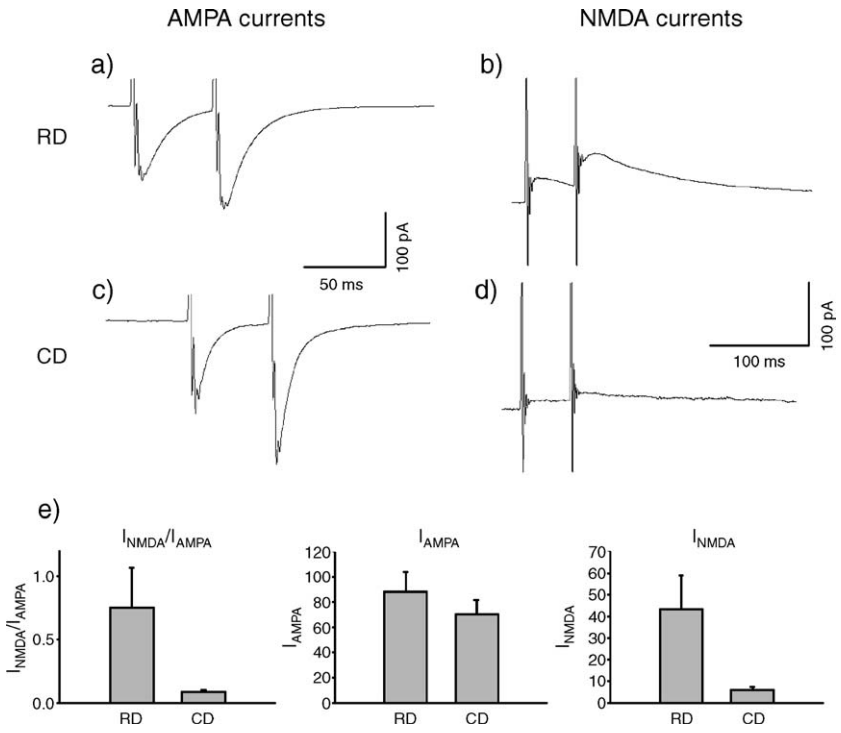


Fig. 4 – Effect of cholesterol diet on glutamatergic currents. Representative recording showing the following: (a) a facilitating, DNQX-sensitive, AMPAR-mediated response is evoked in a CA1 neuron following stimulation of the Schaffer collaterals at V_r = –60 mV; (b) recording from the same cell in a control-fed rat after shifting V_r to +40 mV allows the detection of a slower current corresponding to the I_{NMDA}; (c and d) same as panels a and b but in a CD-fed rat. I_{AMPA} is similar in the two recordings. I_{NMDA} is much smaller in the cholesterol-rich diet fed animal. (e) Average I_{NMDA}/I_{AMPA} and amplitude of each of the two components of the glutamatergic currents in control-fed or in cholesterol-rich diet fed rats.

Morris water-maze using distal visual cues, after 2 or 4 months of cholesterol diet (CD), compared their performances to control animals fed with a regular diet (RD) and measured pattern of learning, proximity distance and thigmotaxis. We found that feeding rats with a cholesterol-enriched diet for 2 months improved their performance in a water-maze paradigm, but after 4 months CD they had a tendency to a weaker retention of memory. Furthermore, animals fed during 4 months with a cholesterol-enriched diet acquired the learning task faster than the controls. In the search for a synaptic change associated with learning, we found that CD rats increased hippocampal paired-pulse facilitation and displayed a reduced ratio I_{NMDA}/I_{AMPA}.

3.1. *Cholesterol diet facilitates learning*

Both controls and cholesterol-fed rats acquired the task (quadrant test after 4 training sessions); meanwhile the comparison of escape latency and proximity distance during the training period showed that CD rats learned faster. Faster learning might be due to an improved capacity to develop a more appropriate strategy to learn the task. Indeed, CD rat thigmotaxis percentage was significantly lower than RD's one, and correlated with better learning performance during the training period. Thigmotaxis may be related to anxiety (Treit and Fundytus, 1988), is usually observed from the very beginning of training (Champagne et al., 2002) and tends to decrease during the next phases of training, when animals switch to a strategy of active exploration to increase their chance of finding the platform (Hoh et al., 1999). In our study, thigmotaxis was similar in both groups during the first 2 sessions, after which cholesterol-fed rats developed a more efficient search strategy. This result suggests that the late difference in thigmotaxis between CD and RD rats reflects a genuine discrepancy in learning, rather than an effect of anxiety. Interestingly, 5-month-old RD rats decreased their thigmotaxis more efficiently than 3-month-old ones. This strategy improved their training pattern, which was similar to age-matched CD rats. However, despite the improvement of 5 month-old RD rat strategies, RD rats performed less accurately than CD rats in the early probe test, regardless of the duration of the cholesterol diet. Altogether, these results suggest a correlation between cholesterol supplementation and the acquisition of a learning task, independent from the age-related improvement in learning strategy.

3.2. *The stimulating effect of cholesterol on memory retention depends on age*

After 2 additional sessions of training, 2-month fed animals improved their performance in the probe test, and CD rats still exhibited a more accurate spatial selectivity. Four-month fed animals (5 months old) also showed better performance in the

probe test with 2 additional sessions of training, but RD rats tended to perform better than age-matched CD ones. Moreover, RD 5-month-old rats performed significantly better than RD 3-month-olds. These results suggest that, despite the fact that cholesterol supplementation facilitates the acquisition of learning, its effect on memory consolidation and/or retention might depend on the age of the animal. Although memory capabilities are generally regarded as inversely correlated to age (Rosenzweig and Barnes, 2003), our results with RD rats partly contradict this tenet, in agreement with the idea that after an improvement of memory capabilities between birth and a peak period, the effectiveness of information storage may be impaired with aging.

Cholesterol may contribute to this age-dependent learning processes. The brain has a relatively self-sufficient supply and transport of cholesterol (Dietschy and Turley, 2001); however, extracerebral cholesterol is transported to the brain via the transport and transcytosis of low-density lipoprotein (LDL) across the blood–brain barrier (Dehouck et al., 1997; Malavolti et al., 1991). Thus, brain cholesterol is potentially acquired through endogenous cellular synthesis and from an external supply. Consequently, feeding animals with cholesterol results in an increase of brain cholesterol content (Sparks et al., 2000; Koudinov and Koudinova, 2001a,b; Boleman et al., 1998; Schoknecht et al., 1994; Malavolti et al., 1991), as we found in the hippocampus after 2 months of cholesterol diet. Two additional months of cholesterol diet did not increase further its concentration in the hippocampus, whereas age-matched controls receiving a regular diet (having a lower basal cholesterol concentration) showed a 36% increase of hippocampal cholesterol compared to younger animals. The concentration of cholesterol in hippocampal homogenates was similar in 5-month-old RD and CD rats, but the origin of the two pools of cholesterol is probably different. In fact, although in our conditions, we could not distinguish between imported and endogenously synthesized cholesterol, the age-dependent increase of cholesterol in RD hippocampus may reflect an increase of endogenous synthesis because their diet contained almost no cholesterol.

In RD rats, the age-related increase of the endogenously synthesized pool of cholesterol correlated with an improvement in the retention of memory (second probe test) but did not enhance the speed of task acquisition (probe test after 2 training sessions). A two-month cholesterol-rich diet might speed up a natural process (i.e., age-dependent increase of cholesterol synthesis in the brain), consistent with the finding that cholesterol fed animals display an increase in cholesterol synthesis (Koudinov and Koudinova, 2001b). This might improve the retention of memory, as we observed in 2-month-older RD rats.

In summary, these data suggest that 2 pools of cholesterol might affect learning at two different levels. An extracerebral supply of cholesterol, possibly via lipoprotein transport, would improve the acquisition of memory, whereas the endogenous synthesis of cholesterol in the brain would affect its retention. Although this hypothesis explains our results, we cannot rule out that the effects of the cholesterol diet on learning may be only indirectly determined by the increase of plasmatic brain cholesterol, possibly by activating a yet unknown biochemical or cellular signaling process.

3.3. *Cholesterol diet modulates the brain synaptic plasticity*

To assess the possibility that changes in synaptic transmission might be associated with the improvement of learning acquisition in CD rats, we tested the influence of a cholesterol diet on a short-term plasticity protocol, induced by paired-pulse stimuli (Debanne et al., 1996). In normal hippocampus, paired-pulse stimuli induce paired-pulse facilitation (PPF) of EPSCs and paired-pulse depression (PPD) of IPSCs (Jiang et al., 2000). In RD rat hippocampus, we observed PPF of excitatory transmission and PPD of inhibitory transmission, in accordance with previous studies (Debanne et al., 1996; Jiang et al., 2000; Andreasen and Hablitz, 1994). Cholesterol-rich diet increased EPSC facilitation and converted pair pulse depression into pair pulse facilitation in IPSCs.

Because changes in PPF are likely to originate in the presynaptic terminal (Thomson, 2000), our data may reflect a change in neurotransmitter release in rats fed with cholesterol, possibly by increasing storage, docking or fusion of synaptic vesicles (Thomson, 2000). The membranes of synaptic vesicles contain high amounts of cholesterol (Breckenridge et al., 1973; Yeagle, 1985; Schmitz and Orso, 2001), which binds to proteins involved in synaptic vesicle structure (Thiele et al., 2000). Cholesterol is in fact required for biogenesis of synaptic vesicles (Thiele et al., 2000) and plays a critical role in docking and fusion process during exocytosis (Lang et al., 2001; Chamberlain et al., 2001; Rohrbough and Broadie, 2005). Likewise, cholesterol strongly increases the efficacy of transmitter release in cultured rat retinal ganglion cells (Mauch et al., 2001; Nagler et al., 2001). Also, cholesterol depletion inhibits calcium-related exocytosis (Kato et al., 2003). In contrast with our observations, Koudinov and Koudinova (2001a) reported that experimental cholesterol efflux from rat brain slices increased PPF magnitude. It is possible that synaptic release rests on an equilibrium with an inverse bell-shaped dependence on cholesterol, whereby any deviation from this equilibrium results in the increase in paired-pulse ratio. Cholesterol might eventually enhance the calcium-dependent release of synaptic vesicles causing an increase in the ratio between second and first response by the enhancement in the rate of endocytotic recycling of synaptic vesicles (Rodal et al., 1999). In conclusion, we found that a cholesterol-enriched diet affects short-term synaptic plasticity in hippocampus, with a cellular mechanism possibly involving the modulation of neurotransmitter release. The participation of cholesterol in presynaptic changes does not rule out the possibility that a cholesterol-rich diet also modifies postsynaptic function.

3.4. *Cholesterol diet affects glutamatergic currents in CA1 pyramidal cells*

AMPAR- and NMDAR-mediated currents are critically involved in synaptic plasticity and learning and memory (Bliss and Collingridge, 1993; Collingridge, 2003; Bennett, 2000; Riedel et al., 2003). Whereas the existence of AMPA channels in the cholesterol-rich lipid rafts is well established, the presence of NMDA receptors in lipid rafts is still controversial (Hering et al., 2003; Suzuki et al., 2001). In addition to this, cholesterol in lipid rafts appears to influence density or distribution of

NMDA channels in postsynaptic densities (Abulrob et al., 2005; Frank et al., 2004). For this reason, we tested the possibility that a cholesterol-rich diet induces a change in the relative proportion of AMPAR- versus NMDAR-mediated currents in naïve RD versus CD animals, by directly measuring and comparing the amplitude of the two components. We found a lower ratio between the amplitude of I_{NMDA} over I_{AMPA} in CD rats compared to RD rats. Because we measured similar amplitude of AMPA currents in RD and CD rats in similar stimulation conditions, this difference is likely due to reduced NMDAR-mediated currents in CD rats, suggesting that cholesterol might affect specifically distribution and/or function of synaptic NMDAR-mediated glutamatergic currents. The recent finding that lipid rafts constitute an anchoring site for nicotinic receptors (Zhu et al., 2006) supports the idea that a cholesterol-rich diet may alter the membrane localization of ionotropic channels.

NMDARs play a pivotal role in the induction of LTP (Malenka, 2003; Bashir et al., 1991; Collingridge, 2003). Recently, Grosshans et al. (2002) demonstrated that a pool of intracellular NMDARs is available for recruitment to the synaptic membrane during the late phase of LTP induction. This LTP-dependent increased expression of NMDAR on the cell surface is accompanied by a functional increase in the number of these receptors in the CA1 region of the hippocampus (Clayton et al., 2002; Grosshans et al., 2002). In our study, NMDA currents were decreased in CD animals, raising the possibility that cholesterol promotes NMDAR turnover and/or the internalization of NMDA receptors to the intracellular pool. Thus, in adult animals, less NMDARs would be functional in CD rats, and the induction of synaptic plasticity, which relies on the effectiveness of NMDAR trafficking from the intracellular pool to the membrane, would account for the increase in membrane excitability. Because AMPA currents were similar in RD and CD rats, cholesterol diet might affect specifically NMDAR-mediated currents. Furthermore, because brain cholesterol concentration increases with age, the effect of this sterol on synaptic plasticity, learning and memory might be age-dependent.

3.5. *The Janus-face of cholesterol*

A considerable amount of data suggest that cholesterol plays a significant role in Alzheimer's disease (AD) (Koudinov, 2003; Hartmann, 2001; Puglielli et al., 2003), the most common neurodegenerative disease associated with early memory loss (Spaan et al., 2003). It has been suggested that inhibiting cholesterol synthesis with statins may lower the risk of developing AD (Jick et al., 2000; Wolozin et al., 2000). On the other hand, memory loss has been related to the use of these drugs (Wagstaff et al., 2003). In the present study, we have shown that a cholesterol diet influenced the learning skills of young adult rats at several levels. Cholesterol enhanced the acquisition of learning regardless of the duration of the diet but tended to impair the retention of memory after a prolonged diet. This double-face of cholesterol and its role in learning should be considered for studies, which aim to develop preventive or curative therapies against Alzheimer's disease, and other dementia. Although we started the cholesterol diet at an age (one months) at which rats are sexually mature, and the major developmental turning points have been passed, we cannot completely rule out that the behavioral, biochemical and synaptic differences detected in CD versus RD rats might be at least in part due to an effect of the cholesterol diet on the neurological and/or systemic development of the animals.

4. Conclusion

For the first time, we have shown that a cholesterol diet improves the acquisition of a learning paradigm in adult rats. This result has been correlated with a permanent change in short-term synaptic plasticity and a decrease in the ratio I_{NMDA}/I_{AMPA}.

Our results indicate that the detrimental effect of cholesterol on memory retention became evident only for older animals and depended on the duration of the diet. Our data also suggests that distinct pools of cholesterol might play specific roles in memory and memory-related diseases. In spite of the fact that the nature of this study does not allow to establish a causal relationship between behavioral and synaptic changes, our data are consistent with the notion that an alteration in synaptic function may represent an important candidate in the induction of behavioral changes.

5. Experimental procedures

5.1. *Animals*

One-month-old male Wistar rats were maintained at 22 °C room temperature under a 12 h/12 h normal light/dark cycle (lights on at 7:00 a.m.) with food and water *ad libitum*. Half of them received a regular rodent diet (RD, Basal diet #5755, TestDyets, Richmond IN), whereas the others were fed with TestDyets #5799, corresponding to the same diet +2% cholesterol (CD) for 2 or 4months. All animal experimentation was performed in accordance with the National Institutes of Health guidelines.

5.2. *Water-maze training*

We used 8 rats per group. Animals were trained to solve a Morris water-maze task after 2 months of diet. The task consisted of finding a non-visible submerged platform (10 cm diameter) in a 1.6-m diameter and 0.6-m depth pool filled with water (23 °C) by using spatial cues located outside the pool, in a room with dim light. To reduce stress, the rats were allowed a 2-min swim in the pool without any platform on day 0; the first training session was performed on the next day. Four training sessions (1 per day) were given to the rats. Each session consisted of 4 trials (1 min each) with a 7-min rest interval between 2 consecutive trials. Once the animal had found the platform, it was left for 15 s to orient, then brought back to its housing cage until the next trial. Each of the 4 cardinal loci (north, south, east and west) was randomly used as a starting location, each one being used at least once per session. If the platform was not found after 1 min, the rat was gently guided to it. On the third (before

the training session) and fifth day, the rats' spatial learning was assessed with a probe test, i.e., a 30-s free swim without any platform. Each trial was monitored and analyzed by a HVS2020 tracker system from HVS IMAGE Ltd., UK. Several parameters were compared between RD and CD groups. Escape latency was defined as the time necessary for the rat to find the platform. Thigmotaxis is the percentage of time spent swimming in the pool peripheral annulus (within 16 cm from the pool wall). Proximity distance was defined as the sum of the 1 s averages of distance between the rat and the platform (Gallagher et al., 1993). The target area was designed as a circular zone (40 cm diameter) centered on the platform. The percentage of time spent in this area is a more stringent measure of spatial bias than the percentage of dwell time in the target quadrant. Spatial selectivity was quantified during the probe test after 2 and 4 sessions of training, using the percentage of time and swimming distance in quadrant. Moreover, to provide a more stringent measure of spatial selectivity, we also monitored the percentage of time in the target area, the number of platform hits and the proximity distance during the same probe tests.

Statistical analysis of the task acquisition kinetics (escape latency, thigmotaxis and proximity) was performed using repeated measures of ANOVA. Fisher's post hoc tests were used for pairwise comparisons. Significance of spatial bias was assessed by analyzing the quadrant dwell time and swimming distance for each group during probe tests using a one-way ANOVA. For each test (day 3 and day 5), swimming speed, percentage of swim distance in the target area, number of target crossings, proximity and thigmotaxis were compared with an unpaired *t*-test. The same test was used to compare rat weight, cholesterol concentrations and electrophysiological data between both groups. A significant difference was set at $P < 0.05$. Statistical analysis was performed by using the software Statview (SAS Institute Inc.). Asterisks in the graph indicate significance levels: one asterisk (*) $P < 0.05$, two asterisks (**) $P < 0.02$.

5.3. *Cholesterol measurements*

Three days after the last probe test, rats were deeply anaesthetized with an intraperitoneal injection of sodium pentobarbital (150 mg/kg) and then decapitated. Blood was collected after death in heparin-coated tubes. Plasma was isolated from erythrocytes after a 800 × *g* centrifugation for 5 min, then stored at –80 °C. The brain was removed rapidly from the skull, the hippocampus was dissected from freshly removed brain, flash frozen in dry ice, then stored at –80 °C. For cholesterol measurements, the hippocampus was homogenized in cold lysis buffer (20 mM Tris–HCl, 1% v/v Nonidet P-40, 10% v/v glycerol, 1% protease inhibitor cocktail, Sigma-Aldrich Co., St. Louis, MO, pH 8.0) with a Dounce homogenizer. Cholesterol concentration was measured in rat blood plasma and hippocampus homogenates with Infinity™ Cholesterol Reagent (Sigma-Aldrich Co., St. Louis, MO). Cholesterol concentration in the hippocampus was normalized with protein concentration. Protein concentration was determined by the Micro BCA™ Protein Assay Reagent Kit (Pierce, Rockford, IL).

5.4. *Slice preparation*

Transverse hippocampal slices (300 μm thick) were prepared from CD and RD naive (non-trained) rats. One-month-old animals were fed with a regular (RD) or cholesterol-enriched diet for 1 month, then sacrificed for brain slices preparation. After being anaesthetized deeply with inhalation of isoflurane, the rat was sacrificed, its brain carefully removed and put into ice-cold artificial cerebrospinal fluid (aCSF) containing the following (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 26 $NaHCO_3$, 2.4 $CaCl_2$, 10 glucose, saturated with 95% O_2, 5% CO_2, at pH 7.4. Transverse slices were dissected at 0–4 °C, cut in modified aCSF, containing 0.5 mM Ca^{2+} and 4 mM Mg^{2+}, with a VT 1000S Vibrating Blade Microtome (Leica Microsystems Inc.), placed on a nylon mesh, submerged in normal aCSF bubbled with 95% O_2, 5% CO_2, and allowed to recover for at least 1 h in 32 °C before recording.

5.5. *Electrophysiological recordings*

Slices were transferred to a perfusion chamber and perfused with aCSF at a rate of 2–3 ml/min at room temperature. CA1 pyramidal cells were identified visually using an Axioskop 2FS microscope (Carl Zeiss, Inc.) equipped with a 40× water immersion DIC objective coupled with an infrared camera system. Whole-cell recordings were made with a patch-clamp amplifier (MultiClamp 700A, Axon Instruments, Inc.) in voltage-clamp mode. Membrane currents were digitized with a Digidata 1322A and analyzed with a pClamp 8.0 system (Axon Instruments Inc.). Patch pipettes of ~5 MΩ were pulled with a Narishige PP-830 puller and fire-polished with a Narishige MF-83 microforge (Narishige International USA, Inc.). Series resistance and membrane capacitance were compensated (~70%) and monitored during experiments. Precise positioning of recording and stimulation electrodes was achieved with PCS-5400 piezoelectric micromanipulators (Burleigh Instruments, Inc.). The stimulation strength was set at 5 μA above the current necessary to evoke just observable responses and the duration was fixed at 200 μs. Electrophysiology experiments were conducted in a blind fashion (the experimenters were not aware of the animal type of diet). Data were compared using *t*-Student tests and reported as different only if $P < 0.05$. Recordings were performed from no more than one cell per slice in number of 1–3 per each animal.

5.6. *Paired-pulse stimulation*

A paired-pulse stimulation protocol was used to evaluate short-term brain plasticity in RD and CD hippocampal slices (6 animals each group). The pipette solution contained the following (in mM): 90 K^+ gluconate, 45 KCl, 1.7 NaCl, 0.1 $CaCl_2$, 2.7 $MgCl_2$, 10 HEPES, 1.1 EGTA, 5 phosphocreatine-Na^+, 3.5 ATP-K^+, 0.3 GTP-Na^+, was titrated at pH 7.2 and 290 mOsm.

GABAergic, inhibitory postsynaptic currents (IPSCs) were evoked using a concentric bipolar stimulation electrode placed in the *stratum pyramidale* about 80 μm from the recording cell, which was kept at a holding potential of $V_r = -75$ mV. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and amino-phosphonovaleric acid (APV, 10 μM each) were included in the perfusion

solution to block glutamatergic currents. A similar protocol was used for evoking glutamatergic postsynaptic excitatory currents (EPSCs) by stimulating the Schaffer collateral pathway in the presence of 10 μM bicuculline. Only one cell was recorded from each brain slice.

5.7. *Glutamatergic currents*

We also used patch-clamp recording for measuring the ratio between *N*-methyl-D-aspartate-receptor-mediated currents (I_{NMDA}) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid-receptor-mediated currents (I_{AMPA}) from hippocampal neurons. In this case, the control solution contained bicuculline methachloride (10 μM) for blocking, γ-aminobutyric-acid A-receptor ($GABA_AR$)-mediated currents.

Postsynaptic currents were recorded with 3–5 MΩ electrodes using a solution containing the following (in mM): 100 CsOH, 100 gluconic acid, 5 1,2-*bis*(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid K (BAPTA-K), 1 lidocaine *N*-ethyl bromide (QX314), 1 $MgCl_2$, 10 *N*-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), 4 glutathione, 1.5 $ATPMg_2$, 0.3 $GTPNa_2$, 8 biocytin. Electrically evoked EPSC were measured by delivering two electric stimuli (90–180 μs, 10–50 μA) 50 ms apart, every 6 s with an isolation unit through a glass stimulation electrode filled with aCSF placed in the *stratum radiatum*. I_{AMPA} were recorded at a holding potential V_r = −60 mV and measured at their peak. I_{NMDA} were recorded in the same cell at V_r = +40 mV in order to remove the Mg^{2+} block at NMDA receptors. I_{NMDA} amplitude measured at a latency of 45 ms after the electric stimulation for minimizing the possible contamination by I_{AMPA}. The stability of the recording was assessed by measuring I_{AMPA} both prior and subsequent to the measurement of I_{NMDA}. Only recordings in which I_{AMPA} measured before and after I_{NMDA} differed by <20% were considered.

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REFERENCES

Abulrob, A., Tauskela, J.S., Mealing, G., Brunette, E., Faid, K., Stanimirovic, D., 2005. Protection by cholesterol-extracting cyclodextrins: a role for *N*-methyl-D-aspartate receptor redistribution. J. Neurochem. 92, 1477–1486.

Andreasen, M., Hablitz, J.J., 1994. Paired-pulse facilitation in the dentate gyrus: a patch-clamp study in rat hippocampus in vitro. J. Neurophysiol. 72, 326–336.

Bashir, Z.I., Alford, S., Davies, S.N., Randall, A.D., Collingridge, G.L., 1991. Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature 349, 156–158.

Bennett, M.R., 2000. The concept of long term potentiation of transmission at synapses. Prog. Neurobiol. 60, 109–137.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Boleman, S.L., Graf, T.L., Mersmann, H.J., Su, D.R., Krook, L.P., Savell, J.W., Park, Y.W., Pond, W.G., 1998. Pigs fed cholesterol neonatally have increased cerebrum cholesterol as young adults. J. Nutr. 128, 2498–2504.

Breckenridge, W.C., Morgan, I.G., Zanetta, J.P., Vincendon, G., 1973. Adult rat brain synaptic vesicles: II. Lipid composition. Biochim. Biophys. Acta 320, 681–686.

Chamberlain, L.H., Burgoyne, R.D., Gould, G.W., 2001. SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis. Proc. Natl. Acad. Sci. U. S. A. 98, 5619–5624.

Champagne, D., Dupuy, J.B., Rochford, J., Poirier, J., 2002. Apolipoprotein E knockout mice display procedural deficits in the Morris water maze: analysis of learning strategies in three versions of the task. Neuroscience 114, 641–654.

Clayton, D.A., Grosshans, D.R., Browning, M.D., 2002. Aging and surface expression of hippocampal NMDA receptors. J. Biol. Chem. 277, 14367–14369.

Collingridge, G.L., 2003. The induction of *N*-methyl-D-aspartate receptor-dependent long-term potentiation. Philos. Trans. R. Soc. Lond., B Biol. Sci. 358, 635–641.

Debanne, D., Guerineau, N.C., Gahwiler, B.H., Thompson, S.M., 1996. Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release. J. Physiol. 491 (Pt. 1), 163–176.

Dehouck, B., Fenart, L., Dehouck, M.P., Pierce, A., Torpier, G., Cecchelli, R., 1997. A new function for the LDL receptor: transcytosis of LDL across the blood–brain barrier. J. Cell Biol. 138, 877–889.

D'hooge, R., De Deyn, P.P., 2001. Applications of the Morris water maze in the study of learning and memory. Brain Res. Brain Res. Rev. 36, 60–90.

Dietschy, J.M., Turley, S.D., 2001. Cholesterol metabolism in the brain. Curr. Opin. Lipidol. 12, 105–112.

Frank, C., Giammarioli, A.M., Pepponi, R., Fiorentini, C., Rufini, S., 2004. Cholesterol perturbing agents inhibit NMDA-dependent calcium influx in rat hippocampal primary culture. FEBS Lett. 566, 25–29.

Gallagher, M., Burwell, R., Burchinal, M., 1993. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav. Neurosci. 107, 618–626.

Grosshans, D.R., Clayton, D.A., Coultrap, S.J., Browning, M.D., 2002. LTP leads to rapid surface expression of NMDA but not AMPA receptors in adult rat CA1. Nat. Neurosci. 5, 27–33.

Hartmann, T., 2001. Cholesterol, A beta and Alzheimer's disease. Trends Neurosci. 24, S45–S48.

Hering, H., Lin, C.C., Sheng, M., 2003. Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J. Neurosci. 23, 3262–3271.

Herz, J., Beffert, U., 2000. Apolipoprotein E receptors: linking brain development and Alzheimer's disease. Nat. Rev., Neurosci. 1, 51–58.

Hoh, T., Beiko, J., Boon, F., Weiss, S., Cain, D.P., 1999. Complex behavioral strategy and reversal learning in the water maze without NMDA receptor-dependent long-term potentiation. J. Neurosci. 19, RC2.

Jiang, L., Sun, S., Nedergaard, M., Kang, J., 2000. Paired-pulse modulation at individual GABAergic synapses in rat hippocampus. J. Physiol. 523 (Pt. 2), 425–439.

Jick, H., Zornberg, G.L., Jick, S.S., Seshadri, S., Drachman, D.A., 2000. Statins and the risk of dementia. Lancet 356, 1627–1631.

Kato, N., Nakanishi, M., Hirashima, N., 2003. Cholesterol depletion inhibits store-operated calcium currents and exocytotic membrane fusion in RBL-2H3 cells. Biochemistry 42, 11808–11814.
Kessler, A.R., Kessler, B., Yehuda, S., 1986. In vivo modulation of brain cholesterol level and learning performance by a novel plant lipid: indications for interactions between hippocampal-cortical cholesterol and learning. Life Sci. 38, 1185–1192.
Koudinov, A., 2003. A link between cholesterol, synapse plasticity, degeneration and neurological disorders: reinvention or integration? BioEssays 25, 736–737.
Koudinov, A.R., Koudinova, N.V., 2001a. Essential role for cholesterol in synaptic plasticity and neuronal degeneration. FASEB J. 15, 1858–1860.
Koudinov, A.R., Koudinova, N.V., 2001b. Brain cholesterol pathology is the cause of Alzheimer's disease. In: 2001. pp. clinmed/2001100005.
Lang, T., Bruns, D., Wenzel, D., Riedel, D., Holroyd, P., Thiele, C., Jahn, R., 2001. SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis. EMBO J. 20, 2202–2213.
Malavolti, M., Fromm, H., Ceryak, S., Shehan, K.L., 1991. Cerebral low-density lipoprotein (LDL) uptake is stimulated by acute bile drainage. Biochim. Biophys. Acta 1081, 106–108.
Malenka, R.C., 2003. Synaptic plasticity and AMPA receptor trafficking. Ann. N. Y. Acad. Sci. 1003, 1–11.
Martin, S.J., Grimwood, P.D., Morris, R.G., 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. Annu. Rev. Neurosci. 23, 649–711.
Mauch, D.H., Nagler, K., Schumacher, S., Goritz, C., Muller, E.C., Otto, A., Pfrieger, F.W., 2001. CNS synaptogenesis promoted by glia-derived cholesterol. Science 294, 1354–1357.
Miller, S., Wehner, J.M., 1994. Cholesterol treatment facilitates spatial learning performance in DBA/2Ibg mice. Pharmacol. Biochem. Behav. 49, 257–261.
Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47–60.
Nagler, K., Mauch, D.H., Pfrieger, F.W., 2001. Glia-derived signals induce synapse formation in neurones of the rat central nervous system. J. Physiol. 533, 665–679.
O'Brien, W.T., Xu, G., Batta, A., Tint, G.S., Salen, G., Dyer, C.A., Kendler, A., Servatius, R.J., 2002. Developmental sensitivity of associative learning to cholesterol synthesis inhibitors. Behav. Brain Res. 129, 141–152.
Pfrieger, F.W., 2003. Role of cholesterol in synapse formation and function. Biochim. Biophys. Acta 1610, 271–280.
Poirier, J., Minnich, A., Davignon, J., 1995. Apolipoprotein E, synaptic plasticity and Alzheimer's disease. Ann. Med. 27, 663–670.
Puglielli, L., Tanzi, R.E., Kovacs, D.M., 2003. Alzheimer's disease: the cholesterol connection. Nat. Neurosci. 6, 345–351.
Riedel, G., Platt, B., Micheau, J., 2003. Glutamate receptor function in learning and memory. Behav. Brain Res. 140, 1–47.
Rodal, S.K., Skretting, G., Garred, O., Vilhardt, F., van, D.B., Sandvig, K., 1999. Extraction of cholesterol with methyl-beta-cyclodextrin perturbs formation of clathrin-coated endocytic vesicles. Mol. Biol. Cell 10, 961–974.
Rohrbough, J., Broadie, K., 2005. Lipid regulation of the synaptic vesicle cycle. Nat. Rev., Neurosci. 6, 139–150.
Rosenzweig, E.S., Barnes, C.A., 2003. Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Prog. Neurobiol. 69, 143–179.
Schmitz, G., Orso, E., 2001. Intracellular cholesterol and phospholipid trafficking: comparable mechanisms in macrophages and neuronal cells. Neurochem. Res. 26, 1045–1068.
Schoknecht, P.A., Ebner, S., Pond, W.G., Zhang, S., McWhinney, V., Wong, W.W., Klein, P.D., Dudley, M., Goddard-Finegold, J., Mersmann, H.J., 1994. Dietary cholesterol supplementation improves growth and behavioral response of pigs selected for genetically high and low serum cholesterol. J. Nutr. 124, 305–314.
Spaan, P.E., Raaijmakers, J.G., Jonker, C., 2003. Alzheimer's disease versus normal ageing: a review of the efficiency of clinical and experimental memory measures. J. Clin. Exp. Neuropsychol. 25, 216–233.
Sparks, D.L., Martin, T.A., Gross, D.R., Hunsaker, J.C., 2000. Link between heart disease, cholesterol, and Alzheimer's disease: a review. Microsc. Res. Tech. 50, 287–290.
Suzuki, T., Ito, J., Takagi, H., Saitoh, F., Nawa, H., Shimizu, H., 2001. Biochemical evidence for localization of AMPA-type glutamate receptor subunits in the dendritic raft. Brain Res. Mol. Brain Res. 89, 20–28.
Teunissen, C.E., De Vente, J., von Bergmann, K., Bosma, H., van Boxtel, M.P., De Bruijn, C., Jolles, J., Steinbusch, H.W., Lutjohann, D., 2003. Serum cholesterol, precursors and metabolites and cognitive performance in an aging population. Neurobiol. Aging 24, 147–155.
Thiele, C., Hannah, M.J., Fahrenholz, F., Huttner, W.B., 2000. Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. Nat. Cell Biol. 2, 42–49.
Thomson, A.M., 2000. Facilitation, augmentation and potentiation at central synapses. Trends Neurosci. 23, 305–312.
Treit, D., Fundytus, M., 1988. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol. Biochem. Behav. 31, 959–962.
Voikar, V., Rauvala, H., Ikonen, E., 2002. Cognitive deficit and development of motor impairment in a mouse model of Niemann–Pick type C disease. Behav. Brain Res. 132, 1–10.
Wagstaff, L.R., Mitton, M.W., Arvik, B.M., Doraiswamy, P.M., 2003. Statin-associated memory loss: analysis of 60 case reports and review of the literature. Pharmacotherapy 23, 871–880.
Wolozin, B., Kellman, W., Ruosseau, P., Celesia, G.G., Siegel, G., 2000. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. Arch. Neurol. 57, 1439–1443.
Yaffe, K., Barrett-Connor, E., Lin, F., Grady, D., 2002. Serum lipoprotein levels, statin use, and cognitive function in older women. Arch. Neurol. 59, 378–384.
Yeagle, P.L., 1985. Cholesterol and the cell membrane. Biochim. Biophys. Acta 822, 267–287.
Zhu, D., Xiong, C., Mei, L., 2006. Lipid rafts serve as a signaling platform for nicotinic acetylcholine receptor clustering. J. Neurosci. 26, 4841–4851.