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Research report

Dietary choline supplementation in adult rats improves performance on a test of recognition memory

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ABSTRACT

In two experiments adult rats (aged at least 6 months at the start of the procedure) received a diet enriched with added choline for a period of 10 weeks; control subjects were maintained on a standard diet during this time. All rats then underwent the spontaneous object recognition (SOR) procedure in which they were exposed to a pair of objects and then tested, after a retention interval, to a display with one object changed. Exploration of the changed object indicates retention and use of information acquired during the exposure phase. All subjects showed retention with a 24-h interval (Experiments 1 and 2) and when retested after a further 24 h (Experiment 1). But when tested for the first time after a 48-h interval (Experiment 2), control subjects showed no evidence of retention, exploring both objects equally, whereas those given the dietary supplement continued to show a preference for the changed object. This supports the conclusion that dietary choline supplementation can enhance performance on a task regarded as a test of declarative memory, and will do so even when the supplementations is given in adulthood.

1. Introduction

Choline (Ch) is a quaternary amine, classified within the vitamin B complex. In some species (including humans) it can be synthesized in the liver, but intake from foodstuffs is also important. It has a number of physiological functions (e.g., it is critical for the synthesis of the phospholipid component of cell membranes) but here we focus on its role as a precursor of the neurotransmitter acetyl choline (ACh) [1]. Increasing the availability of choline in the diet increases the level of cerebral choline, promoting synthesis and emission of ACh in the brain [2–4]. This prompts the notion that dietary supplementation might influence (perhaps enhance) those cognitive functions that are taken to depend on ACh [5,6].

ACh has long been implicated in cognitive functioning, especially in aspects of attention and memory [7–9]. The relation between Alzheimer's disease and dysfunction of the cholinergic system has been influential in establishing this notion [10], and it has been supported by studies of animals subjected to direct manipulation (by surgery or drug treatment) of the forebrain cholinergic system. These studies have provided evidence of a role for this system in the acquisition of new information [11,12,9], and also in its consolidation and use ([13,14], but see also [15]). The behavioral tasks used in these studies have been varied. They have included: tests of spatial learning and contextual conditioning (see, e.g., [16]), behavior thought to depend on hippocampal functioning; procedures sensitive to the modulation of attentional processing of conditioned stimuli [17], thought to depend on the functioning of the amygdala; and tests of recognition memory [18,19], thought to depend on the functioning of the perirhinal cortex [20,21]. Studies of manipulations of dietary choline have made use of the same set of tasks. Thus Meck and Williams have looked at both spatial learning [22] and at attentional learning in conditioning [23]; and Moreno, de Brugada, Carias, and Gallo [24] investigated recognition memory in the following experiment, which forms the basis for the new work to be reported here.

Moreno et al. [24] studied the effects of manipulating the prenatal availability of dietary choline for rats on their performance on a test of object-recognition memory in adulthood. Different groups of pregnant dams were given a period of exposure to a diet that was deficient in choline, or in which choline was supplemented, or they remained on the standard laboratory diet. At the age of 3 months the pups born of

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these dams were tested with the procedure known as spontaneous object recognition (SOR). In this procedure the rats are allowed to explore two identical objects (call these: A, A). Then, after a retention interval, they are given access to a pair of objects, one of the originals, and one new (A, B). If rats tend preferentially to explore the new object, we may conclude that they have retained information about the first training session over the interval. This procedure has been taken to be a test of, at least some of, the processes involved in declarative memory [18].

The original experiments of Ennaceur and Delacour [25] on SOR demonstrated retention over intervals of up to an hour; later studies (giving more initial exposure and using different objects) have demonstrated effects over intervals of one or two days (see e.g., [26,27]). Moreno et al. [24] used a retention interval of 24 h, and found a preference for the novel object (i.e., performance indicating memory for the original) in all three of their dietary groups. However, in a further test with a new novel object (an A, C test), given 48 h after the initial training, no preference was evident in rats that had suffered prenatal choline deficiency. Rats that had received the standard diet retained some tendency to explore the novel object; and rats that had experienced choline supplementation prenatally, showed the biggest preference of all. Moreno et al. concluded that maternal dietary choline can modulate the learning and memory processes involved in SOR.

In the studies of the effects of dietary choline just mentioned [22–24], and indeed in the bulk of work on the topic, the availability of choline has been manipulated prenatally or perinatally (for a review see [5]). And although it is likely that the availability of choline during early development will have long-lasting effects throughout the lifespan, there is growing evidence to indicate that, even for adult animals, a period of exposure to a diet in which choline availability is modified, can influence performance on tasks of the sort used to test animals given the treatment early in life. Moreno, Gil, Carias, Gallo, and de Brugada [28] studied context conditioning, measuring the aversion shown to a context in which nausea had been experienced. They found that rats given access to a choline-supplemented diet for 7 weeks at 3–4 months of age developed a greater aversion than controls given only standard rodent diet. Moreno, de Brugada, and Hall [29] used a similar dietary manipulation and demonstrated an effect on a test of attentional learning akin to that used by Meck and Williams [23]. It remains to investigate the effects of dietary manipulation in adulthood on a test of recognition memory. Encouraging results come from a recent report by Tabassum et al. [30] who found that choline supplementation improved short-term recognition memory in young rats aged about 8 weeks. The experiments to be described look at SOR performance exploring long-term recognition memory in rats that had received chronic choline supplementations in mature adulthood.

2. Experiment 1

In Experiment 1 we explored the effects of choline supplementation in adult rats using test procedures essentially identical to those used previously in our study of prenatal choline supplementation and object recognition memory [21]. The rats were about 6 months old at the start of the study, and had been raised on a standard laboratory diet. One group of rats was then given a period of 10 weeks on a supplemented diet; control subjects remained on the standard laboratory diet. They then were given the object recognition test. This consisted of a familiarization phase in which the subjects were allowed to explore an arena containing two identical objects (A, A). After 24 h they were tested in the arena with one object replaced by a novel object (A, B). This test was repeated after a further 24 h with another new object (A, C). The behavioral measure was the time spent exploring the objects. A tendency to explore the changed object (e.g., B on the first test) would indicate that learning about the initial arrangement (A, A) had been retained over the interval. The question of interest was whether experience of the dietary supplement would enhance performance (increase the likelihood of exploring the novel object) on this test.

2.1. Method

2.1.1. Subjects and diet

The subjects were 16 male Wistar rats, aged 6–7 months, and with a mean weight of 545 g (range: 470–618 g), at the start of the experiment. They were assigned to one of two equal-sized groups: SUP (given the choline supplemented diet) and STA (remaining on the standard diet). During the 10 weeks supplementation period SUP group received a modified AIN-76 diet containing 5.0 g/kg choline chloride; subjects assigned to the STA group were fed with the standard AIN-76 diet that provides 1.1 g/Kg choline chloride. At the end of the supplementation period and during the behavioural procedure that followed, all the animals were fed the standard AIN-76 diet. Water and food were available ad libitum throughout the experiment. The animals were kept in a room with constant temperature (22–24 °C) and a 12 h light-dark cycle (lights on at 8:00 a.m. and off at 8:00 p.m.), the behavioural testing being conducted during the light phase of the cycle. The procedures were approved by the University of Granada Ethics Committee for Animal Research.

2.1.2. Apparatus

The testing arena was a square open-topped box (50 cm × 50 cm, made of black plastic, with walls 40 cm high. The size of the arena, although less than that sometimes used in the SOR procedure, is similar to that used in many other studies (45 cm × 45 cm × 45 cm [30–32], and 40 cm × 40 cm × 40 cm [32,33]). The use of these dimensions has the advantage of reducing the spatial component of the task, thus favoring the exploration of the objects and eliminating other external influences. The objects presented in the arena were positioned centrally, 9 cm, apart. The objects used in familiarization and the first test were pairs of porcelain jars. Each was about 10 cm high and 5 cm wide. Two sets were available, differing in shape, one pair rounded and one pair elongated. A third object, a yellow plastic apple, 5 cm high, was used in the second test. A video camera mounted above the chamber allowed us to record the sessions.

2.1.3. Procedure

The procedure consisted of three phases: habituation to the test situation, object familiarization, and recognition memory tests. In the habituation session all the subjects were acclimated to the testing room for 30 min, spending 5 min in the empty arena. On the next day, during the familiarization session, the rat was allowed to explore the chamber containing two identical objects for 10 min. For half the subjects in each group, these were the elongated jars and for half they were the rounded jars. Twenty-four hours later, subjects were given the first test, in which one of the jars was replaced by one of the other shape. For half the subjects this was the object on the left; for half, the object on the right. The test session lasted 5 min. A second, 5-min, test was given after a further 24 h. In this, the jar familiar from initial training was paired with the novel plastic apple. This new object was in the location that was not used for the novel object in the previous test.

Behaviour during the testing sessions was scored from the video recordings by an experimenter blind to the experimental conditions. Object exploration was measured by scoring time spent in contact with an object ("contact" being defined as having the snout within 2 cm of an object, with the vibrissae moving).

2.1.4. Statistical analysis

We used General Linear Model analyses followed, where appropriate by *t*-tests. Ratio scores (computed as time spent exploring the novel object over the total exploration time; see below) were compared against the chance level (of 0.5) by one-sample *t*-tests. Partial eta squared ($\eta^2 p$) and Cohen's *d* were used as measures of effect size. A significance level of $p < .05$ was adopted for all statistical analyses.

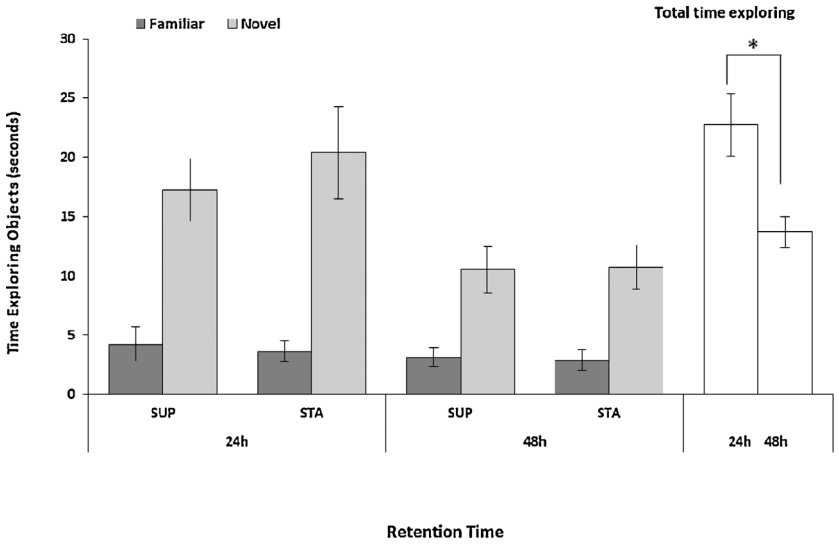

Fig. 1. Mean time in seconds (± SEM)that supplemented (SUP) and standard (STA) animals spent exploring two objects (familiar or novel), and total time exploration of objects (familiar plus novel) by both dietary groups (right panel) during test sessions given 24 h and 48 h after familiarization.

2.2. Results and discussion

During the familiarization phase the mean time spent exploring the objects was 34.0 s for the SUP group and 35.1 s for the STA group. Both objects were explored equally. The SUP group spent a mean of 17 s exploring each; the mean times for the STA group were: object 1, 18.4 s, and object 2, 16.7 s. An analysis of variance (ANOVA), with dietary condition (SUP or STA) and object (1 or 2) as the variables, revealed no significant differences: all Fs < 1.

Group mean scores for the two test sessions are shown in Fig. 1. As shown in the right panel of the figure, the total amount of time spent in object exploration was reduced on the second as compared to the first test; comparing the two tests yielded a significant difference, $t_{(15)} = 3.08$. $p = .008$, $d = .77$. It is evident that on both tests, however, more time was spent in contact with the novel objects than with the familiar object, and that there was no difference between the groups. A 2 × 2 × 2 ANOVA, with the between-group variable of diet (SUP or STA) and two within-subject variables, retention interval (24 h vs. 48 h), and object novelty (familiar vs. novel), revealed significant effects of novelty, $F(1, 14) = 125.9$, $p < .01$, $\eta^2p = .90$; of retention interval, $F(1, 14) = 9.27$, $p = .01$, $\eta^2p = .40$; and of the Retention × Novelty interaction ($F(1, 14) = 4.69$, $p = .048$, $n^2p = .25$); for all other main effects and interactions, ps $> .05$. An analysis of the interaction using paired samples t-tests showed non-significant differences in exploration of the familiar object between 24 (3.94 s) and 48 h (3.00 s), $t(15) = .97$, $p = .35$; but significant differences in exploration of the novel object (24 h = 18.88 s vs 48 h = 10.69 s), $t(15) = 2.79$, $p = .014$, $d = .70$.

In our previous study of SOR [24] we made use of an exploration ratio (ER), calculated as time spent exploring the novel object over the total exploration time for both familiar and novel objects during each testing session. To maintain comparability, we report equivalent scores for this experiment.[1] As Fig. 2 shows, both groups had high scores at both test intervals. Comparing against the chance level of 0.5 by means of one-sample t-tests showed all scores differed significantly from the chance level (SUP-24: $t_{(7)} = 6.77$, $p < .001$; SUP-48 $t_{(7)} = 5.79$, $p = .001$; STA-24: $t_{(7)} = 3.02$, $p = .02$; and STA-48: $t_{(7)} = 2.58$; p = .036). This analysis confirms the conclusions drawn on the basis of the raw scores – in this procedure the test shows evidence of an effective recognition memory at both retention intervals, and this is as true for animals raised on a standard diet as for animals given the supplement. There is nothing in these data to support the view that supplementation of dietary choline can be beneficial when given to mature adult animals.

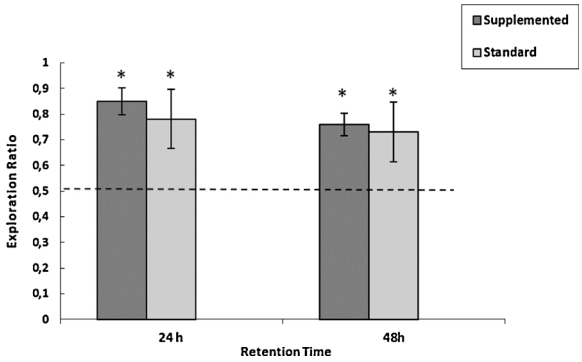

Fig. 2. Mean (± SEM) exploration ratios (ERs) during the testing session at 24 and 48 h retention intervals for groups receiving a choline supplemented or standard diet. The dotted line indicates the chance level of .5.

3. Experiment 2

In spite of the results of Experiment 1 it remains the case that Moreno et al. [29] were successful in finding an effect of choline supplementation in adult rats on their subsequent performance on a cognitive task. This may reflect the different demands of the task used in that experiment, which was principally concerned with attentional processes, compared with the memory-based task used in Experiment 1. But given that Moreno et al. [29] have shown that choline adult supplementation can affect behavior, and given the sensitivity of the SOR procedure to manipulation of (prenatal) choline [24], it seems surprising that no effect was found in Experiment 1. Accordingly, it is appropriate to consider the possibility that the failure to find an effect in Experiment 1 was simple a consequence of the insensitivity of the test used. In particular, the exploration ratio score for the rats raised on the standard diet in the experiment by Moreno et al. [24] was about .60, a score that leaves scope for detecting a diet-induced enhancement of performance. In the present Experiment 1, performance was rather better, with animals raised on the standard diet having a score as high

[1] This score is slightly different from the discrimination ratio (DR) reported by Ennaceur and Delacour [25], who used the difference between the time spent exploring the novel and familiar over the total time spent exploring. We have computed DRs for our data and have obtained exactly the same pattern of results as with our exploration ratio.

as .76, even in the test given after 48 h. It is not clear why performance should be so much better in this case, but a consequence is that the test is not likely to be sensitive to factors tending to enhance performance.

Accordingly, in the present experiment we modified the testing procedure in the hope of reducing the overall level of performance so that any advantage of the special diet might be more readily evident. The general procedures were the same as those described for Experiment 1, and we included a group tested after a 24-h retention interval that would, we anticipated allow replication of the results previously obtained in this condition. A separate group of rats was used for the 48-h test. In Experiment 1, there was surprisingly little reduction in the preference comparing the 24-h and 48-h tests. One possible reason for this is that the same animals were used for both. Thus the test given after 24 h (with the objects A, B) constituted a further familiarization trial in which the rats could further explore object A. This might be expected to boost performance on the next test (with objects A and C), thus obscuring any beneficial effect of the supplementation. By modifying the procedure to ensure a true retention interval of 48 h for the relevant animals we hoped to avoid this problem.

3.1. Method

This experiment was performed in the Experimental Psychology laboratories of the University of York. All the procedures were approved by the Ethics Committee for Animal Research of the University of York. Thirty-two 7-month-old male hooded Lister rats (mean weight: 639 g; range: 550–760 g) were assigned to one of two conditions (n = 16 per condition): SUP (choline supplemented diet) and STA (standard diet). The dietary treatment was identical to that described for Experiment 1; that is the SUP subjects spent 10 weeks on the special diet, and were returned to the standard diet at the start of behavioural testing. Half of the animals in each dietary group were assigned at random to the 24-h retention interval condition; half to the 48-h condition. There were thus 4 groups (n = 8 per group): SUP-24, SUP-48, STA-24, and STA-48.

As in Experiment 1, all subjects first received a session of habituation to the test situation, followed, on the next session, by familiarization with two identical objects (two porcelain jars). Two groups (one SUP and one STA) were tested 24 h later with one familiar and one novel object (jar). The rest of the subjects received the same test after a period of 48 h. In details not specified here the procedure followed that described for Experiment 1.

3.2. Results and Discussion

Data of two animals (one outlier during the test belonging to SUP-24 group, and one belonging to SUP-48 group which failed to explore the objects during the familiarization phase) were excluded from further analysis. In the familiarization session both objects were explored, and dietary condition was without effect at this stage. The group mean scores for total exploration time for the SUP and STA groups were 44.71 s and 44.25 s respectively, for those to be tested at the 24 h retention interval, and 59.14 s and 49.88, for those to be tested at the 48 h retention interval. The two objects were explored equally, both by the SUP (object 1 = 22.0 s and object 2 = 22. 71 s) and by STA groups (object 1 = 230 s and object 2 = 21.25 s) to be tested at 24 h; and by the SUP (object 1 = 31.86 s and object 2 = 27.29 s) and STA groups (object 1 = 26.0 s and object 2 = 2388 s) tested the 48 h retention interval. A 2 × 2 × 2 ANOVA, with diet, retention interval condition, and object as the variables, was conducted on the scores for time spent exploring each objects during the familiarization session yielded no significant main effects or interaction: all $Fs < 1$, apart from that for the main effect of interval where $F(1, 26) = 1.62$, $p = .22$.

The results of the test sessions are presented in Fig. 3, which shows group means for time spent exploring each object. The results for the groups tested after 24 h match those of Experiment 1; both groups showed preferential exploration of the novel object, and did so to the same extent. Subjects tested after 48 h showed more total exploration that those tested after 24 h ($t_{(28)} = 2.89$, $p = .007$, $d = .48$; right panel of Fig. 3). This contrasts with the results found in Experiment 1 (less exploration at 48 h), but in that experiment animals given the 48-h test had experienced another test

24 h previously. Importantly, for subjects tested after 48 h in this experiment, there was a difference according to dietary condition. No preference for the novel object was found in the STA group (although both objects were explored); by contrast the SUP group tested at 48 h showed a clear preference for novelty. An ANOVA, with dietary condition and retention interval as between-group variables and object novelty as a within-subject variable, revealed significant effects of retention interval, $F(1, 26) = 8.01$, $p = .009$, , $\eta_p^2 = .24$; novelty, $F(1, 26) = 52.37$, $p < .001$, $\eta_p^2 = .67$; and of the interactions of Diet x Novelty, $F(1, 26) = 9.48$, p = .005, $\eta_p^2 = 0.27$; and of Diet × Retention × Novelty, $F(1, 26) = 4.25$, $p = .049$, $\eta_p^2 = .14$. No other variables or interactions were significant ($Fs < 2$). The three-way interaction was explored by means of separate (Diet x Novelty) ANOVAs at each retention interval. At 24 h, only the novelty variable produced a significant effect, $F(1, 13) = 33.64$, $p = 0$, $\eta_p^2 = .72$; other $Fs < 1$. In contrast, at 48 h, there was a significant effect of novelty, $F(1, 13) = 19.13$, $p = .001$, $\eta_p^2 = .60$; no significant main effect of diet, $F(1, 13) = 1.\ 11$, $p = .31$, $\eta_p^2 = .08$ but, critically, a significant interaction of diet and novelty, $F(1, 13) = 14.75$, $p = .002$, $\eta_p^2 = .53$.

As in Experiment 1, we also computed exploration ratios, and Fig. 4 shows group mean scores for the SUP and STA groups tested at the 24-h and 48-h intervals. Comparing each score against the chance level of 0.5 by means on one-sample *t*-tests showed that at 24 h both SUP ($t_{(6)} = 6.86$, $p < .001$) and STA ($t_{(7)} = 4.25$, $p = 0.004$) groups were significantly higher than chance. At the 48-h interval the SUP group differed from the chance level ($t_{(6)} = 5.2$; $p = .002$), but the STA group did not ($t_{(7)} = 0.55$; $p = .59$). Analysis of the ratio scores confirmed the difference between the dietary conditions at the 48-h interval. An ANOVA with diet and retention interval as

the variables yielded significant main effects of diet, $F(1, 26) = 5.\ 9$, p = .02, $\eta_p^2 = 0.18$ and of retention interval, $F(1, 26) = 9.1$, $p = .006$, $\eta_p^2 = 0.26$ and a significant interaction, $F(1, 26) = 4.7$, $p = .04$, $\eta_p^2 = 0.15$. One-way ANOVAs at each retention interval found no significant difference between the SUP and STA groups at 24 h ($F < 1$), but a significant difference at 48 h, $F(1, 13) = 15.00$, $p = .002$, $\eta_p^2 = 0.54$.

The results of this experiment are quite clear in showing that giving dietary choline supplementation to mature adult rats can influence performance on the SOR test, promoting the tendency to explore a novel rather than a familiar object. No effect was seen (as in Experiment 1) after a retention interval of 24 h; but when tested after 48 h, a condition in which rats raised on the standard diet show no preference for the novel, a preference was still found in those given the supplementation. It seems that the failure to find an effect in Experiment 1 was a consequence of the insensitivity of the test procedure. The procedure used in that experiment generated good performance on the 48-h test, even in control subjects, making it difficult for any effect of dietary supplementation to show itself. With a true retention interval of 48 h the performance of control subjects was poor enough for the advantage conveyed by supplementation to be seen.

The difference between the groups in this experiment is not to be explained in terms of a direct effect on their behavior during the training (familiarization) phase; they did not differ in the exploratory behavior they showed at this stage. We conclude, therefore that dietary supplementation influences one or more of the cognitive processes involved in SOR performance – in the ability to acquire information during familiarization, the ability to retain this information over a retention interval, to use it during the test, or all of these. The possible contributions of these various processes will be taken up next.

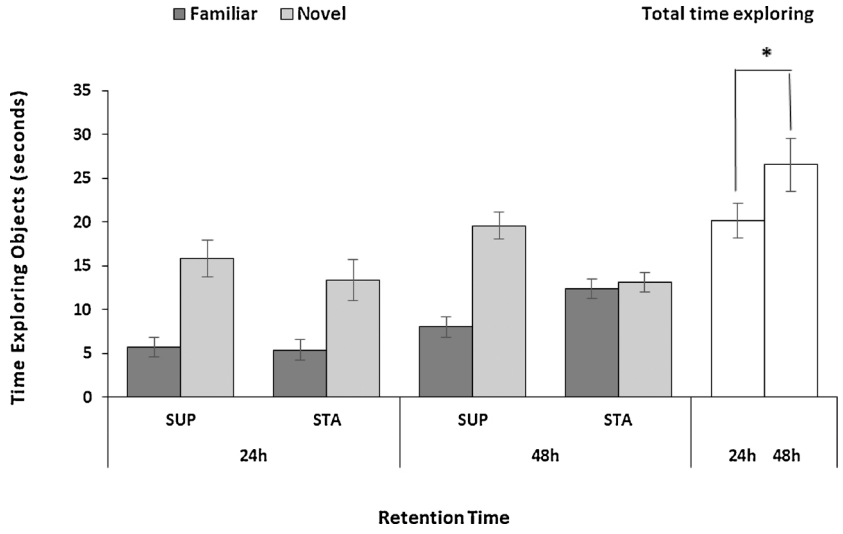

Fig. 3. Mean time in seconds (± SEM) supplemented (SUP) and standard (STA) animals spent exploring two objects (familiar or novel, left panels), and total time exploration of objects (familiar plus novel) by both dietary groups (right panel) during test sessions at 24 h or 48 h.

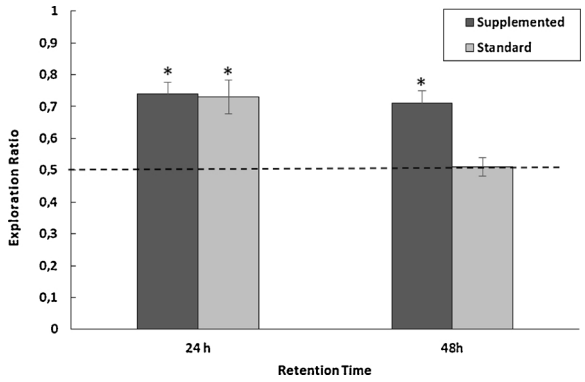

Fig. 4. Mean (± SEM) exploration ratios during the testing sessions at 24 or 48-h retention intervals for the groups receiving a choline supplemented (SUP) or standard (STA) diet in Experiment 2.

4. General discussion

Our experiments have shown that, given appropriate testing conditions, it is possible to demonstrate that choline supplementation given to mature adult rats will enhance performance on a long-term test of recognition memory (the SOR procedure). In contrast to the results reported by Tabassum et al. [30], the improvement could be detected only in a 48-h retention test since both supplemented and standard groups showed good recognition memory in the 24-h retention test. Tabassum et al. [30] found an effect of supplementation in a retention test applied 20 min after familiarization. This difference may indicate that supplementation has different effects on short-term and long-term memory mechanisms. Alternatively, the fact that Tabassum et al. [30] used much younger rats may be relevant. Although the authors did not specify the age, they used male Wistar rats weighing 150–200 g a weight that corresponds to 6–8 weeks of age. These cannot be considered to be adult as 3 months is usually considered the lower limit for young adulthood. In fact, we have previously demonstrated that 2-month-old male Wistar rats do not exhibit the adult pattern of behavior [34]. Our results better parallel those reported by Melichercik, Elliott, Biancha, Ernst, and Winters [35]. Their experiment used a long retention interval (72 h) such that performance of control subjects fell to chance on test; but in rats given an infusion of nicotine designed to activate the nicotinic ACh receptors of the perirhinal cortex, performance was much enhanced.

More generally, our results accord with those from a range of experiments (see, e.g., [18,36]) showing that disruption of the cholinergic forebrain system impairs SOR performance (see [19,20] for reviews). We can be confident, then, that cholinergic systems are involved in object recognition memory, but it remains to specify their exact role. Good performance on the SOR task requires that the rat acquires information about the objects during familiarization, retains this information over the retention interval, and is able to use it on test. The results obtained in our experiments (and in the other experiments just cited) could be by way of effects of the treatment on any of these stages.

A role for ACh in attentional processes is well established (see, e.g., [8,37]), and if choline supplementation enhances attention this might allow efficient acquisition of relevant information during familiarization and promote appropriate responding on test. Support for the proposal that cholinergic mechanisms play a modulatory role in stimulus processing comes from lesion studies in rats [38] and primates [39] showing that selective ablation of basal forebrain cholinergic neurons by IgG-saporin induces impairment of some forms of attention and perceptual learning. It should be acknowledged, however, the interpretation of such lesion studies can be debatable given the anatomy of the cholinergic systems with its widespread projections and close interactions with other neurotransmission systems [40]. A role for ACh in initial acquisition is also a feature of the Encoding versus Retrieval Scheduling (ERS) framework [e.g.,9], which holds that cholinergic mechanisms promote the response to novelty, enhance synaptic plasticity for the encoding of novel associations, and reduce proactive interference from previously formed associations on this new learning.

Equally influential has been the proposal that ACh is critical in supporting memory consolidation [13,41,42], and maintenance of what was learned during familiarization is clearly necessary for appropriate test performance. Pharmacological interventions provide evidence for a role of cholinergic systems in both long and short term recognition memory. Nicotinic receptor antagonists produce impairments at long delays (e.g., 24 h) between familiarization and test, whereas muscarinic receptor antagonists impair recognition memory at shorter delays (of the order of minutes) [20, for a review]. Finally, of course, ACh could have multiple functions [32,33], influencing cognitive processing at all stages in the procedure [14].

To determine which of these cholinergic mechanisms is influenced by our dietary treatment, and is responsible for the performance observed on our SOR task, requires a closer analysis of the cognitive mechanisms that are engaged by this task. In general terms the learning process is an instance of habituation. When it is first encountered, a

novel stimulus (an object in these experiments) evokes its normal unconditioned response of exploration. Experience of the object results in the formation of some central representation of it. When the object is experienced again there is a match between the input and the central representation, and the exploratory response no longer occurs [45]. For a more detailed specification of the processes involved in this form of habituation we may turn to the theory of memory developed by Wagner [46,47]. Robinson and Bonardi [48] in a recent review the psychological processes involved in SOR concluded that Wagner's theory supplies a satisfactory account of performance on various versions of the SOR task; also that it generates unique predictions that have largely been confirmed.

Wagner's [46] theory proposes that the central, cerebral representation of a stimulus (an object or event), which is activated by presentation of the stimulus, suffers a refractory period after presentation of the stimulus. Its sensitivity to further presentation of the stimulus will be reduced until the after-effects of an earlier presentation have decayed away. Thus, with sustained or repeated presentation of an object, the tendency of that object to elicit an exploratory response will diminish. When rats are presented with objects A and B after previous exposure to A and A, a tendency to explore B is to be expected if the after-effects of the initial presentation of A are still present. Another way of putting this is to say that the representation of A has been "primed" and is still active in memory. The duration of this priming effect is not specified; it may be just a matter of a few seconds for a brief simple stimulus, such as tone or light, in which case this mechanism would not be relevant to an SOR procedure in which the test follows after a period of hours. It is possible, however, that the effect could be much more prolonged when exposure is protracted and the stimulus is a complex object or event.

The process just described has been called "self-generated priming". Wagner's [46] theory also allows the possibility of "retrieval-generated priming". Exposure to a stimulus will engage associative learning processes so that, for example, a particular object might become associated with the context in which it is presented. When the subject is next put into that context, the representation of the object will be activated by way of the association (in other terminology, it will be retrieved and primed into memory) and response to it will be attenuated. Such effects need not be restricted to cueing by contextual cues. In the version of the theory developed by McLaren, Kaye, and Mackintosh ([49]; see also [50]) it is pointed out that exposure to complex stimuli having many features will allow links to be formed among these features – a process sometimes referred to as *unitization*. A unitized (i.e., familiar) stimulus will have a reduced capacity to evoke responding because the network of links among its features will allow detection of one to activate all the others, priming them into memory. Given that associative links are assumed to be long lasting, retrieval-generated priming (either by way of contextual cues or as a consequence of unitization) will allow the effects of prior exposure to an object to be found even after a long retention interval.

Applying this theoretical analysis to the present results suggests the following possibilities. First, with our stimuli and training procedures, the memory of the training experience (and critically of object A) is well activated after a 24-h retention interval – both control and supplemented subjects showed rather little exploration of the familiar object. If we assume that the self-generated priming process will not operate with a retention interval of this duration, this implies that retrieval-generated priming is operating in both groups – that both can retrieve the unitized representation of the familiar stimulus. But since control subjects failed to show a preference for the novel object with a 48-h interval we may conclude that information about this object has not been retained in these subjects over the longer interval. By contrast, that supplemented subjects still showed the preference after 48 h suggests that additional dietary choline has helped them to maintain or consolidate memory established by the original familiarization training.

To the extent that retrieval-generated priming may be taken to depend on associative learning, we might expect that such learning would be facilitated generally in animals that have received choline supplementation in adulthood. Although there are studies of the effects of prenatal diet on conditioning [51], there are rather few studies that allow us to assess the course of acquisition in animals given in adult animals given supplementation. Such evidence as is available does not allow any firm conclusion, but supplies no evidence of a general facilitation. Moreno et al. [29] looked at the acquisition of conditioned suppression in two experiments in which rats that had received supplementation in adulthood were given presentations of a light or tone followed by footshock. In one study the level of suppression was identical in supplemented and control animals; in the other, there was an indication of a difference, but with the supplemented subjects apparently learning slightly *less* readily. Such null results cannot, of course, be decisive, but if it could be shown that choline supplementation affects SOR performance but not simple conditioning this would challenge the theoretical framework being used here; it would lend support to the view that recognition memory engages processes other than those involved in simple associative learning.

An alternative account is possible within the theoretical framework supplied by Wagner's [46] theory. This proposes, not that supplementation helps the rats better maintain or consolidate the information acquired during familiarization; rather that it promotes acquisition of the information in the first place. When tested after 24 h, rats given the standard diet show evidence of a memory of the preexposed object (A) by showing a preference for the novel object. This could be because A's representation is activated associatively (the hypothesis we have just been considering): alternatively it may be because the effects of the initial presentation have not decayed away completely. If their performance on the 24-h test is principally a consequence of self-generated priming, and retrieval-generated priming is relatively unimportant for these subjects, then it might be expected that, with a longer retention interval that allows more opportunity for decay, the rats given the standard diet would show no evidence of a memory of object A. The fact that rats given the supplemented diet show perfectly good performance, even after 48 h, suggests the hypothesis that for these subjects the associative mechanism responsible for retrieval-generated priming is operating effectively.

Why should choline supplementation promote the occurrence of retrieval-generated priming? As we have said, there is little evidence to support the view that simple associative learning is facilitated in rats given supplementation in adulthood. What the study of Moreno et al. [29] did show, however, was an effect of supplementation on an aspect of attentional learning, and consideration of this factor allows a hypothesis about the source of the present results. Moreno et al. were using the conditioned suppression procedure to monitor the effects of prior exposure to a stimulus (a light or tone) on it ability to serve as a conditioned stimulus in classical conditioning. They demonstrated that such exposure, when the stimulus was followed consistently by a given event (Experiment 1) or by no event at all (Experiment 2), resulted in slowed subsequent conditioning for animal raised on the standard diet. Such effects have been obtained repeatedly in rats raised under standard conditions. They are to be expected on the basis of the account of conditioning put forward by Pearce and Hall ([52]; see also [53,54]), according which, training of this sort will bring about a reduction in the "associability" of the stimulus (of its readiness to enter into associations). The new finding of Moreno et al. was that these effects were not present in rats given supplementation; that is, these animals learned readily in the test stage even with stimuli that had been preexposed. Moreno et al. concluded that the mechanism that reduces the attention

paid to uninformative stimuli (more precisely, reduces the associability of the stimuli that have consistent consequences) was not functional after choline supplementation.[2] (See also [23]). Such a change in attentional processing could have relevance to the performance of rats on the SOR task.

We have argued that rats given supplementation showed good performance on the SOR test, even after an interval of 48 h, because, for them, the retrieval-generated priming process was particularly effective. This would occur if these rats had a strong association between the training context and the objects presented in it, whereas the control subjects did not. In fact, a weak association in the control subjects might be expected on the basis of standard theories. In our procedure (as is usual; see, e.g., [25]) the rats received an initial phase of exposure to the arena to be used in training and test. That is, they received preexposure to the context. Attentional learning processes would thus lead to a reduction in the associability of contextual cues; association with the context and the objects would form poorly during the training stage, and context-based retrieval would contribute little to performance on test. The hypothesis that the rats that have received choline supplementation lack the normal mechanism for reducing associability leads to the prediction that they will be able to learn about the context and thus show retrieval-generated priming when tested after a long interval. There is nothing in the present data to allow a choice among the various hypotheses presented above, and indeed it may be inappropriate to try to choose among them. It is entirely reasonable to suppose that manipulation of choline levels by way of the diet will have a widespread effect on a range of cholinergic systems and that the behavioral effects will have a range of sources [43], involving all or many of stages of information processing that lie between initial exposure to the stimuli and a later test.

Finally we should conclude by restating the positive conclusion to be drawn from this work, which is the confirmation that supplementation of dietary choline can have positive cognitive effects even when it is given only in adulthood. We may hope this will have relevance to the search for interventions that might be used to alleviate cognitive decline in humans. Recent work on the effects of acute administration of a choline supplement on human cognitive functioning in healthy young adults [55] is encouraging in this regard. Although supplementation did not facilitate the performance of subjects who already performed well on the cognitive tasks employed, the supplement was found to enhance processing speed, working memory, verbal learning, verbal memory, and measures of executive function in individuals whose initial performance on these tasks was relatively poor.

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