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Research report

Chronic dietary choline supplementation modulates attentional change in adult rats

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HIGHLIGHTS

- Adult rats were maintained on a supplemented with choline.
- Experiment 1 investigated the effects of prior training with a stimulus on subsequent acquisition of conditioned suppression.
- Experiment 2 investigated the effect of prior nonreinforced exposure (latent inhibition).
- In both experiments, choline supplementation disrupted the loss of stimulus associability normally produced by preexposure.
- Chronic exposure to a choline-supplemented diet alter the behavior of adult rats.

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ABSTRACT

In two experiments adult rats were maintained on a diet enriched with added choline for 12 weeks prior to behavioral testing; control rats remained on the standard diet during this time. In Experiment 1 all rats received training in the Hall-Pearce negative transfer paradigm in which prior training with a conditioned stimulus (CS) paired with a small reinforcer retards further learning when the size of the reinforcer is increased. This effect, which has been attributed to a loss of associability by the CS, was obtained in control subjects but not in those given the supplement. Experiment 2 investigated the effect of prior nonreinforced exposure of the to-be-CS (latent inhibition). Such exposure retarded subsequent learning in control subjects, but latent inhibition was not obtained in those given the supplement. We conclude that the mechanism that reduces the attention paid to a stimulus that accurately predicts its consequences does not operate effectively after choline supplementation. These results are consistent with a role for the cholinergic system of the basal forebrain in modulation of attention.

1. Introduction

Choline, is a quaternary amine classified within the vitamin B complex, present in many foods. It is regarded as an essential nutrient [1,2]. It is necessary for the normal functioning of all cells due to the role that it plays in the synthesis of phospholipid components of the membrane; it is also a precursor of the neurotransmitter acetylcholine (ACh) [3]. The availability of this precursor can determine the speed of production and liberation of the neurotransmitter and will be influenced by diet. Choline transport at the blood-brain barrier depends on plasma concentration; in basal conditions this is between 8 and 11 μM of free choline in humans and experimental animals, but this can increase to about 40 μM in humans and up to 50 μM in rats [4,5] after the ingestion of choline-rich food. Dietary supplementation will thus increase levels of cerebral choline, and promote the synthesis and emission of ACh in the brain [4,6,7]. Conversely, choline restriction reduces serum concentration, and diminishes the production of ACh in cholinergic neurons [8–10].

Studies in which levels of dietary choline have been manipulated have shown an effect on cognitive functioning in experimental animals given tasks taken to depend on the cholinergic system of the forebrain. For the most part these studies have focused on the role played by choline availability very early in development, usually perinatally (see Ref. [11], and Ref. [12], for reviews; also Ref. [13]). Evidence on the effects of choline in older subjects is sparse and contradictory (see Ref. [14], for a review), although there is some evidence for effects in rats that might be classified as adolescent or young adult at the time of the dietary manipulation. Thus, rats

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given supplementary choline for 21 weeks from the age of 5 weeks have been found to show improved performance on a temporal discrimination task [15], and rats given a choline-deficient diet for 12 weeks from the age of 2 months showed a memory deficit in a task of passive avoidance [10]. There are few studies available examining cognitive functioning after dietary manipulation exclusively in adulthood. However, Teather and Wurtzman [16] have shown that 12 weeks of access to a high-choline diet for 3-month-old rats attenuated a memory deficit caused by exposure to an impoverished environment, and Moreno et al. [17] found better retention of a context aversion in rats of the same age given 7 weeks of supplement. These findings were enough to encourage us to investigate the effects of supplementation on fully adult subjects.

We chose to investigate the effects of dietary choline on behavioral tasks designed to assess an aspect of attention. The ability of manipulations of choline levels to influence cognitive functioning may be assumed to operate by way of an effect in the basal forebrain cholinergic system, the main source of cholinergic input to the cortex and the limbic system. Lesions of this system have been found to generate a wide range of effects, but, according to Sarter and Bruno [18], they are largely consistent with the hypothesis that cholinergic input to the cortex mediates the subject's ability to select stimuli for processing (see also Ref. [19]). This form of attention has been intensively studied by Holland and Gallagher and their collaborators, using a set of behavioral paradigms that allows specification of the detailed mechanisms involved (see Ref. [20], for a review). One paradigm of particular interest assesses the ability of rats to resume attending to a stimulus with which they have grown familiar, when the consequences of that stimulus are changed. In this procedure (devised by Wilson et al. [21]) rats are trained initially with a target stimulus (a light) reliably followed by another (a tone). When, in the test phase, the light is used to signal the immediate availability of food, conditioned responding develops slowly. This is taken to indicate that, during the first phase, the light, being a reliable predictor of its consequences, loses the power to govern attention (suffers a decline in its *associability*, [22]); subsequent conditioning is thus retarded. This retardation can be eliminated, however, if, between the two phases, the rats experience some trials on which light-tone trials are intermixed with light alone trials. The surprising change in the consequence of the light restores its lost associability, allowing learning to occur normally on the test.

Holland and Gallagher [23] investigated the effects of lesions of the central nucleus of the amygdala on the task just described. They found that after such lesions the interpolated "surprise" trials were without effect, so that learning in the test phase remained slow. They concluded that the lesions had disrupted the mechanism responsible for restoring lost associability. Their interpretation was that the central nucleus regulates the surprise-induced increase in associability by way of its interaction with the basal forebrain cholinergic system. Direct support for this interpretation came from a study by Chiba et al. [24] who gave rats given central infusions of a form of saporin that produces selective lesions of cholinergic neurons. When tested in the procedure of Wilson et al. [21], subjects with saporin-induced lesions in the caudal region of the forebrain (the region that provides the primary cholinergic input to the cortex) behaved like rats with lesions of the amygdala, in that they failed to show the normal, surprise-induced restoration of associability.

In a subsequent study, Baxter et al. [25] reported a parallel investigation of the effects of saporin-induced lesions of the rostral region of the basal forebrain, a region that projects primarily to the hippocampus. Rats given this treatment learned readily in the test phase of the Wilson et al. [21] procedure, and did so whether they had experienced the surprise trials or not. This result suggests that in these animals the normal loss of associability produce by the first phase of training had failed to occur. Han et al. [26] have observed a similar effect in rats given neurotoxic lesions of the hippocampus itself. These and related results have been taken to support the general conclusion that increase and decreases in associability are mediated by distinct and separate brain mechanisms (see, e.g., Ref. [20], for a review).

Prompted by these observations Meck, Jones, Williams, Paul dine, and Holland (1997; reported in Ref. [11]) examined the effects of variation in dietary choline on performance in the Wilson et al. [21] procedure. Choline was manipulated prenatally (i.e., via the diet of pregnant dams, whose offspring were the experimental subjects). There were three groups: one in which the mothers were maintained on a standard diet, one in which they were given a diet with supplementary choline for 7 days during the second half of gestation, and one in which they were given a choline-deficient diet during this period. When tested in adulthood the offspring of mothers in the choline-deficient condition, like rats with lesions of the amygdala and rats treated with saporin in the caudal region of the basal forebrain, showed slow learning in the test phase even after the surprise trials–in these animals the mechanism responsible for reducing the associability of a consistent predictor appeared to work normally, but that responsible for restoring lost associability did not. Performance on this task was also influenced by supplementation of choline. Subjects in this condition learned readily in the test phase, and did so whether they had experienced the surprise trials or not; that is, like rats with lesions of the rostral region of the basal forebrain or with hippocampal lesions, these 2.1.1subjects appeared to be resistant to the loss of associability normal induced by the first phase of training. Thus, choline deficiency disrupts the processes necessary for an increase in associability when this has fallen to a low level, but choline supplementation prevents loss of associability on the first place.

Accordingly, in our initial studies of the effect of choline supplementation in adult subjects, we decided to examine training procedures that are effective in producing decrements in stimulus associability in normal animals. The first of these, used in Experiment 1, and sometimes known as Hall–Pearce negative transfer [27], has something in common with the well-established latent inhibition effect [28] – the retardation of conditioning produce by prior nonreinforced exposure to the to-be-conditioned stimulus. However, it shares with the procedure of Wilson et al. [21] that in the initial phase of training, the target stimulus is followed by a consistent consequence. According to Pearce and Hall [22] subsequent poor learning about this stimulus results from a loss of stimulus associability during the first phase of training. The second procedure (used in Experiment 2) was latent inhibition itself. This effect may be multiply determined (see Ref. [29], for a review) but an important component is the loss of associability generated by the preexposure treatment [30].

2. Experiment 1

In this experiment we compared rats that had been maintained throughout their lives on a standard laboratory diet with rats given a diet containing supplementary choline for 12 weeks from the age of 8 months. The rats were tested using the conditioned suppression procedure. The design of the experiment is summarized in Table 1. The rats received an initial phase of training in which the conditioned stimulus (CS; a tone for half the subjects, a light for the rest) was followed by a relatively weak footshock, the intensity of the shock being chosen to generate a moderate level of suppression of the baseline response (food-reinforced lever pressing). The second stage assessed the acquisition of further suppression with a shock of increased intensity, all the rats now experiencing the tone as the CS. The control subjects can be expected to show the

Table 1
Experimental designs.

Experiment 1		
Group	Phase 1	Phase 2
SUP-T	T → US_{Weak}	T → US_{Strong}
SUP-L	L → US_{Weak}	
CON-T	T → US_{Weak}	
CON-L	L → US_{Weak}	

Experiment 2		
Group	Preexposure	Conditioning
SUP	T or L	T → US_{Shock} and L → US_{Shock}
CON		

Note: SUP = supplemented diet, CON = standard diet; T and L = tone and light CSs; US_{weak} = electric footshock of 0.25 mA; US_{strong} = electric footshock of 0.5 mA; US_{Shock} = electric footshock of 0.25 mA.

negative-transfer effect of Hall and Pearce [27], with subjects pretrained with the tone learning more slowly than those pretrained with the light. According to Pearce and Hall [22] this effect occurs because the initial phase of training produces a reduction in the associability of the CS used in that stage. The question of interest was whether rats given the choline supplement would show this effect. If supplementation disrupts the mechanism responsible for reducing associability, we might expect the negative-transfer effect to be absent in these subjects.

2.1. Method

2.1.1. Subjects and diet

The subjects were 32 male Lister hooded rats supplied by Harlan Laboratories UK. After arriving in the York laboratory at the age of 3 months, they were housed in pairs in an environmentally controlled colony room, with a 12 h light/dark cycle. Experimental sessions occurred during the lit periods of the cycle. Before the start of the present experiment the rats were maintained with ad libitum access to Certified Rodent Diet 5002 (supplied by LabDiet; PMI Nutrition International). This is the standard diet used in our laboratory; it contains 2 g/kg of choline chloride. The rats were initially used in a study of flavor preference conditioning, but were naïve with respect to the procedures of the present experiment, which commenced when they were aged 8 months (when the rats had a mean weight of 608 g; range: 470–710 g).

Previous work on perinatal [11] and adult [16,17,31] supplementation has demonstrated effects with choline concentrations between 2.6 and 5 times higher than that of the standard diet. We made use, therefore, of a supplemented version of the rodent diet of the American Institute of Nutrition (AIN) which produces a 4.5-fold increase of choline with respect to the AIN-76A standard diet. (The standard AIN 76-A diet supplies 1.1 g/kg of choline; the supplemented diet supplies 5 g/kg.) Sixteen rats were assigned to the AIN 76-A standard diet and the remainder were given the supplemented formulation. For the next 12 weeks of dietary manipulation the appropriate food was provided ad libitum. At the end of this period, all were transferred to a restricted feeding regime with the AIN 76-A standard diet, and were maintained at 85% of their free-feeding body weights until behavioral testing was complete.

2.1.2. Apparatus

Eight Skinner boxes (Med Associate Inc, St. Albans, VT) were used. These measured 30 cm × 24 cm × 21 cm and were housed in sound-attenuating chests. The ceiling and two longest sides of the chamber were made of clear plastic, and the front and back walls of stainless steel. A houselight, set high on the rear wall, provided dim illumination. Each box was equipped with a response lever on the front wall. Situated to the right of the lever was an aperture (5 cm × 5 cm), that gave access to a food cup to which 45-mg Noyes food pellets could be delivered. The floor of the chamber consisted of stainless steel rods to which a scrambled shock could be delivered from a Coulbourn Instruments (Allentown, PA) shock source. Two different stimuli were used as CSs. Set above of the lever was a 100-mA 28-V lamp, which provided the light stimulus. The second CS was 2.5 kHz tone, at 80 dB, generated by a speaker adjacent to the houselight. Both stimuli had a duration of 60 s.

2.1.3. Procedure

Training consisted of daily 40-min sessions. The baseline response of lever pressing was established over the first five sessions. In the first session, food pellets were delivered on a variable-time (VT) 30-s schedule while lever press responses were continuously reinforced by delivery of a food pellet; in the second, each lever press again yielded a single food pellet again but in addition a delivered VT 60-s schedule was in effect. Reinforcement was delivered according to a variable interval (VI) 30-s schedule in Session 3 and a VI 60-s schedule in Sessions 4 and 5. The VI 60-s schedule remained in force throughout the rest of the experiment.For the first phase of conditioned suppression training, half the rats in each of the main groups (supplemented, SUP, and control, CON) were assigned to conditioning with the light (L) as the CS and half to the tone (T), making the four groups of 8 (SUP-T, SUP-L, CON-T, CON-L) of Table 1. There were five sessions of Phase-1 training, each containing 4 trials, consisting of presentation of the CS followed immediately by a 0.5-s, 0.25-mA footshock. Stimulus presentations occurred 5, 15, 25 and 35 min after the start of the session. All subjects were treated identically during the five sessions of Phase 2. The four groups received 2 trials per session in which the tone was followed by a shock of 0.5 mA for 0.5 s. Presentations of the tone occurred 5 and 25 min after start of the session. Responding was recorded during the CS and during the 60-s period (the preCS period) that preceded each trial. Suppression ratios were calculated for each session after pooling all CS and preCS scores for a given animal. These ratios took the form $a/(a+b)$, where a represents the rate of response in the CS and b the rate in the preCS.

2.2. Results and discussion

The rats readily acquired the baseline response during the pretraining sessions. Unexpectedly, an effect of diet was evident at this stage, with rats in the supplemented condition responding much more frequently than the control subjects. On the last day of pretraining rats in the SUP groups had a mean rate of response of 25.65 responses per min; those in the CON groups had a rate of 10.76 responses per min. These scores differed significantly, $F(1,29) = 15.51$, $p < .01$ (here and throughout a significance level of $p < .05$ has been adopted).

This difference in baseline responding was maintained throughout conditioned suppression training. The left panel Fig. 1 shows

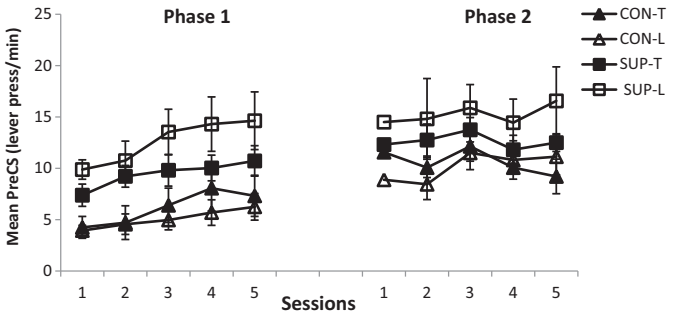

Fig. 1. Experiment 1: Mean (±SEM) baseline (PreCS) response rates recorded during Phases 1 and 2. SUP = supplemented; CON = control; T = tone; L = light.

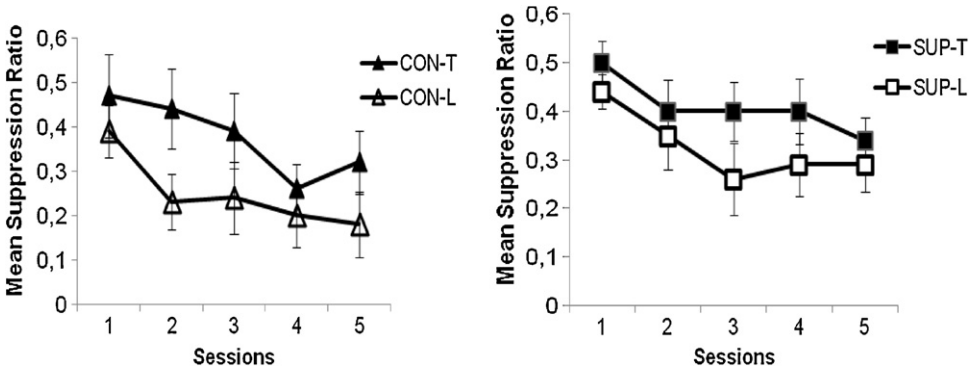

Fig. 2. Experiment 1: Mean (±SEM) suppression ratios during Phase 1 for supplemented (SUP) and control (CON) groups; T = tone; L = light.

group mean responses per preCS period over the five sessions of Phase 1. For all groups, response rate tended to increase, but a higher rate was consistently shown by the SUP than the CON groups. An analysis of variance (ANOVA) with session, diet (CON or SUP), and type of CS (T or L) as the variables, showed there to be significant effects of session, $F(4,108) = 9.38$, $p < .01$, and of diet, $F(1,27) = 14.97$, $p < .01$. There was no significant effect of CS-type, and none of the interactions among variables achieved significance; largest $F(1, 27) = 2.30$, for the Group × Diet interaction.

Fig. 2 shows the development of conditioned suppression during Phase 1. It is evident that all four groups acquired a moderate level of suppression by the final session and it appears that the light was somewhat more effective as a CS than was the tone, in that means for the L groups were consistently lower than those for the T groups. The overall levels of suppression were less in the SUP than in the CON groups, but a direct comparison of the suppression ratios of these groups would not be legitimate given that they are derived from substantially different baseline response rates. Accordingly we conducted separate analyses for the CON and SUP groups, with CS-type and session as the variables. One subject in the CON-T group lost baseline responding during Phase 1, making it impossible to compute a suppression ratio; this subject was excluded from further analysis, resulting in $n = 7$ for the CON-T group. For both the CON and the SUP groups, there was a significant effect of session: $F(4,56) = 8.69$, $p < .01$, for the SUP groups, and $F(4,52) = 5.32$, $p < 0.01$ for the CON groups. The difference between tone and light turned out to be nonsignificant; there was no significant effect of CS type in either dietary condition ($F_s < 1.2$), and in neither was the interaction of the variables significant ($F_s < 1.5$).

The acquisition of suppression in Phase 2 (in which all animal received the tone followed by the stronger shock) is shown in Fig. 3; the baseline preCS scores, from which the ratios were derived, are shown in the right panel of Fig. 1. An error on the part of the experimenter meant that data were lost on Session 1 for four of the subjects in groups SUP-L and CON-L (although the rats experienced the events as scheduled). Accordingly the mean scores shown in the figure for the first session for groups SUP-L and CON-L are derived from the remaining four subjects in each of these groups. A full set of data was available for the remaining four sessions, and statistical analysis was confined to the data from these sessions. Introduction of the stronger shock resulted in a reduction in the baseline response rate, but the rate of the SUP groups remained higher than that of the CON groups (Fig. 1). An ANOVA conducted on the data shown in the figure with CS-type, dietary condition and session as the variables revealed only a significant main effect of diet, $F(1,27) = 5.01$, $p < .05$; for other main effects and interactions, $F < 1$. Given this difference, analyses were again conducted separately on the suppression ratios of the CON and the SUP groups.

As Fig. 3 (left panel) shows, the CON group that had received Phase-1 training with the tone acquired suppression rather poorly compared with the CON group pretrained with the light, thus replicating the negative transfer effect of Hall and Pearce [27]. An ANOVA with group (Phase 1 with T or with L) and session as the variables revealed significant main effect both of group, $F(1,13) = 9.18$, $p < .05$, and of session $F(3,39) = 5.30$, $p < .01$; the interaction between the variables was not significant, $F(3,39) = 1.1$. The SUP groups by contrast (Fig. 3, right panel) both learned readily in Phase 2 and at much the same rate. For these groups the equivalent ANOVA revealed only a significant main effect of session, $F(3,42) = 42.8$, $p < .01$; neither the effect of group, $F(1,14) = 1.8$, nor the Group × Session interaction, $F(3,42) = 1.5$ was significant.

The results of this study demonstrate that chronic dietary supplementation with choline can produce behavioral effects even in fully adult rats. This is shown both in the elevated rate of food-reinforced lever pressing shown by rats given the supplement and also by that fact that these rats failed to show retarded acquisition

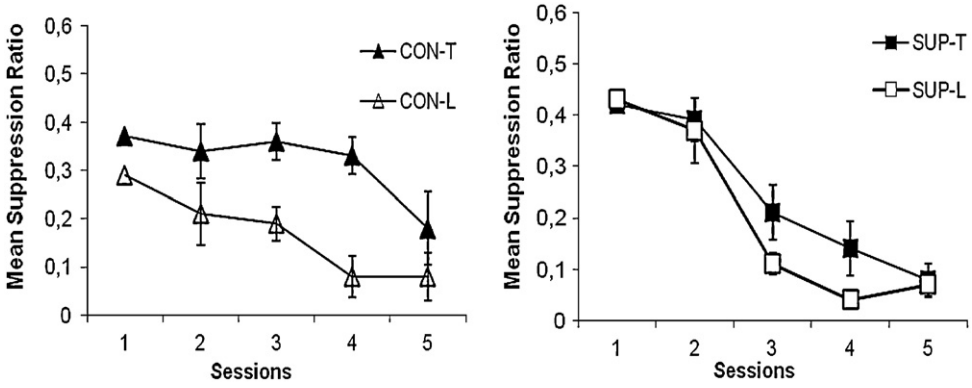

Fig. 3. Experiment 1: Group mean (±SEM) suppression ratios during Phase 2 for choline supplemented (SUP) and control (CON) groups. (T = tone; L = light.)

of conditioned suppression after pretraining with the CS. The first effect is novel and needs to be confirmed by further work; the second is consistent with the results reported by Meck and Williams [11], suggesting that choline supplementation disrupts the process by which the associability of a consistent predictor is normally reduced. This proposal was examined further in Experiment 2, which also allowed a further examination of the effects of supplementation on food-reinforced leverpress responding.

3. Experiment 2

According to Pearce and Hall ([22]; see also Ref. [30]) the negative transfer effect of Experiment 1 and the latent inhibition effect have the same source. In both, it is suggested, experience of a stimulus followed by a consistent consequence (the absence any event, in the case of latent inhibition) leads to a loss of associability, so that subsequent conditioning is retarded. On the basis of the results of Experiment 1, therefore, we might expect that choline supplementation would abolish or attenuate the latent inhibition effect.

The design of the experiment is shown in Table 1. As before, there were two main groups of subjects, those given choline supplementation in adulthood (SUP), and those maintained on a standard diet (CON). During the first phase of training all received nonreinforced presentations of a stimulus (a tone for half of each group; a light for the rest) that was to be used as a CS in conditioned suppression training in the test phase. During the test all subjects received reinforced trials both with the tone and with the light. Latent inhibition should be evident as slower acquisition to the stimulus preexposed in the first phase; the question of interest was whether such an effect would be obtained in the SUP group.

3.1. Method

The experiment was conducted at the University of Granada. The subjects were 16 male Wistar rats supplied by Harlan Laboratories. After arriving in the Granada laboratory at the age of 3 months, they were housed four to a cage in an environmentally controlled room under a 12-h light/dark cycle, with ad libitum access to the standard diet, AIN 76-A. At age 8 months (mean weight 519 g; range: 422–614 g) eight subjects were assigned to the SUP condition and for 12 weeks were given access to the supplemented formula of the AIN 76-A diet; the CON group remained on the standard diet. At the end of period of dietary manipulation all were given the standard diet but feeding was restricted to reduce the animals to 80% of their free-feeding weights, prior to behavioral testing. The apparatus consisted of four Med Associates operant chambers. These measured 32 cm × 25 cm × 34 cm, and the speaker supplying the auditory stimulus was located on the front wall above the stimulus light; otherwise they were identical to those described for Experiment 1.

Pretraining established a baseline of food-reinforced leverpressing on a VI 60-s schedule, and this schedule was maintained throughout the experiment. The preexposure phase consisted of five 40-min sessions with a 60-s stimulus being presented four times, 5, 15, 25, and 35 min after the start of the session. For half the animals in each group this was the light, and for half it was the tone. The conditioning phase consisted of a single session of four trials in which presentation of the CS was followed by a 0.5-s, 0.25-mA footshock. On two of the trials the CS was the tone and on two it was the light, the trial sequence was counterbalanced, being presented in the sequence TTLL for half the subjects in each group and in the sequence LLTT for the remainder. For each individual subject, the scores for both trials of a given type were pooled to compute a suppression ratio for that session. Details not specified here were the same as those described for Experiment 1.

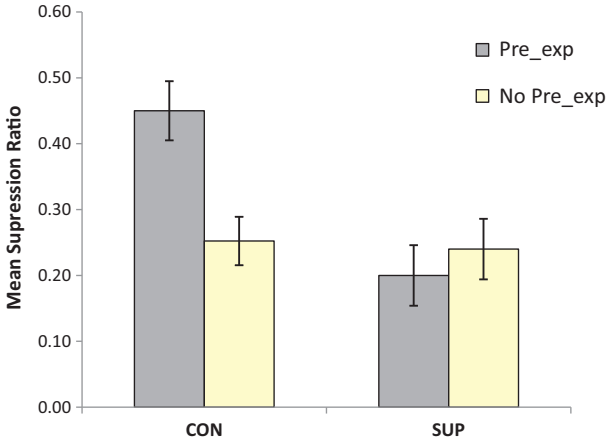

Fig. 4. Experiment 2: Mean (±SEM) suppression ratios on the conditioning session shown to a CS that had been presented in the preexposure phase (Pre_Exp) and to a novel CS (No Pre_exp). Subjects in the SUP group had received choline supplementation; control (CON) subjects had not.

3.2. Results and discussion

Apparatus failure meant that data were lost for two subjects in the CON group (one preexposed to the light. the other to the tone), reducing the group size to six.

The leverpress responding established by pretraining was maintained at a high rate throughout preexposure and conditioning. In contrast to Experiment 1, however, there was no substantial difference in rates between the SUP and CON groups. Thus on the last day of the preexposure phase the mean response rates recorded during the preCS periods were 29.3 responses per min for the CON group and 30.6 responses per min for the SUP group; these rates did not differ reliably ($F < 1$). The mean baseline response rates pooled over all preCS periods for the conditioning phase were 16.77 responses per min for the CON group and 17.34 responses per min for the SUP group; again, these did not differ reliably ($F < 1$). The marked difference between this and the previous experiment in the effect of diet on baseline responding raises the possibility that the different strains used in this investigation (Lister rats in Experiment 1, Wistar rats in Experiment 2) are differently sensitive to the effects of diets. However this may be, the lack of a difference between the SUP and CON groups in this experiments has a positive feature in that it is possible to make a direct comparison of the suppression ratios of the two groups that is not complicated by differences in baseline levels of responding.

Presentations of the stimuli (the tone and light) during the preexposure phase generated some unconditioned suppression of responding, that persisted throughout the phase. Thus on the last session of preexposure the mean suppression for subjects exposed to the tone was .39 in the SUP group and .44 in the CON group; suppression scores for the light were .33 for the SUP group and .37 for the CON group. An ANOVA with stimulus (tone or light) and dietary group as the variables showed there to be no significant effect of diet, $F(1,10) = 3.70$, $p > .05$, but there was a significant effect of stimulus, $F(1,10) = 9.48$, $p < .05$. The interaction was not significant ($F < 1$.)

The results of the conditioning test session, group mean scores for suppression to the preexposed and the novel CS, are presented in Fig. 4. It is evident that, for the CON group, suppression was acquired readily to the nonpreexposed stimulus but not to the preexposed stimulus; that is, the standard latent inhibition effect was

obtained. The SUP group, by contrast failed to show latent inhibition, suppressing to both the preexposed and the nonpreexposed CS, at a level similar to that shown by CON subjects to the nonpreexposed CS. Statistical analysis confirmed this description of the results. We conducted an ANOVA with dietary condition and stimulus novelty (preexposed or not) as the variables; and given the difference between tone and light observed during preexposure we also included stimulus-type (light or tone) as a variable. This yielded a significant main effect of diet, $F(1,10) = 8.17$, $p < 0.05$, and of the interaction between diet and stimulus novelty, $F(1,10) = 11.53$, $p < .01$. No other effects or interactions were significant; largest, $F(1,10) = 4.74$. Analysis of the interaction confirmed that the score for the preexposed condition differed from that for the nonpreexposed condition in the CON group, $F(1,5) = 13.95$, $p < .05$, but not in the SUP group ($F < 1$). Further, the CON and SUP groups differed for the preexposed condition, $F(1,13) = 15.67$, $p < 0.01$, but not for the nonpreexposed condition ($F < 1$).

Although these results are consistent with the proposal that the CON group shows latent inhibition (i.e., retarded acquisition to the preexposed CS) and the SUP group does not, other possibilities should be mentioned. First, (for those conditions that showed it) suppression was acquired very rapidly; and it was not evident at all in the preexposed CON condition, whose test performance was closely similar to the level shown at the end of preexposure. One interpretation of this pattern of results is that the test scores reflect not conditioned suppression, but an enhancement of the unconditioned suppression evoked by the stimuli as a consequence of the introduction of shocks. The performance of the preexposed CON condition would thus reflect the fact that the unconditioned response to the CS had habituated during preexposure; in this case the occurrence of suppression in the preexposed SUP condition would indicate not an absence of latent inhibition but a failure to habituate the unconditioned response to the preexposed stimulus in the first phase of training. Although this possibility would be of interest in itself, the results of the preexposure phase argue against it. In that phase unconditioned suppression was observed, but it was seen to the same extent in both groups, suggesting that there was no difference between them in the degree of habituation they showed.

A second possible alternative interpretation arises from our use of a within-subject testing procedure. With this procedure, for subjects to show latent inhibition to just one of the test stimuli, it is obviously necessary that they be able to discriminate between the stimuli. Similar levels of suppression to the two stimuli, as shown by the SUP group, might thus indicate an inability to discriminate between them rather than the absence of latent inhibition to the preexposed stimulus. Evidence against this interpretation comes from a comparison of the levels of suppression shown by the two groups in Fig. 4. If the SUP subjects do suffer from latent inhibition but fail to discriminate the preexposed from the nonpreexposed stimulus, then we would expect that learning about both these stimuli would be retarded. Thus the performance of the SUP subjects to both stimuli should be similar to that shown by the CON subjects to the preexposed stimulus. But in fact their performance matched that of the CON subjects to the nonpreexposed stimulus; that is they learned readily to both cues, consistent with the suggestion that latent inhibition influenced acquisition to neither of them.

4. General discussion

The experiments reported here demonstrate that chronic exposure to a choline-supplemented diet can alter the behavior of fully adult rats. In both experiments the subjects were 8 months old at the start of the dietary manipulation (and 12 weeks older than that at the start of behavioral testing). Both experiments produced results suggesting that choline supplementation modifies the processes responsible for learned changes in attention to significant environmental stimuli – specifically that the mechanism responsible for reducing the associability of stimuli in certain circumstances fails to operate normally after choline supplementation. Our results thus confirm that an attentional effect previously observed after perinatal supplementation can be obtained in adults, and do so using behavioral assays that complement that used in previous research.

Previous work (e.g., [20]) has suggested that there are separable cholinergic mechanisms in the basal forebrain for producing learned changes in associability, with the rostral region being responsible for loss of associability and the caudal region being responsible for restoration of lost associability. In these circumstances there are no grounds for predicting what the effects of a general increase in ACh levels, such as will be produced by choline supplementation, are likely to be. The results reported by Meck and Williams [11], however, showed that supplementation prevented the loss of associability normally seen in the appetitive training paradigm of Wilson et al. [21]. In this paradigm, control rats that have had training in which a light is reliably followed by a tone learn poorly in a subsequent test in which the light is followed food, a result interpreted as indicating that experience of the tone as a reliable predictor on a consequence produces a loss of associability. Our Experiment 1 used a related procedure [27] in which initial training with the target CS reliably signaling a weak shock was followed by a test stage in which the shock intensity was increased. Control subjects showed retarded learning in the test stage, interpreted as being the consequence of loss of associability in the first phase. Subjects given the dietary supplement learned readily in the test stage, suggesting that the initial loss of associability had failed to occur.

Support for this interpretation came from Experiment 2 in which conditioning was assessed after prior nonreinforced presentations of the event to be used as the CS. According to Pearce and Hall [22] the retarded learning produced by such preexposure (latent inhibition) is also a consequence (in part; see Ref. [30]) of a reduction in the associability of the preexposed stimulus. Latent inhibition was obtained in our control subjects, but not in the subjects given choline supplementation, supporting the view that the mechanism responsible for reducing associability is dysfunctional in the latter.

Before pursuing the implications of these findings it would be worthwhile to establish that the effects obtained are specific to attentional learning and are not a consequence of some more general learning deficit. The ready acquisition of food-reinforced lever press responding by the SUP group of Experiment 1 may seem to suggest quite the opposite, but these results need to be treated with caution. A high rate of response may reflect an effect on performance rather than on the acquisition of the relevant association. As for central neurons, ACh synthesis and liberation in motor neurons will depend on choline availability and some studies have shown that small doses of choline will improve neuromuscular transmission [32] and increase liberation of ACh at the neuromuscular junction [33]. The heightened activity shown by the SUP group of Experiment 1 could thus be a peripheral effect. A further reason for caution is that the effect was obtained only with the rats used in Experiment 1 and not with those used in Experiment 2. This result is interesting in itself and highlights the possibility the effects of dietary choline levels might interact with genotypic differences; it does, however, preclude us from concluding that choline supplementation enhances lever press acquisiton generally.

When it comes to classical conditioning, there is no reason to think that choline availability modifies simple acquisition. Thus, for example, Lamoureaux et al. [34] found no effect of prenatal supplementation on the acquisition and extinction of a noise → food

association (although the sensitivity of the rats to contextual factors was modified). In our aversive conditioning procedure the overall level of suppression established by phase-1 training in Experiment 1 was somewhat less in SUP than in CON subjects, but the difference in baseline response rates makes a direct comparison of the suppression ratios of the two groups difficult to interpret. In Experiment 2, however, where baseline rates were comparable, there was no difference between the SUP and CON groups in acquisition of suppression to the nonpreexposed stimulus, supporting the conclusion that the effect of supplementation was confined to the learning process responsible for retarded learning about the preexposed stimulus in the CON group (i.e., for the latent inhibition effect).

It remains to explain why long-term choline supplementation should impair the ability to reduce attention. The results reported here parallel those reported for the effects of lesions of the cholinergic system of the basal forebrain [25] prompting the speculation that this form of supplementation produces compensatory changes in that system making it less able to operate effectively. Although there is empirical support for this possibility (e.g., the demonstration by Li et al. [35], for knockout mice lacking acetyl cholinesterase, of a down-regulation of muscarinic receptors) it will need further research to confirm its applicability to the present case.

An inability to reduce attention to stimuli that do not deserve or require it has often been taken as a hallmark of schizophrenia, and, indeed, the latent inhibition phenomenon has been advocated as a model system for study of the mechanism that is dysfunctional in schizophrenia (e.g., [36]). Accordingly, our present findings lend support to the developing hypothesis that a disturbance of the normal functioning of cholinergic mechanisms plays a role in schizophrenia (see, e.g., [37,38]). Relevant observations include the fact that neuropathological investigation has demonstrated a decrease in muscarinic receptors in the prefrontal cortex of patients with schizophrenia (see, e.g., [39,40]) and the early observation [41] that chronic exposure to high levels of extracelleuar ACh can produce an increase in some of symptoms associated with schizophrenia.

It is true that much previous research in this area has focused on the role of mesolimbic dopaminergic mechanisms; for example the observation that treating rats with amphetamine disrupts normal latent inhibition has been taken to reflect an effect on the dopaminergic system of the nucleus accumbens (NAcc) (see, e.g., [42], for a review). But the dopaminergic and cholinergic systems are intimately linked; the NAcc projects to the basal forebrain and the activity of the corticopetal cholinergic system of the basal forebrain has been found to vary according to the level of activity in the NAcc [43]. Such observations have led to the hypothesis that the symptoms of schizophrenia derive from dysfunctions in a chain of mechanisms that ultimately influence a cholinergic cortical process that selects or discards certain stimuli for attentional processing [44]. Our results prompt the suggestion that choline supplementation may provide a useful model system in which this hypothesis could be tested further.

Finally we should comment on the fact that cholinergic mechanisms have often been linked not so much with attention as with memory, primarily as a consequence of the suggestion (e.g., [45]) that a dysfunction of the cholinergic neurons of the basal forebrain is responsible for the memory deficits of aging. This characterization has been disputed (see, e.g., Voytko [46], who specifically assesses the alternative view that the basal forebrain controls attention rather than memory). The issue is not easy to resolve, partly, we suggest, because terms like “memory” and “attention” are relatively ill-defined. Each covers a covers a range of psychological processes and aspects of each will be involved in generating most behavioral phenomena. Thus, although we have used the term attention in summarizing our findings, our behavioral analysis has been concerned with a specific psychological process – that responsible for reducing the associability of stimulus followed by consistent consequences – a process that involves learning, and thus a contribution from some aspect of memory. It remains to investigate if such changes in associability can be shown to be occurring in other experimental paradigms that have been said to show cholinergic involvement in attention and memory.

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