Original research paper

Effects of dietary choline availability on latent inhibition of flavor aversion learning

Fernando Gámiz[1]*, Sergio Andrés Recio[2]*, Adela Florentina Iliescu[2], Milagros Gallo[1], Isabel de Brugada[2]

[1]Department of Psychobiology, Institute of Neurosciences, Center for Biomedical Research (CIBM), University of Granada, Spain, [2]Department of Experimental Psychology, Mind, Brain and Behavior Research Center (CIMCYC), University of Granada, Spain

Objective: It has been previously reported that dietary choline supplementation might affect latent inhibition (LI) using a conditioned suppression procedure in rats. We have assessed the effect of dietary choline on LI of flavor aversion learning.

Method: Adult male Wistar rats received a choline supplemented (5 g/kg), deficient (0 g/kg), or standard (1.1 g/kg) diet for 3 months. After this supplementation period, all rats went through a conditioned taste aversion (CTA) procedure, half of them being pre-exposed to the conditioned stimulus before the conditioning.

Results: The results indicated that choline deficiency prevents LI of conditioned flavor aversion to cider vinegar (3%) induced by a LiCl (0.15 M; 2% body weight) intraperitoneal injection, while choline supplementation enhances CTA leading to slower extinction.

Discussion: The role of the brain systems modulating attentional processes is discussed.

Keywords: Attention, Choline, Latent inhibition, Learning, Flavor aversion, Rats

Introduction

In a variety of Pavlovian conditioning procedures, it has been demonstrated that learning is retarded by prior exposure to either of the stimuli to be associated during conditioning. Previous exposure to the conditioned stimulus (CS) produces a retardation in subsequent learning known as latent inhibition (LI), or the CS pre-exposure effect. This retardation in acquisition of a conditioned response after CS pre-exposure when compared with a non-pre-exposed group has usually been explained in terms of attentional changes. A given CS followed by a consistent consequence (the absence of consequence in this case) will lose associability or salience hindering their subsequent learning.[1] LI has been demonstrated in a variety of learning tasks, conditioned flavor aversion (CFA) being one of the most frequently used.[2] A number of learning theories explain LI in terms of attention processes modulated by novelty, and so the phenomenon has been employed as a model for the study of attention disorders.[3,4] The search for the neural substrates of LI has focused on the dopaminergic activity in the nucleus accumbens.[5,6] In this area, either reduction or enhancement of LI is induced by increased or decreased dopaminergic transmission, respectively. In addition, opposite effects of damaging the shell and core accumbens subregions have been reported, along with similar opposing effects of lesioning different brain areas sending afferents to the nucleus accumbens. Consequently, a neural network involved in LI modulation has been proposed, with a relevant role for the basal forebrain cholinergic system.[7–9]

We have previously demonstrated that changes in dietary choline availability might modulate attention,[10] as well as learning and memory.[11,12] Chronic dietary choline supplementation for 12 weeks was shown to disrupt LI using the conditioned emotional response (CER) procedure in adult rats.[10] The authors discussed these findings in terms of the close relationship between the nucleus accumbens and the basal forebrain cholinergic systems and the disturbance induced by choline supplementation in those cholinergic mechanisms modulating attention.

*Both authors contributed equally to this work and should be considered as first authors of the article.

Correspondence to: Sergio A. Recio, Department of Experimental Psychology, Mind, Brain and Behavior Research Center (CIMCYC), University of Granada, Campus Cartuja, Granada 18071, Spain.
Email: sergioandres@ugr.es

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In the present experiment, we examine the effect of chronic dietary choline supplementation and deficiency for 3 months on LI of CFA in adult rats. The main aim was to assess if choline supplementation has a disruptive effect on CFA, that is, comparable to that previously reported in CER. In addition, since the disruption of LI by choline supplementation has been attributed to an imbalance of cholinergic transmission,[9] a group receiving a choline-deficient diet was added, with the expectation that we might find similar effects to those induced by the supplemented diet. Finally, we investigated the potential impact of dietary choline availability on CFA itself, that is, independently of the effects of pre-exposure (LI).

Material and methods

The subjects were 48 adult male Wistar rats (mean weight = 524.3 g, range: 410–650), previously used in conditioned preference experiments. None of these experiments involved the flavor solution used in the present procedure, nor any kind of malaise induction, and they were conducted in a completely different context (room, cages, and bottles). The rats were housed in groups of four in a room with constant temperature (22–24°C) and a light–dark cycle of 12 hours (from 8 a.m. to 8 p.m.). They had *ad lib* access to water and food during the dietary treatment lasting for 3 months. According to the dietary treatment, the rats were randomly assigned to one of the three groups ($n = 16$ each) receiving different choline chloride content diets (Harlan Teklad Custom Research Diets): supplemented (5 g/kg), standard (1.1 g/kg), and deficient (0 g/kg). These concentrations were chosen following a previous series of experiments.[9–11] The behavioral procedure began 1 week after the end of the supplementation period, the animals having *ad libitum* access to the standard laboratory diet during the behavioral procedure in order to keep similar conditions in all the groups. Half of the rats in each dietary group were assigned either to the pre-exposed (PRE) or to the control non-pre-exposed (CNT) group, thus leading to six groups ($n = 8$ each): PRE-SUP, CNT-SUP, PRE-STN, CNT-STN, PRE-DEF, and CNT-DEF. They were individually housed and adapted to a water deprivation schedule consisting of two daily 30-minute drinking sessions at 10:00 and 17:00 hours. The behavioral procedure was performed during the morning sessions, while during the afternoon sessions *ad libitum* water was available throughout the experiment. All of the behavioral procedures took place in the home cages.

The dependent variable we choose was consumption. The amount ingested was recorded weighing the bottles before and after each morning session throughout the behavioral procedure. The water consumption baseline was established over 3 days. On the next 4 days, 10 ml of a cider vinegar solution (3% v/v) was available to the PRE groups while the CNT groups received the same amount of water. Conditioning took place during the morning session of day 5, in which all the rats received an intraperitoneal injection of LiCl (0.15 M; 2% body weight) as the US immediately after drinking the vinegar solution. After a recovery day, one-bottle extinction tests were applied over the following eight morning sessions. During these tests, the rats had *ad lib* access to the vinegar solution during the 30 minutes session in order to explore the extinction patterns exhibited by the groups.

The data were analyzed using general lineal model contrasts with the SPSS software. A confidence interval of $P < 0.05$ was adopted for all the statistical analyses, and the Greenhouse–Geisser correction was used on repeated-measure contrasts.

Results

There were no differences among the groups in terms of baseline water intake. One subject belonging to the group CNT/DEF was discarded from further analyses because it had outlier scores on most of the sessions.

The intake of the pre-exposed (PRE) and the non-pre-exposed (CNT) groups during the pre-exposure phase was analyzed by a mixed $3 \times 2 \times 4$ (diet × pre-exposure × session) analysis of variance (ANOVA) with diet and pre-exposure the between-group factors and session a within-subject factor. There was a significant effect of the main factor pre-exposure, $F(1, 41) = 11.63$, $P < 0.05$, indicating lower consumption of the vinegar solution in the PRE groups than water in the CNT groups (see Table 1). There were no significant effects of the main factors diet and session, nor were any of the interactions significant (all $P \geq 0.20$), showing that the intake remained stable over the pre-exposure phase without any effect of the previous dietary treatment (see Table 1). The apparent lack of vinegar neophobia, indicated by the absence of the effect of session, could be explained by the earlier exposure to different flavors, as showed in previous reports.[13] However, this is not critical for exploring the effect of dietary supplementation on CFA and LI.[14]

A 2×3 (pre-exposure × diet) ANOVA analysis of the amount ingested by the different groups during the conditioning trial revealed no significant effects of the main factors pre-exposure, $F(1, 41) = 2.77$, $P > 0.10$; diet ($F < 1$); or an interaction between these factors ($F < 1$), thus showing no differences between the groups (see Table 1, CFA).

Fig. 1 shows mean (±SEM) consumption of the vinegar solution during the extinction sessions. A $2 \times 3 \times 8$ (pre-exposure × diet × sessions) mixed ANOVA

Table 1

Groups	P1	P2	P3	P4	CFA
PRE-SUP	6.08 (*0.93*)	6.23 (*0.94*)	4.45 (*0.75*)	5.19 (*0.79*)	4.88 (*0.62*)
CNT-SUP	6.06 (*0.55*)	5.92 (*0.54*)	6.93 (*0.61*)	6.76 (*0.54*)	4.81 (*0.59*)
PRE-STN	5.85 (*1.04*)	5.34 (*1.09*)	6.33 (*0.63*)	4.96 (*0.67*)	5.09 (*0.68*)
CNT-STN	7.11 (*0.45*)	6.96 (*0.74*)	7.35 (*0.73*)	7.88 (*0.52*)	4.17 (*0.51*)
PRE-DEF	4.92 (*0.77*)	5.38 (*0.78*)	5.27 (*0.42*)	4.47 (*0.42*)	4.97 (*0.50*)
CNT-DEF	6.53 (*0.41*)	7.15 (*0.49*)	6.93 (*0.43*)	6.20 (*0.48*)	3.62 (*0.43*)

including the extinction trials as a within-subject factor yielded significant effects of session, $F(7, 287) = 6.72$, $P < 0.05$; and pre-exposure, $F(1, 41) = 8.90$, $P < 0.05$; but no effect of diet, $F(2, 41) = 2.01$, $P > 0.14$. A triple interaction between session, pre-exposure, and diet was found, $F(14, 287) = 2.29$, $P < 0.05$. In addition, the interaction between session and pre-exposure was marginally significant, $F(7, 287) = 2.47$, $P = 0.053$. To assess LI in each dietary group, further analyses with planned comparisons were performed. In the DEF group, we found a significant effect of session, $F(7, 91) = 3.57$, $P < 0.05$, indicating extinction, but no effect of pre-exposure ($F < 1$) or an interaction, $F(7, 91) = 1.40$, $P > 0.20$. Since there was no significant effect of pre-exposure, the aversion was similar in the PRE and CNT groups, thus showing an absence of LI in those rats fed with a choline-deficient diet. In the STN group, there was a significant pre-exposure × session interaction, $F(7, 98) = 2.92$, $P < 0.05$. Analyses of this interaction by separate one-way ANOVAs for each session showed a lower aversion in the pre-exposed group in the first three extinction tests, consistent with there being a LI effect. These differences disappeared from the fourth day ($P > 0.20$) due to extinction in the non-pre-exposed group. With respect to the SUP group, there were significant effects of pre-exposure, $F(1, 14) = 19.22$, $P < 0.05$ (thus indicating LI), and session, $F(7, 98) = 3.44$, $P < 0.05$. The pre-exposure × session interaction did not reach significance, $F(7, 98) = 1.65$, $P > 0.20$. Although the interaction is not significant, a visual inspection of the data (see Fig. 1A) suggests that there was slower extinction in the non-pre-exposed group. To test this, we ran simple comparisons between the first and the last trials. This analysis showed a significant difference in the pre-exposed group, $F(1, 7) = 6.99$, $P < 0.05$ (mean consumptions 2.38 ± 0.38 vs. 3.90 ± 0.74 ml), but not in the non-pre-exposed group, $F(1, 7) = 1.23$, $P = 0.30$ (mean consumptions 0.77 ± 0.07 vs. 1.16 ± 0.33 ml). These results suggest impaired extinction in the supplemented non-pre-exposed group.

Discussion

The main finding is that unexpectedly our results do not confirm a similar disruption of LI by choline supplementation in CFA to that found in CER.[10] While dietary choline supplementation disrupted LI in a conditioned suppression paradigm using a CER task, we found no such effect in the present experiment. In spite of employing an identical choline supplementation regime during the same 12-week period, we found that the group receiving pre-exposure exhibited a weaker learned vinegar aversion than the non-pre-exposed group, thus indicating LI. This was evident from the first extinction test and the differences between PRE and CNT remained evident throughout the first three extinction sessions, as in the groups receiving the standard diet. Therefore, it can be concluded that, at least with our CFA procedure, choline supplementation had no effect on LI.

An alternative explanation in terms of an inefficient supplementation procedure is not feasible. The choline supplementation regime that we used was chosen in accordance with the previous work and it has proven to modulate cognitive functions both in LI and other learning and memory phenomena.[10–12] In fact, choline concentrations between 2.6 and 5 times higher than the standard diet, such as that used in the present experiment, have been shown to effectively increase choline plasma levels and acetylcholine (ACh) synthesis in the brain.[15] Moreover, such increase seems to be within the physiological levels. Taking into account the variety of foods in the adult rat diet, it has been estimated around 0.5–0.9 g as the mean daily intake. However, due to the occasional consumption of food rich in choline at a given day it might increase up to 5 g, thus inducing variations up to tenfold with respect to the mean.[16]

Instead, the different effects of dietary choline on LI using CER vs. CFA are likely to be due to the different tasks used, since they involve specific behavioral procedures that rely on different brain areas. This could be a plausible explanation of our results since there is evidence that dietary choline can induce long-term effects on specific brain areas and receptors, without affecting others.[17]

Although the role of the learning task used to study the neural mechanisms of LI has often been neglected, previous data point to marked discrepancies between the results obtained using conditioned taste/flavor aversion (CTA/CFA) and other learning tasks.

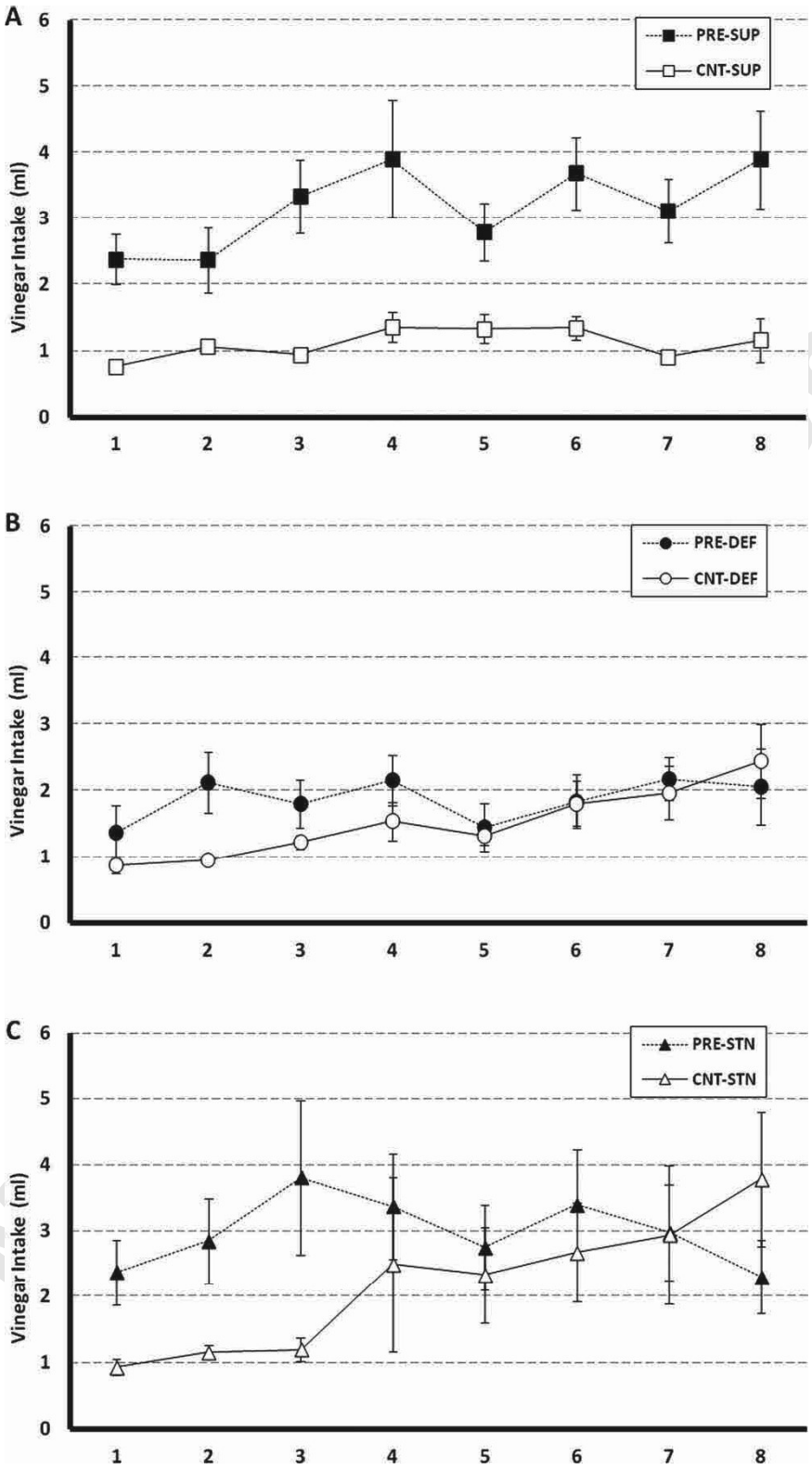

Figure 1 Mean (±SEM) vinegar intake during the eight extinction tests. (A) Choline supplemented groups (SUP): pre-exposed group (black square) and control non-pre-exposed group (white square). (B) Choline-deficient groups (DEF): pre-exposed group (black circle) and control non-pre-exposed group (white circle). (C) Choline standard groups (STN): pre-exposed group (black triangle) and control non-pre-exposed group (white triangle). The pre-exposure groups received four flavor pre-exposures prior the conditioning phase (PRE; dashed lines and black markers) and the non-pre-exposed groups received water during the same pre-exposure phase (CNT; continuous lines and white markers). LI was evident in the supplemented and the standard groups, but not in the deficient groups.

Unlike with other tasks, bilateral intra-striatal but not intra-accumbens injections of amphetamine disrupt LI of CTA.[18] Likewise, lesions that have been found to impair LI using other learning tasks leave intact LI of CTA.[19] Moreover, contradictory effects of nucleus accumbens lesions have been reported using CER

4 and CTA. Selective nucleus accumbens shell NMDA lesions abolished LI using a CER but not a CTA task.[19] Similar findings have also been reported with lesions of other brain areas, which appear to generate different results depending on the behavioral procedures.[20] This is due to the fact that LI of different tasks might depend on dissociable neural circuits,[18,21] for which the cholinergic system plays an important role in these circuits. However, divergent effects of the same pharmacological intervention have been reported. Impairment, enhancement, and no effect of cholinergic antagonism and agonism on LI have been found (for a review[8]). Different proposals have contributed to the explanation of these discrepancies based on the so-called cholinergic hypothesis regarding the role of ACh in attentional processes. Specifically, models of the neural circuit involved in LI that include relevant modulation by basal forebrain cholinergic projections have been proposed.[7,22–24] According to these proposals, ACh would play an important role in processing relevant stimuli and suppressing non-relevant cues in attentional tasks.[25] Subtle changes in ACh levels might change this modulatory role, thus leading to LI blockade or enhancement. Therefore, we could envisage that different ACh levels can be required for inducing similar effects using different learning tasks. This might explain the apparently contradictory consequences of the same dietary choline supplementation when using different behavioral protocols.

With respect to the effect of the choline-deficient diet, the results clearly indicate an absence of the LI effect, since the only significant effect was that of conditioning session, there being no effect of pre-exposure or an interaction between them. This suggests the need for choline dietary availability in order to observe the effect of previous flavor pre-exposure on later learning. This is consistent with the disruption of LI by cholinergic antagonists, such as scopolamine, which has been found using other learning tasks.[26,27]

In addition to our main finding, some other issues merit mention. First, the animals drank low amounts of the vinegar solution throughout the behavioral procedure. This reduced intake can be attributed to a reduced thirst due to water availability during the afternoon drinking sessions. This might also be related to the limited extinction seen in the groups that show LI, which seem to reach an asymptote at 4 ml. Thus, the reduced thirst and the low palatability of the vinegar could have prevented higher levels of consumption in spite of the extinction of the conditioned aversion. Second, the results point to the fact that the supplemented diet slows extinction of CFA in the non-pre-exposed group. The absence of an effect of session in the SUP-CNT group, unlike that found in the STN-CNT group, indicates slower extinction of the learned vinegar aversion in the non-pre-exposed supplemented rats. This does not seem to be due to the impairment of flavor familiarity processes, since LI was evident in the supplemented pre-exposed group. It cannot be attributed to the impairment of extinction by the supplemented diet, since the pre-exposed group did show extinction. Rather it is conceivable that the supplemented diet had enhanced CFA, thus slowing extinction. This is consistent with previous reports indicating that increases of the cholinergic activity in hyper-innervated p75 − /− knockout mice 5 favours robust taste aversion,[28] and the suggested role for the cholinergic system in the formation and retention of learned taste aversion using non-familiar tastes.[29,30]

Taken together, our results point to the importance of dietary choline supply in the modulation of the cholinergic function involved in attentional and learning processes. This adds to previous results showing the effects of exogenous choline on learning and memory[31–34] and it is consistent with those reports indicating that dietary choline increases the cholinergic metabolism in memory brain systems, by favouring ACh synthesis and release.[35,36] The present data therefore highlight the need for further research on the potential impact of dietary interventions for the treatment of attentional disorders.

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Disclaimer statements

Contributors

F.G.: Conceiving and designing the study, collecting the data, analyzing the data, interpreting the data, writing the article in whole or in part, revising the article. S.A.R.: Conceiving and designing the study, collecting the data, analyzing the data, interpreting the data, writing the article in whole or in part, revising the article. A.F.I.: Collecting the data, analyzing the data, interpreting the data, revising the article. M.G.: Conceiving and designing the study, analyzing the data, interpreting the data, writing the article in whole or in part, revising the article, obtaining funding and/or ethics approval. I.B.: Conceiving and designing the study, analyzing the data, interpreting the data, writing the article in whole or in part, revising the article, obtaining funding and/or ethics approval.

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Conflicts of interest

None.

Ethics approval

All the procedure was approved by the Animal Experimentation Ethics Board (CEEA) from the University of Granada.

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