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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