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Effects of Chronic Manipulations of Dietary Choline on Locomotor Activity, Discrimination Learning and Cortical Acetylcholine Release in Aging Adult Fisher 344 Rats[1]

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BENINGER, R. J., S. A. TIGHE AND K. JHAMANDAS. *Effects of chronic manipulations of dietary choline on locomotor activity, discrimination learning and cortical acetylcholine release in aging adult Fisher 344 rats.* NEUROBIOL AGING 5(1) 29–34, 1984.—Dietary levels of choline have been shown to influence central cholinergic neurotransmission. To further examine the behavioral and neurochemical effects of dietary choline, adult Fisher 344 rats were maintained on choline deficient (n=6), enriched (n=6) or usual lab chow (n=6) diets for 38 weeks. During 14 weeks of free access to these diets, controls gained little weight whereas the deficient group increased and the enriched group decreased. For the remaining 24 weeks the weights of all groups were maintained at 90% of their original level. Locomotor activity did not differ significantly in a 90 min session but during a 20 hr session controls were most active, deficient rats least with the enriched group in between. The groups showed no significant differences in the acquisition or reversal of a discrimination. Spontaneous and evoked cortical release of acetylcholine was enhanced in the enriched group and decreased in the deficient group relative to controls. These data suggest that chronic manipulations of dietary choline may significantly influence locomotor and neurochemical activity but not discrimination learning.

Dietary choline Choline Locomotor activity Discrimination learning Acetylcholine Cortical acetylcholine release

SYSTEMIC pharmacological manipulations of the brain's cholinergic systems [7] influence locomotor activity [1] and various forms of learning and memory [22]. For example, the acquisition and maintenance of a discrimination was enhanced by cholinergic agonists and impaired by cholinergic receptor blockers [19]. The observation that some cholinergic markers decreased with age [14, 15, 21, 23] suggested that cholinergic function may be subnormal in the aged. This hypothesis was supported by the finding of similar memory deficits in young subjects treated with anticholinergics and drug-free aged subjects [5].

Currently there is interest in improving cognitive function by adding choline, a precursor of acetylcholine to the diets of aged animals [2]. Dietary choline increased levels of biochemical markers for cholinergic activity in the brains of animals [24] and reduced memory impairments in aged mice [3]. Similarly, old mice receiving dietary supplements of lecithin, a substance which generates choline in the brain showed enhanced maze learning [9]. Moreover, locomotor activity of aged rats was lower than that of younger rats [23] and dietary choline increased activity in such animals [20]. Thus, adult rats receiving choline enriched diets might be more active than those receiving deficient diets.

Chronic dietary choline is of interest in the possible treatment of age related behavioral deficits in humans [2]. Little work has been done to investigate possible behavioral and neurochemical changes that occur in adult animals following dietary choline manipulations. The present study was conducted to examine the influence of choline enriched and deficit diets on locomotor activity, discrimination learning and cortical release of acetylcholine *in vivo*.

METHOD

Subjects

Eighteen retired breeder male albino rats of the Fisher 344 strain, approximately 35 weeks old and weighing from 320–390 g were housed individually in wire-bottomed cages in a

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climatically controlled colony kept on a 12 hr light (0700–1900)/dark cycle. Water was available ad lib.

Diets

The choline enriched (No. 785-M) and deficient (No. 785) diets were purchased from Bio-Serv, Inc. (Frenchtown, NJ). Independent blind assays by Raltech Scientific Services (Madison, WI) showed the enriched diet to contain approximately 70 mg/g and the deficient diet 0.5 mg/g of choline chloride. All of the remaining ingredients of the Bio-Serv diets were identical. The control group was fed standard rat chow (Purina) containing approximately 1.6 mg/g of choline; however levels of the other ingredients were not determined. Thus, differences between rats fed Purina versus those fed Bio-Serv diets may not be attributed solely to differences in choline content.

Apparatus

General activity was measured in 3 similar running wheels (35 cm diam) constructed of Plexiglas sides with grid floors. Operant conditioning experiments were conducted in 3 similar test chambers (23×30×19 cm high) with Plexiglas sides and top, aluminum plate ends and a grid floor. Each chamber was outfitted with a lever that was 5 cm wide and located to the left of centre on one of the end walls at a height of 5 cm; a 2.5 cm high feeder cup was located in the centre of the same end wall. Each wheel and chamber was located in a sound-attenuating box, outfitted with a light and ventilated by a fan that also provided constant masking noise. Environmental control and data collection were achieved with the use of solid state switching, timing and counting devices (BRS/LVE) and cumulative recorders located in an adjacent room.

Procedure

Rats were assigned randomly to one of 3 groups (n=6) receiving control, choline deficient or enriched diets ad lib. After 6 weeks the enriched group was losing weight (see below and Fig. 1); therefore, their diet was supplemented with 10 g of regular chow each day. As these animals required more than 15 g of food per day, they were receiving on average approximately 20–25 mg of choline per g of food eaten. For planned operant experiments, food deprivation was begun on week 15. Weights were reduced to 90% of their original free-feeding value by daily feeding with measured rations. Animals in the enriched group continued to receive about 10 g of regular chow per day.

Locomotor activity. During weeks 23 and 24, rats were tested 3 at a time (one from each group), each being placed alone in a wheel with wheels counterbalanced as additional squads were tested. Activity was assessed for 21.5 hr beginning at 1330 each day, the rats being in darkness without food or water. The first 90 min was treated as an acute phase and the subsequent 20 hr as a chronic phase. Each quarter wheel turn was registered as a response and activity was monitored continuously with cummulative recorders.

Discrimination learning. During weeks 28 to 30, 16 rats were trained to lever press for reinforcement consisting of one 45 mg food pellet (Bio Serv). Each received several 30-min sessions of continuous reinforcement, one on a variable interval (VI) 15-sec schedule and 6 on a VI 30-sec, response dependent reinforcment becoming available every 30 sec on the average with intervals ranging from 15 to 60 sec.

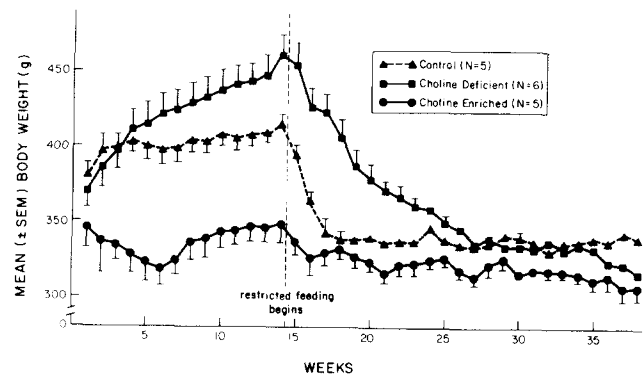

FIG. 1. Mean (±SEM) body weight (g) of control, choline deficient and choline enriched dietary groups over 38 weeks. The vertical broken line between weeks 14 and 15 indicates the beginning of restricted feeding after which weights were reduced to 90% of their initial free-feeding values for each group.

Thirty-min discrimination sessions occurred at approximately the same time each day, 5 days a week during weeks 31 to 34. Each session consisted of alternating 1-min periods of S+ and S−: the S+ stimulus was a 2900 Hz tone (3 sec on, 3 sec off, etc.) signalling the availability of reinforcement according to the VI 30 schedule; during the S− period the tone remained off and no reinforcement occurred. This is termed a multiple VI 30-sec extinction schedule.

Discrimination reversal. During weeks 35 to 38 rats continued to receive daily discrimination sessions except that the tone stimulus now was the S− and the 1-min tone-off period now was the S+. The dependent variables for both acquisition and reversal were total number of responses in the S+ and S− periods per session.

Release of cortical acetylcholine. The spontaneous and evoked release was measured using the cortical cup technique [12,13]. Briefly, the rat was anesthetized lightly by intraperitoneal injection of pentobarbitone sodium (30 mg/kg) and urethane (400 mg/kg). The trachea was cannulated and the femoral vein catheterized. The rat was placed in a stereotoxic head holder, the muscle, bone and dura overlying the cerebral cortex was removed, and a perspex cup (2.5 mm cross-sectional area) was placed on the parietal cortex. All exposed areas were covered with a 2–3 mm layer of 4% agar in saline. The cup was filled with 0.3 ml of Ringer-Locke solution containing neostigmine (50 μg/ml) and atropine (0.5 μg/ml). The solution was removed every 20 min and bioassayed for acetylcholine using the hearts of Mercenaria mercenaria as described earlier [11]. This assay has been shown to be highly specific for acetylcholine [8]. To induce release electrical stimulation (10 volts, 0.3 msec, 0.5 Hz) was applied to the forepaws through bipolar electrodes inserted under the skin using a Grass stimulator (Model S-88). Release was also induced by injecting *d*-amphetamine (2.5 mg/kg) through the femoral vein.

RESULTS

The mean (±SEM) weights per week are shown in Fig. 1. Because of sampling error, the initial weights of the groups differed (week 1). The weights of the control group increased from week 1 to 2 and then showed little further change over the 14 weeks of free feeding. The enriched group lost weight

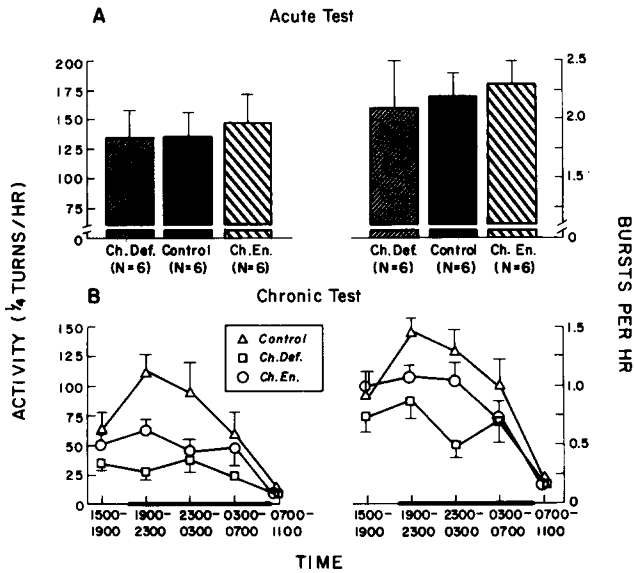

FIG. 2. Mean (±SEM) locomotor activity (quarter wheel turns per hr) and bursts per hr (see text) for each dietary group over the acute, 90 min test session (A) and the chronic, 20 hr test session (B). The darkened portion of the horizontal axis in B indicates the usual light-off period from 1900 to 0700 hr.

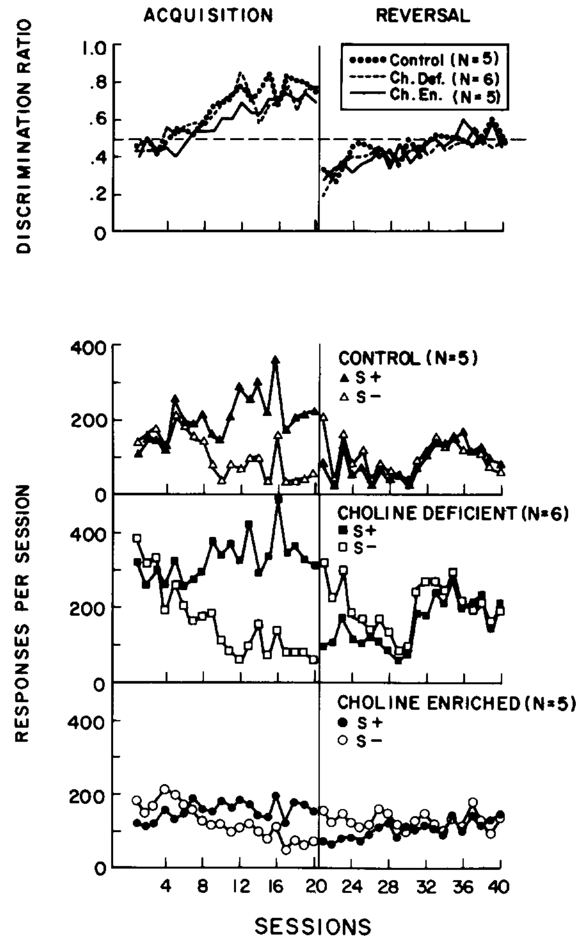

FIG. 3. Mean discrimination ratios (upper panel) and responses per session in S+ and S− components for each dietary group over the 20 sessions of acquisition and the 20 sessions of reversal of the auditory discrimination.

over the first 6 weeks but once the supplement was begun (week 7), regained their initial level. The deficient group continued to gain weight. Once food deprivation was initiated on week 15, all groups lost weight, approaching the 90% target level by about week 25.

Locomotor activity. The results were analysed in two ways. One was to look at the mean response rate per hr during the acute test and during each 4 hr period of the chronic test. The other examined the possibility that the pattern was different. This was done by calculating response bursts from the cumulative records; bursts were defined as at least 12 consecutive responses occurring in a period both preceded and followed by at least 5 min without a response.

Responses and bursts per hr during the acute activity test (Fig. 2A) were analysed separately using one way analyses of variance. Group differences were not significant for either variable, $F(2,15)<1$, $p>0.05$ in both cases.

For the 20 hr activity tests (Fig. 2B), response and burst rates were analysed separately using two variable analyses of variance with repeated measures on one variable; the variables were groups and time (4 hr blocks), the latter being the repeated measure. Response rates differed, $F(2,15)=9.54$, $p<0.003$, and the interaction between groups and time was significant, $F(8,120)=5.21$, $p<0.001$. The burst measure similarly revealed a main effect of groups, $F(2,15)=5.54$, $p<0.02$, and a group by time interaction, $F(8,120)=3.36$, $p<0.002$. Tests of simple main effects of groups at each time revealed response rate differences at 1900–2300 hr, $F(2,75)=13.22$, $p<0.01$ and at 2300–0300 hr, $F(2,75)=7.33$, $p<0.01$ and burst rate differences also at only these times, $F(2,75)=4.11$, $p<0.05$ and $F(2,75)=7.71$, $p<0.01$, respectively. These results indicate the source of the significant interactions. Further pairwise tests (Scheffe) showed that at 1900–2300 hr response rates of the deficient and enriched groups different from control, $F(2,15)=26.11$, $p<0.01$ and $F(2,15)=9.30$, $p<0.05$, respectively; similarly at 2300–0300 hr, $F(2,15)=12.35$, $p<0.05$ and $F(2,15)=9.46$, $p<0.05$, respectively. For the burst measure, deficient differed from control at 1900–2300 hr, $F(2,15)=8.00$, $p<0.05$, and at 2300–0300 hr, $F(2,15)=14.75$, $p<0.01$.

Discrimination. For each rat each day, the accuracy of discrimination performance was calculated by expressing the total number of lever press responses during the S+ periods as a ratio of the total number of responses during the S+ and S− periods combined, i.e., discrimination ratio: $S+ \div [(S+)+(S-)]$. A ratio of 0.5 would reveal no discrimination, values greater than 0.5 indicating that more responses occurred in the S+ periods and 1.0 indicating perfect discrimination (Fig. 3).

Ratios were analysed separately for acquisition and reversal using two variable analyses of variance with independent groups and repeated measures on the sessions variable. Results revealed improvement during acquisition, $F(19,228)=24.56$, $p<0.001$ but no significant group differences, $F(2,12)<1$, $p>0.05$ or interaction, $F(38,228)=1.05$, $p>0.05$. Over the 20 reversal days, there also was a session effect, $F(19,228)=5.18$, $p<0.001$ and no significant group effect, $F(2,12)<1$, $p>0.05$, or interaction, $F(38,228)<1$, $p>0.05$. Thus, although the rats learned the discrimination

TABLE 1

MEAN (±SEM) ACETYLCHOLINE LEVELS (ng, 0.25 cm^{-2}, 20 MIN^{-1}) IN CORTICAL PERFUSATE COLLECTED OVER PERIODS OF TWENTY MINUTES

	N	Basal 1	Basal 2	Following brief electrical stimulation (0.5 Hz) of forelimb¶ 1	2	3	Following *d*-amphetamine (2.5 mg/kg)**
Control	4	2.37 ± 0.06	2.37 ± 0.05	4.09 ± 0.30	3.04 ± 0.11	2.78 ± 0.07	5.93 ± 0.05
Choline deficient	4	2.26 ± 0.01	2.30 ± 0.02	3.69 ± 0.26	2.69 ± 0.04	2.63 ± 0.04	4.60 ± 0.41*
Choline enriched	6	2.54 ± 0.03*§	2.57 ± 0.03†§	4.43 ± 0.14‡	3.07 ± 0.20	2.90 ± 0.18	6.04 ± 0.27‡

*$p<0.05$, †$p<0.01$ Control vs. Enriched or Control vs. Deficient (F test).
‡$p<0.05$, §$p<0.01$ Deficient vs. Enriched.
N = Number of rats.
¶Electrical stimulation was applied during collection No. 1.
**Represents release during the 20 min period immediately following injection.

and reversal, there was no significant effect of diet on this learning.

It is possible that overall response rates in the S+ and S− components differed. For this reason, mean S+ and S− rates were analysed separately (Fig. 3). For each group a two way analysis of variance with repeated measures on both variables (component and time) was conducted separately for acquisition and reversal.

During acquisition, S+ rates differed from S− rates for the control and deficient groups, $F(1,4)=7.29$, $p<0.05$ and $F(1,4)=11.41$, $p<0.03$, respectively, but not for the enriched group, $F(1,4)=1.48$, $p>0.05$. However, the interaction of component and session was significant for all groups: $F(19,76)=5.03$, $p<0.001$ for control, $F(19,76)=7.37$, $p<0.001$ for deficient and $F(19,76)=5.45$, $p<0.001$ for enriched. These interactions reflect the initial similarity of S+ and S− rates and their subsequent divergence (see Fig. 3). The results show that response rates and changes in rates in the S+ and S− components over sessions differed for the 3 groups.

During the reversal, S+ rates were lower than S− rates for the choline deficient and enriched groups, $F(1,4)=12.52$, $p<0.02$ and $F(1,4)=8.72$, $p<0.04$, respectively, but not for the controls, $F(1,4)=4.13$, $p>0.05$. The interaction of component and session was significant for control and deficient groups, $F(19,76)=5.01$, $p<0.001$ and $F(19,76)=5.54$, $p<0.001$, respectively, but not for the enriched group, $F(19,76)=1.33$, $p>0.05$. Thus, as was the case during acquisition, rates and changes in rates in S+ and S− components over sessions differed among groups.

Release of cortical acetylcholine. The results of the biochemical assays are shown in Table 1. Analysis of variance for the two 20-min periods when basal levels were determined revealed a group effect, $F(2,11)=16.59$, $p<0.001$ and a group by period interaction, $F(2,11)=4.87$, $p<0.05$. Tests of simple main effects showed groups to differ in both the first, $F(2,22)=16.20$, $p<0.001$ and second period, $F(2,22)=15.78$, $p<0.001$. Individual comparisons (Scheffe) further showed that enriched differed from control and deficient in both the first, $F(2,11)=11.38$, $p<0.05$ and $F(2,11)=30.44$, $p<0.01$, and second period, $F(2,11)=14.57$, $p<0.01$ and $F(2,11)=27.66$, $p<0.01$, respectively. Analysis of variance for the 3 20-min periods following electrical stimulation revealed a significant effect of period, $F(2,22)=136.66$, $p<0.001$ and a group by period interaction, $F(4,22)=3.03$, $p<0.05$. Tests of simple main effects showed the groups to differ during the first period, $F(2,33)=4.43$, $p<0.05$ and pairwise comparisons showed the deficient to differ from enriched, $F(2,33)=8.83$, $p<0.05$. When d-amphetamine was injected there was a group effect, $F(2,11)=7.27$, $p<0.01$. Pairwise comparisons showed that control and enriched groups had higher release than the deficient group, $F(2,11)=9.17$, $p<0.05$ and $F(2,11)=12.95$, $p<0.05$, respectively.

These results can be summarized as follows. Under basal conditions, animals with a history of dietary choline deficiency show a slight reduction in cortical acetylcholine release relative to controls whereas enriched diets produce an increase. Under conditions of enhanced stimulation, on the other hand, all animals show an increase in release. However, animals receiving enriched diets show a slightly greater level of release than controls whereas the deficient group shows a reduced response.

DISCUSSION

The results can be summarized as follows. Animals free feeding on choline enriched diets lost weight relative to controls fed usual laboratory chow whereas those on deficient diets gained weight over 14 weeks. Wheel running of the 3 groups did not differ significantly over a 90 min test session. When assessed over 20 hr, all groups showed a circadian rhythm of activity, number of wheel turns and bursts being least in the first 4 hr of morning light; during the usual 12 hr of darkness, the controls showed the highest level of activity, the deficient group the least with the enriched group in between. Groups did not differ significantly in their acquisition or reversal of a discrimination although response rates in S+ and S− differed. Cortical acetylcholine release was increased in the enriched group but insignificantly decreased in the deficient group relative to controls under basal conditions. Enhanced stimulation resulted in a large increase in all groups; however, the deficient group showed lower levels of release than the enriched and control groups.

The effects of diet on body weight were unexpected and, to our knowledge, have not been reported previously. As behavioral and acetylcholine release experiments were conducted after weights had been adjusted to approximately

90% of their original level, the possible confounding effect of this variable was reduced in the present study. Perhaps the enriched group lost weight because the diet was unpalatable; indeed, it was necessary to supplement this group's diet with regular chow to prevent further weight loss. However, even with the supplement, this treatment produced elevated levels of acetylcholine release. The deficient group, on the other hand, may have eaten greater amounts to compensate for the deficit or the diet may have been more palatable.

The significant effects of diet on locomotor activity depended on the duration of testing. It has been reported that the locomotor activity of aged mice was less than that of young mice tested for 1 hr but no dietary manipulations were made in that study [23]. A preliminary report has shown that young rats placed for 2 weeks on a choline enriched diet were more active than controls whereas a group on a choline free diet showed no significant change during the 30 min test session [20]. These results show that manipulations of dietary choline may influence activity of young rats but the relationship between these and the present results remains unclear as age, species, diet, duration of exposure to the diet, duration of testing, apparatus for testing activity, etc. varied among experiments. Many pharmacological studies have shown that cholinergic agents influence locomotor activity, e.g., [1]; however, most of these studies used acute treatments making comparison with the present results difficult. One recent study reported that the circadian rhythm of activity in senescent rats was flattened relative to younger controls, locomotion being reduced during darkness and enhanced somewhat during light [16]. The present observation of a flattened circadian rhythm of activity in the deficient group might reflect a possible similarity between senescence and choline deficiency. However, as the enriched diet group also showed a flattening, albeit less than the deficient group, this possibility must be viewed with caution.

Many studies have shown that acute treatments with cholinergic agents can influence discrimination learning. Thus, anticholinesterases enhance whereas anticholinergics disrupt discrimination performance [19]; one study reported that maze learning of old mice was enhanced by dietary lecithin [9]. However, in the present study no significant effect of chronic dietary choline was found. Although this result is inconsistent with the report of enhanced learning with lecithin, insignificant effects of choline or lecithin on human memory often have been reported [6, 17, 18]. As these human and animal studies differed in task sensitivity, memory requirements, duration of treatment, age at onset of treatment etc., further studies will be needed to specify the possible role of cholinergic neurons.

The observation that an increase of dietary choline produced a significant increase in *in vivo* cortical acetylcholine release is in agreement with an extensive literature showing that treatment with choline or lecithin can enhance levels of acetylcholine in the brain [4, 10, 24]. In addition the present results showed that chronic exposure to choline deficient diets could lead to a reduction in release.

In conclusion, chronic dietary manipulations of choline that produce significant changes in cortical acetylcholine release influence locomotor activity but fail to significantly alter discrimination learning. The possibility that the observed effects were produced by changes in other neurotransmitter systems cannot be ruled out from the present data (cf., [3]). Clearly, more work is needed to examine these possibilities. However, the present results provide further evidence that diet may produce both neurochemical and behavioral changes.

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