Pre- and Postnatal Choline Supplementation Produces Long-term Facilitation of Spatial Memory

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Although research has demonstrated that short-term improvement in memory function of adult rats can occur when the availability of precursors for the neurotransmitter acetylcholine is increased, little is known about whether memory function of adult rats can be permanently altered by precursor supplementation during early development. In the present study, male albino rats were exposed to choline chloride supplementation both prenatally (through the diet of pregnant rats) and postnatally (subcutaneous injections). At 60 days of age rats were tested on a 12- and 18-arm radial maze task. Results indicated that compared to control littermates, perinatal choline-treated rats showed more accurate performance on both working and reference memory components of the task. This performance difference was apparent on the first block of sessions and continued throughout training. Further analysis revealed that the difference between choline and control rats is not due to use of differential response or cue-use strategies. Instead, it appears that choline induced performance differences are due to long-term enhancement of spatial memory capacity and precision.

In cholinergic (Ch) neurons free choline is the immediate precursor of the neurotransmitter acetylcholine (ACh). A number of investigators have demonstrated that an increase in exogenous choline enhances transmission at Ch synapses in a variety of species (Barry & Gelperin, 1982, 1984; Blusztajn & Wurtman, 1983; Cohen & Wurtman, 1975; Haubrich, Wang, Clody, & Wedeking, 1975). Until recently there was some controversy over the conditions under which exog-

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enous choline will enhance the synthesis of ACh in the mammalian brain (Leathwood, 1986). It has now been established that an increase in plasma choline *per se* is not a sufficient condition for increased synthesis and release of ACh. Rather, it appears that increased plasma choline will augment central Ch function to the extent that Ch neurons are activated concurrent with choline treatment or have been depleted of ACh stores previously (Trommer, Schmidt, & Wecker, 1982; Jope, 1982). These findings have been extended using the rat phrenic nerve/hemidiaphragm preparation (Bierkamper & Goldberg, 1980). Addition of choline to the medium produced no significant increase in ACh release when the nerve was at rest but caused a marked increase during electrical stimulation. London and Coyle (1978) have also demonstrated activity dependent utilization of choline. Unilateral destruction of striatal Ch neurons with kainic acid increases the firing frequency of spared neurons on that side of the brain. Systemic administration of choline has no effect on ACh levels in the intact striatum but significantly increases ACh levels on the lesioned side.

It has also been demonstrated that dietary intake of choline contributes to high serum choline concentrations in the neonatal rat (Zeisel & Wurtman, 1981) and that the neonatal blood–brain barrier readily transports choline into the brain (Pardridge, Cornford, Braun, & Oldendorf, 1979). Therefore, in the infant, as in the adult, high serum choline concentrations should be reflected in high brain choline levels and subsequently in high brain ACh concentrations. Furthermore, at high concentrations choline may also function directly as a Ch agonist and thereby modify neuronal organization through effects on developing feedback regulation mechanisms (Krnjevic & Reinhardt, 1979; Wurtman, 1983).

Dietary choline supplementation appears to affect the central control of some forms of adaptive processes in animals and humans. This conclusion is based on the finding that choline or related compounds administered to normal adult subjects produced activational changes in cholinergic function which could be related to improvement in certain cognitive processes (Bartus, Dean, Goas, & Lippa, 1980; Bartus, Dean, Pontecorvo, & Flicker, 1985; Bartus, Dean, Sherman, Friedman, & Beer, 1981; Meck & Church, 1987b). The observed effects of choline supplementation on behavior have been similar to the memory-enhancing effects of anticholinesterases such as physostigmine (Meck & Church, 1987a; Thal, Fuld, Masur, & Sharpless, 1983). In addition, Gelperin and his colleagues have shown that increased dietary intake of choline elevates blood choline levels, amplifies Ch transmission at a peripheral neuromuscular junction, and prolongs the duration of memory retention in the mollusc *Limax maximus* when the appropriate neuronal systems are activated by environmental contingencies (Barry & Gelperin, 1982a,b, 1984; Sahley, Barry, & Gelperin, 1986).

Despite our knowledge of the effects of dietary choline supplementation during adulthood, the behavioral and biochemical effects of early choline exposure are virtually unknown. During ontogeny, the central nervous system is rapidly undergoing cellular proliferation, differentiation, and growth and is, therefore, particularly sensitive to environmentally induced alterations. Choline is rapidly incorporated into developing brain tissue as acetylcholine (Atterwill & Prince, 1978) and can act to influence maturation of cells in the central nervous system (Prives & Quastel, 1969). In the present study we have examined the organizational effects of giving choline supplementation to rats during pre- and postnatal stages of development. The long-term effects of this treatment on spatial memory processes were assessed by performance in the radial arm maze when the animals reached adulthood.

The radial arm maze consists of an elevated central platform that has a number of arms (e.g., 12) radiating from it at equal angles. One paradigm using this maze allows analysis of both working and reference memory. Animals are normally given one test a day which lasts 5–10 min. In each test, on the first approach to each arm the rat ought to choose that arm and find food at the end of baited (S+) arms and find an empty food well at the end of unbaited (S−) arms. In all subsequent approaches in that test, the rat should not choose a previously chosen arm because the food has been removed or was never present. Consequently, the rat must remember each choice for the duration of each test, but at the end of each test he ought to forget these choices so that they will not interfere with the memory he will establish in subsequent tests. Working memory is used to retain trial-specific information, such as the locations already visited on a particular test, and reference memory is used to retain information that is relevant to all trials, such as which arms are consistently unbaited. Thus, revisits to arms (S+ or S−) during a trial are defined as working memory errors and visits to S− arms are defined as reference memory errors. (See Olton, Becker, & Handelmann, 1979 for a review of these distinctions.)

In the present study the acquisition and maintenance of maze performance for rats given pre- and postnatal choline supplementation and for rats given control treatments were analysed using a 12-arm maze with 8 S+ arms and 4 S− arms. After each group reached steady-state performance on the 12-arm maze, a maze rotation test was conducted to determine whether the rats were differentially controlled by intramaze versus extramaze cues. In a second phase of the experiment, the level of difficulty of the task was increased proportionally by transferring the rats to an 18-arm maze with 12 S+ arms and 6 S− arms. Rats were trained on this maze until they reached steady-state performance and completed the experiment.

Methods

Subjects

The subjects were 16 male albino rats from 4 litters born in our breeding colony. Two groups (n's = 4) of Sprague-Dawley CD strain female rats were exposed to either a 0 or 5 mL/liter solution of 70% choline chloride (Syntex Agribusiness Inc., Springfield, Missouri). The choline chloride was delivered in a 0.05 M saccharin solution in tap water that was given to all of the rats as their only source of drinking water. Choline supplementation for these breeding females began approximately 2 days prior to conception and was maintained throughout pregnancy. Females in the control group (0 mL/liter choline chloride) consumed 73 ± 9 mL/day of solution and females in the experimental group (5 mL/liter choline chloride) consumed 53 mL ± 7 mL/day of solution; $t(6) = 1.19$, ns.

At birth, litters were culled and pups born to control and choline chloride-treated dams were divided evenly among two untreated foster-mothers. During the next 24 days prior to weaning, pups ($n = 8$) from choline treated dams received choline chloride in concentrations of 25.0 mL/liter saline once per day at a volume of 0.05 mL for the first 5 days of life and 0.1 mL thereafter. Pups ($n = 8$) from control dams received injections of saline in the same volumes. Pups were hand held while the solutions were injected subcutaneously through a needle entered at the nape of the neck and inserted parallel to the spine for 4 cm. The injection procedure took approximately 15 sec to complete and appeared to be relatively

stressless for the young pup. When animals were weaned at approximately 24 days of age, choline supplementation was terminated and the animals were placed in individual cages and given free access to food and water.

Behavioral testing began when the rats reached approximately 60 days of age. At this time the rats were placed on a 24-hr food deprivation schedule during which they were fed approximately 12 g/day of Purina Rat Chow at the completion of daily behavioral testing. This food source contains about 2.3 mg of choline chloride/g. A light-dark cycle (LD) cycle of 12 : 12 hr was maintained in the vivarium with fluorescent lights on from 06.00–12.00 hrs eastern standard time.

Apparatus

12-Arm Radial Maze

The elevated radial maze was similar to the one described by Olton and Samuelson (1976). Two configurations of the maze were used. One maze was composed of 12 arms extending away from a central platform at equal angles. A stand placed under the central platform raised the maze 80 cm above the floor of the room and allowed rotation of the maze. Each arm of the maze was 83 cm long and 7.6 cm wide, with an edge 1.2 cm high along each side. At the far end of each arm, a hole, 2.5 cm in diameter and 0.06 cm deep, served as a food well. The central platform was 36.5 cm in diameter and the entire maze was painted flat gray. Rats could travel between different arms of the maze only by returning to the central platform. The maze was placed in a test room (4.6 × 4.8 m) in which the rats lived. The room contained a single rack of rat cages arranged along one of the walls, a window, and a variety of other extramaze stimuli that remained in a relatively constant position throughout the experiment.

18-Arm Radial Maze

The second maze configuration was composed of 18 arms extending away from a central platform at equal angles. A stand placed under the central platform raised the maze 80 cm above the floor of the room and allowed rotation of the maze. Each arm of the maze was 83 cm long and 7.6 cm wide, with an edge 1.2 cm high along each side. At the far end of each arm, a hole, 2.5 cm in diameter and 0.06 cm deep, served as a food well. The central platform was 45.6 cm in diameter and the entire maze was painted flat gray. Rats could travel between different arms of the maze only by returning to the central platform. The maze was placed in another test room (12.0 × 8.2 m) in which the rats lived. The room contained a single rack of rat cages located approximately 2.5 m from the maze, three rows of black laboratory tables, and a variety of other extramaze stimuli that remained in a relatively constant position throughout the experiment.

Procedures

Shaping

Beginning at approximately 60 days of age, rats were trained to run out the arms of the maze to obtain food by baiting the food well at the end of each arm with 10–15 45-mg Noyes food pellets. A number of rats (n = 3–5) were then

placed on the maze together after a period of 24-hr food deprivation and allowed to explore the maze for 20–30 min. This shaping procedure was conducted for 2 days, after which all rats failing to obtain food were forced to the ends of the arms and allowed to eat. When each rat ran readily to the ends of the arms and ate the food pellets, training began.

12-Arm Maze Training

To investigate the nature of choline's effects on a spatial memory task, a mixed-pattern paradigm of baited (S+) and unbaited (S−) arms was used to distinguish between working and reference memory components of the radial arm maze task. One session was given each day, 7 days a week for 30 days. At the beginning of each session, two pellets of food were placed in the food well at the end of 8 of the 12 arms. Eight different patterns of S+ and S− arms were randomly selected and the 8 rats in each of the two treatment groups were each randomly assigned to one of these 8 patterns. Once assigned, each rat's pattern was maintained throughout training.

At the beginning of each test session the rat was placed on the central platform and allowed to choose arms in the maze until all baited arms were visited at least once. All rats typically completed the maze by finding all 8 baited arms within 5–10 min. The order of arms chosen was recorded; a choice was defined as a rat advancing more than half of the way down an arm. Rats from each of the two treatment groups were randomly assigned to a particular chronological test order (1–16) that was kept constant across training.

Rotation Test

During the maze rotation test the rat was placed on the maze and allowed to make 4 choices as described above. After the fourth choice the rat was briefly removed from the maze. While the rat was being held in one hand, the experimenter rotated the maze 180° and the rat was placed back on the central platform of the maze and allowed to make 11 additional choices for a total of 15 choices. Thus, maze rotation put intramaze and extramaze cues in conflict with each other. In order to determine which cues the rats used to guide their behavior we estimated the number of errors made by each rat in two ways. First, working memory and reference memory errors were calculated based on the assumption that the rats only used intramaze cues to guide their behavior during the entire test and second, errors were calculated based upon the assumption that the rats only used extramaze cues to guide their behavior during the entire test. The strategy that was the most successful in locating the food was the one presumed used by the rats.

18-Arm Maze Training

To further investigate the nature of choline's effects on a spatial memory task, a mixed-pattern paradigm of baited (S+) and unbaited (S−) arms was again used to distinguish between working and reference memory components of the radial arm maze task. Animals trained on the 12-arm maze were transferred to an 18-arm maze which was located in a different room which contained quite different spatial cues. Each rat was given one test/day, 7 days a week for 30 days beginning when the rats were approximately 180 days old. At the beginning of each session, two

pellets of food were placed in the food well at the end of 12 of the 18 arms. Eight different patterns of S+ and S− arms were randomly selected and the 8 rats in each of the two treatment groups were each randomly assigned to one of these 8 patterns. Once assigned, each rat's pattern was maintained throughout training.

At the beginning of each test session the rat was placed on the central platform and allowed to choose arms in the maze until all baited arms were visited at least once. All rats typically completed the maze by finding all 12 baited arms within 5–10 min. The order of arms chosen was recorded; a choice was defined as a rat advancing more than half of the way down an arm. Rats from each of the two treatment groups were randomly assigned to a particular chronological test order (1–16) that was kept constant across training.

Results

Accuracy

Two measures of maze performance were analyzed: 1) The number of choices required to obtain all food items, and 2) the number of arms chosen before the first working memory error. The minimum score for the first measure would be 8 choices for the 12-arm maze and 12 choices for the 18-arm maze. For the second measure, the maximum score would be 12 choices for the 12-arm maze and 18 choices for the 18-arm maze.

12-Arm Maze Training

The median number of choices required to obtain the food pellets in each of the 8 S+ arms for the two groups (Control and Choline) is plotted as a function of blocks of 3 sessions in Figure 1. The general pattern seen in the number of choices to criterion was a constant difference in performance produced by choline treatment and an improvement over blocks of sessions at approximately equal rates for both groups. The animals treated both pre- and postnatally with choline showed the most accurate performance beginning with the first block of sessions and continuing throughout the 30 days of training.

An A × (B × S) analysis of variance with treatment condition as one factor and subjects nested with blocks of 3 sessions as another indicated a significant effect of both treatments and sessions; $F(1,14) = 6.37$, $p < 0.025$ and $F(9,126) = 75.15$, $p < 0.001$, respectively. For additional analysis the combined performances of rats were broken down into working memory and reference memory components as illustrated in Figures 2 and 3 respectively. A two-way analysis of variance again indicated a significant effect of both treatments and sessions for both types of memory. For working memory, $F(1,14) = 6.77$, $p < 0.025$ and $F(9,126) = 71.6$, $p < 0.001$ as a function of treatment condition and blocks of sessions, respectively, and for reference memory, $F(1,14) = 4.73$, $p < 0.05$ and $F(9,126) = 64.3$, $p < 0.001$ as a function of treatment condition and blocks of sessions, respectively.

The capacity of working memory was assessed for 12-arm maze performance by determining the number of arms chosen before the first working memory error. Control animals made 7.8 ± 0.4 choices before committing their first working memory error and Choline animals made 8.2 ± 0.2 choices before committing their first working memory error, a nonsignificant difference, $t(14) < 1$, ns.

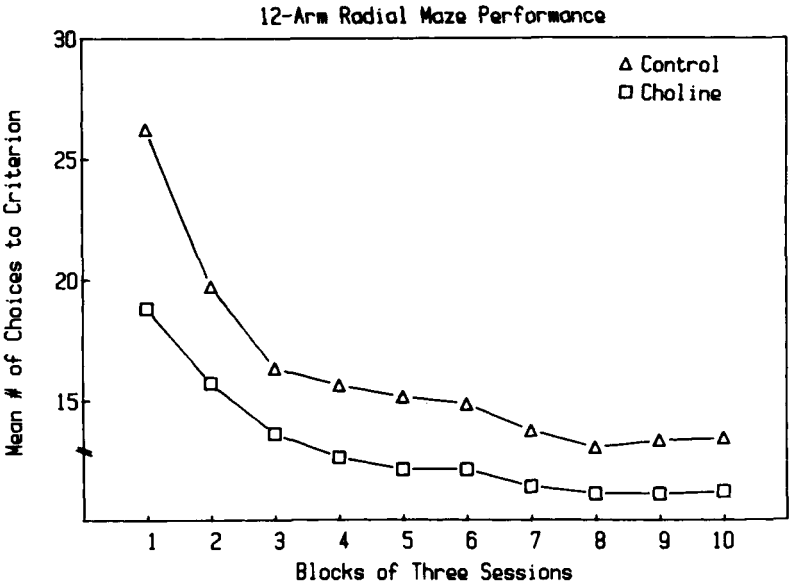

Fig. 1. Median overall 12-arm radial maze performance (combined working and reference memory errors) represented by the mean number of choices to criterion as a function of blocks of three sessions for rats in the Control and Choline treatment conditions.

18-Arm Maze Training

The median number of choices required to obtain the food pellets in each of the 12 S+ arms for each of the groups (Control and Choline) is plotted as a function of blocks of three sessions in Figure 4. The general pattern seen in the

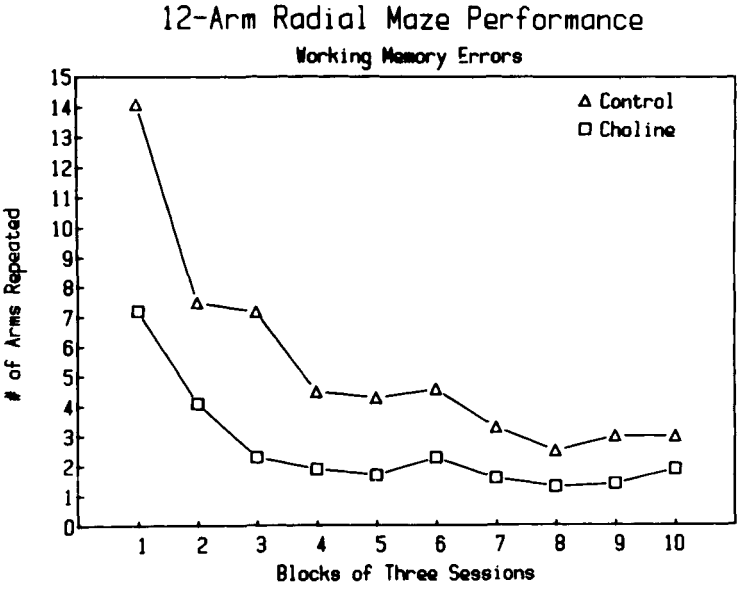

Fig. 2. Working memory performance on the 12-arm radial maze represented by the mean number of arms repeated during a session as a function of blocks of three sessions for rats in the Control and Choline treatment conditions.

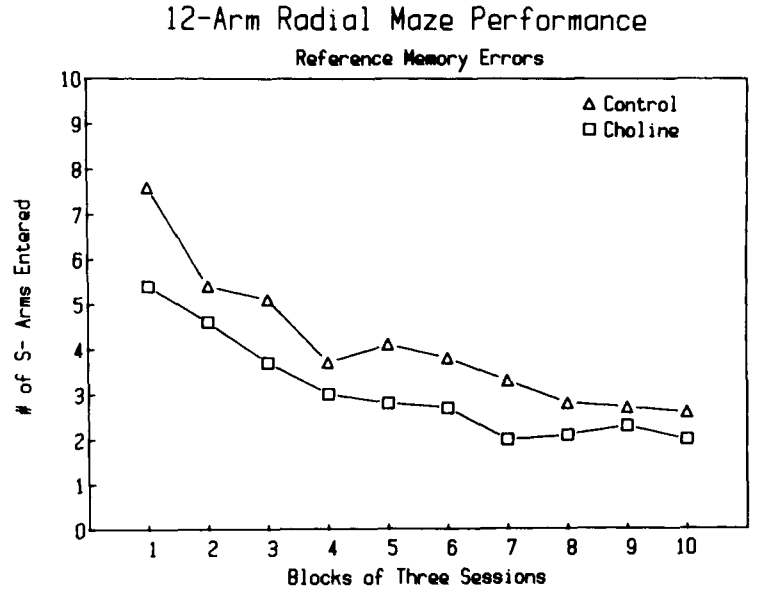

Fig. 3. Reference memory performance on the 12-arm radial maze represented by the mean number of S- arms entered during a session as a function of blocks of three sessions for rats in the Control and Choline treatment conditions.

number of choices to criterion was a separation produced by choline treatment and an improvement over blocks of sessions at approximately equal rates for both groups. Again, the animals treated both pre- and postnatally with choline showed the most accurate performance beginning with the first block of sessions and continuing throughout training.

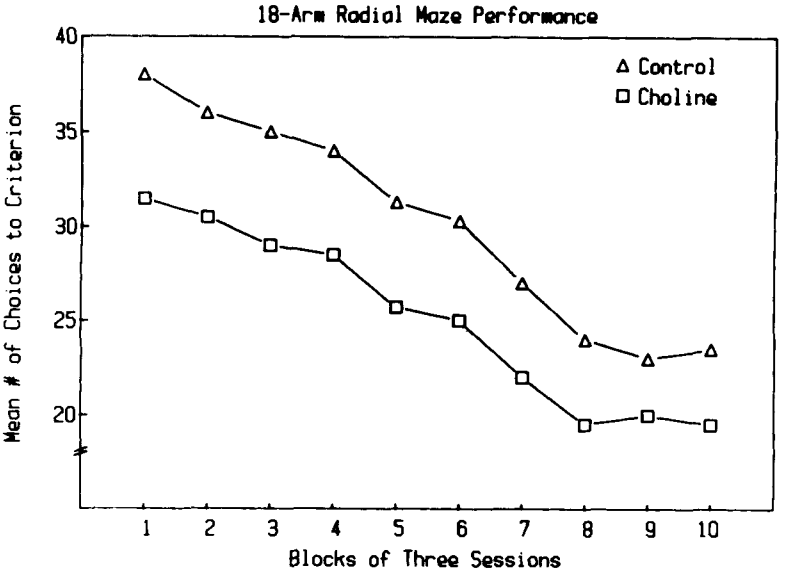

Fig. 4. Median overall 18-arm radial maze performance (combined working and reference memory errors) represented by the mean number of choices to criterion as a function of blocks of 3 sessions for rats in the Control and Choline treatment conditions.

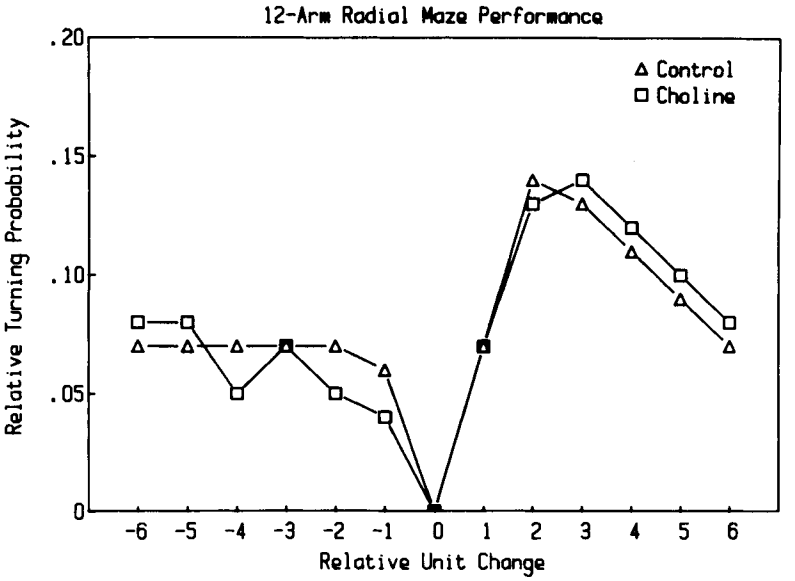

Fig. 5. Mean turning probabilities as a function of the relative unit change in the 12-arm radial maze task for rats in the Control and Choline treatment conditions. Negative values indicate leftward turns and positive values indicate rightward turns.

An A × (B × S) analysis of variance with treatment condition as one factor and subjects nested with blocks of 3 sessions as another indicated a significant effect of both treatments and sessions; $F(1,14) = 5.38$, $p < 0.05$ and $F(9,126) = 81.37$, $p < 0.001$, respectively.

The capacity of working memory was assessed for 18-arm maze performance by determining the number of arms chosen before the first working memory error. Control animals made 7.6 ± 0.2 choices before committing their first working memory error and Choline animals made 9.1 ± 0.2 choices before committing their first working memory error, a highly significant difference, $t(14) = 4.7$, $p < 0.001$.

Position of Chosen Arms

12-Arm Maze Training

An analysis of the sequence of choices made during the first 15 sessions was conducted for 12-arm maze performance. Each choice made on a test trial was scored as 0, 1, 2, 3, 4, 5, or 6; 0 corresponded to reentrance into the arm just exited, and 1, 2, 3, 4, 5, and 6 corresponded to entrances into arms 1, 2, 3, 4, 5, or 6 units removed from the arm just exited. The left (−) or right (+) direction of each turn was recorded. The mean proportions for the relative turn magnitudes are shown for each of the groups in Figure 5. As the figure illustrates, rats in each of the groups generally turned more frequently to the right into arms two or three units from the arm just departed. These findings are in general agreement with other data on turning tendencies in both rats and pigeons (Dale, 1982; Roberts & Dale, 1981; Roberts & Van Veldhuizen, 1985).

18-Arm Maze Training

An analysis of the sequence of choices made during the first 15 sessions was conducted for 18-arm maze performance. Each choice made on a test trial was scored as 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9; 0 corresponded to reentrance into the arm just exited, and 1, 2, 3, 4, 5, 6, 7, 8, and 9 corresponded to entrances into arms 1, 2, 3, 4, 5, 6, 7, 8 or 9 units removed from the arm just exited. The left (−) or right (+) direction of each turn was recorded. The mean proportions for the relative turn magnitudes are shown for each of the groups in Figure 6. As the figure illustrates, rats in each of the groups generally turned more frequently into arms two or three units from the arm just departed. These findings are in general agreement with the 12-arm maze data reported above.

The fact that the frequencies of arm entrances for both 12-arm and 18-arm maze performance were not evenly distributed over turn magnitudes suggests that simple calculations of expected chance performance are not entirely appropriate for comparison with the observed empirical performance of the rats. That is, a rat could score much higher than chance by entering adjacent arms in succession. However, such behavior would require only a response algorithm and no memory for previously entered arms. In order to deal with this problem we performed Monte Carlo runs, in which sampling from the pool of responses was biased according to the rat's turning preferences, but was independent of any working or reference memory information (see Eckerman, 1980). Thus, if a rat entered the +1 arm 50% of the time, then half of the responses in the response pool were +1. For each of our rats, we performed 500 similar computerized Monte Carlo runs. The mean *observed* and *expected* levels of accuracy generated by this procedure for both 12-arm and 18-arm maze performance as a function of the experimental

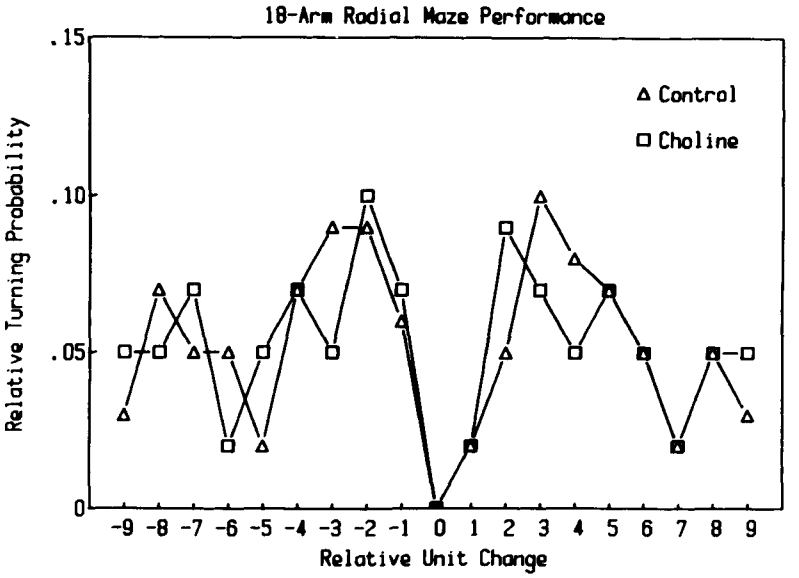

Fig. 6. Mean turning probabilities as a function of the relative unit change in the 18-arm radial maze task for rats in the Control and Choline treatment conditions. Negative values indicate leftward turns and positive values indicate rightward turns.

TABLE 1. Monte Carlo Simulations: Observed and Expected Choices to Criterion.

Group	Observed	Expected
12-Arm maze performance		
Choline	13.3 ± 0.6	28.7 ± 0.5
Control	16.5 ± 0.8	29.4 ± 0.3
18-Arm maze performance		
Choline	28.1 ± 1.3	44.3 ± 2.2
Control	35.5 ± 2.1	46.7 ± 2.8

Note: Numbers are means ± the standard error of the mean. Observed values are the mean number of obtained choices to criterion averaged over individual subjects in each group for the first 15 sessions of maze performance. Expected values are the mean number of predicted choices to criterion averaged over the 500 Monte Carlo simulations/subject based on the turning biases obtained for individual subjects in each group during the first 15 sessions.

treatments are shown in Table 1. There were no reliable differences in the *expected* levels of accuracy between the groups; $t(14)$s < 1, ns.

Rotation Test

Rats were considered to have reached steady-state levels of performance when they showed no further improvement in performance for at least 9 sessions. Based on this criterion all subjects in both the Control and the Choline groups reached a steady-state level of 12-arm maze performance prior to the maze rotation test. Rats in the Control group made 7.8 ± 0.3 errors based on an intramaze cue strategy and 4.8 ± 0.5 errors based on an extramaze cue strategy, a significant difference in favor of the extramaze cue strategy, $t(7) = 4.4$, $p < 0.01$. Rats in the Choline group made 8.3 ± 0.6 errors based on an intramaze cue strategy and 2.9 ± 0.8 errors based on an extramaze cue strategy, a significant difference in favor of the extramaze cue strategy, $t(7) = 4.15$, $p < 0.01$.

Choice Latency

The time taken to make the first 8 choices of each trial did not differ significantly for the Control and Choline treatment groups on either the 12 arm maze (89.8 ± 4.4 s and 93.3 ± 6.4 s, respectively) or the 18 arm maze (92.4 ± 5.0 s and 94.1 ± 4.9 s, respectively) during the last 3 training sessions. The similarity of these choice latency measures suggests that all animals were at comparable motivational levels at this stage of training.

Body Weight

At the completion of the experiment there was no difference between the mean weights of the rats in the Control group (362.0 ± 7.0 g) and rats in the Choline group (357.2 ± 6.4 g); $t(14) < 1$, ns.

Discussion

The present study demonstrates that rats given supplemental choline pre- and postnatally perform more accurately on both the 12-arm and 18-arm radial maze when they reach adulthood than control rats not given supplemental choline perinatally. This performance difference reflects a modification of both working and reference memory and is not due to differential response or cue-use strategies used by the Control and Choline rats. Instead, it appears that performance differences are due to long-term enhancement of spatial memory capacity and precision. These conclusions are supported by the observation that Choline rats perform approximately 20% more accurately than Control rats at all phases of training as illustrated in Figure 7. Furthermore, the capacity of working memory as assessed by the number of choices made before the first working memory error is significantly greater in Choline rats than in Control rats, when training is conducted on the 18-arm maze. This difference in working memory capacity was probably not observed in the 12-arm maze performance because of a ceiling effect. Taken together, these results lend support to the idea that exposure to choline during early development can increase working memory capabilities for processing spatial information.

One question that must be considered when interpreting these results is the specificity of the observed behavioral effects. Might any dietary enhancement early in life influence performance later in life? For example, would tyrosine (a catecholamine precursor) or tryptophan (a serotonin precursor) have similar effects or is the effect demonstrated here specific to cholinergic systems? Is choline supplementation increasing arousal or attention, thereby setting the stage for enhanced performance later on? Since control animals in this study did not receive a different dietary supplement, but rather no supplement at all, the observed

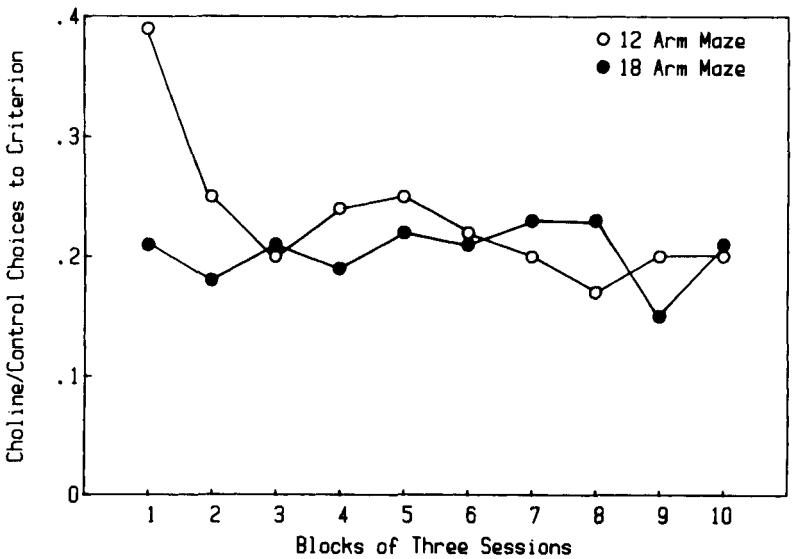

Fig. 7. Ratio of Choline : Control overall group performance on the 12-arm and the 18-arm radial maze as a function of blocks of three sessions.

effects may have occurred because of a generalized increase in performance due to enhanced early nutrition rather than because of a specific effect on memory.

However, recent work with tyrosine administration to pregnant rats has shown behavioral modifications in the male offspring that reflect a marked increase in both spontaneous locomotor activity and open field locomotion as adults (Arevalo, Castro, Palarea, & Rodriguez, 1987). These long-term behavioral effects of tyrosine supplementation do not seem consistent with our findings of no long-term changes in locomotion in the radial-arm maze following choline supplementation. In addition, there are several reports suggesting that in humans a transitory increase of plasma tyrosine levels during the first postnatal days decreases intellectual performance (Menkes, Welcher, Levi, Dallas, & Gretsky, 1972) and induces learning disabilities 6 or 8 years later (Mamunes, Prince, Thornton, Hunt, & Hitchcock, 1976). However, as far as we know, there are no reports of spatial memory testing following perinatal tyrosine supplementation in animals or humans. Although these data suggest that the effects of early dietary supplementation of choline on spatial memory reported here are specific, further research utilizing other dietary supplements and other behavioral tests are necessary.

The long-term facilitative effects of perinatal choline supplementation on spatial memory ability have recently been replicated and extended (Meck, Smith, & Williams, 1988; Smith, Meck, & Williams, 1986; Meck & Williams, 1988). This work has further examined the behavioral and neurochemical specificity of added dietary choline on 12-arm radial maze performance. In the Meck et al. (1988) study, choline chloride was given both prenatally (to the diet of pregnant rats) and/or postnatally (intubed directly into the stomachs of rat pups). Working memory and reference memory functions were correlated with neurochemical measures of brain cholinergic activity in the hippocampus and frontal cortex of male albino rats when they became adults. The data demonstrate that perinatal choline supplementation causes: (1) Long-term facilitative effects on both working memory and reference memory components of a 12-arm radial maze task as a function of pre- and/or postnatal choline administration. Within the dose range employed, pre- plus postnatal treatment produced the largest behavioral facilitation, followed by prenatal treatment alone, postnatal treatment alone, and finally the no choline (control) group. Clearly the critical period for choline exposure spans both pre- and postnatal development. However, the fact that prenatal treatment was more effective than postnatal treatment may be a dose, rather than a timing effect. (2) Choline supplementation produced long-term increases in muscarinic receptor density as indexed by [^{3}H]-quinuclidinyl benzilate (QNB) binding in both the hippocampus and the frontal cortex and lowered choline acetyltransferase (ChAT) levels in the hippocampus but had no effect on ChAT levels in the frontal cortex. An analysis of the relationship between these organizational changes in brain and memory function indicated that the ratio of ChAT : QNB in the hippocampus is highly correlated with working memory ability and the ratio of ChAT : QNB in the frontal cortex is highly correlated with reference memory ability.

Notes

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