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Effects of Folic Acid and Folinic Acid on Cognitive and Motor Behaviors in 20-Month-Old Rats

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LALONDE, R., C. C. JOYAL AND M. I. BOTEZ. *Effects of folic acid and folinic acid on cognitive and motor behaviors in 20-month-old rats.* PHARMACOL BIOCHEM BEHAV **44**(3) 703–707, 1993.—Old rats had lower plasma concentrations of folates but not of vitamin B12 than young rats. Old rats injected with pharmacological doses of folic acid (5 mg/kg) or folinic acid (2.5 mg/kg) every 2 days for a 32-day period spontaneously alternated above chance levels at the 0-min retention interval whereas old rats injected with placebo did not. Rats injected with folinic acid that had plasma folate concentrations above the median for that group alternated at the 3-min retention interval whereas none of the other subgroups did. These results indicate that supplementation with folinic acid at pharmacological doses may decrease perseverative responding and improve spatial memory in old rats. However, neither vitamin group was improved in motor coordination, grip strength, or spatial learning in a water maze. There was no hyperactivity or loss of body weight following vitamin supplementation.

Folic acid Folinic acid Aging Spontaneous alternation Spatial learning

ELDERLY persons are liable to develop folate deficiency (12). This condition is rarely encountered in a pure form and there are many possible causes, including malnutrition, malabsorption, alcoholism, drugs, depression, and illness (12). In both humans and animals, there is an age-related folate malabsorption in polyglutamate, but not monoglutamate forms (2,15). In old rats, Kesavan and Noronha (13) reported low levels of folyl conjugase of pancreatic origin, an enzyme that converts dietary polyglutamates into absorbable monoglutamates.

Learning impairments in young rats (1) and neuropsychological deficits in humans (7) have been described following folate deficiency. Moreover, alterations in neurochemical processes may be related to folate deficiency such as a reduction of serotonin and its main metabolite, 5-hydroxyindoleacetic acid (4,6,8), although a negative report exists (9). Because folate deficiency is associated with behavioral deficits and neurochemical alterations, it is possible that folate deficiency in old age causes behavioral dysfunctions that are reversible with folate supplementation.

The main goals of the present study were to determine whether plasma folate levels are lower in older rats and if so whether administration of folic acid or the more potent analog, folinic acid (5-formyltetrahydrofolate, leucovorin) improves behavioral performances in these rats.

Many behavioral functions undergo an age-related decline in rodents, including motor activity (17), motor coordination, grip strength (16), and spatial learning and memory (11,18). In the present study, we chose behavioral tasks particularly sensitive to aging effects for the purpose of evaluating the possible beneficial effects of folic acid and folinic acid.

METHOD

Subjects

Fifty-seven old (20 months old at reception) male Fischer-344 and 10 younger (9 months old at reception) rats were obtained from Harlan-Sprague-Dawley (Indianapolis, IN). Upon arrival at the laboratory, old rats were daily handled during 10 consecutive days prior to behavioral testing. All animals were housed two per cage with food (Purina Rat Chow) and water available at all times in a temperature- and humidity-controlled room. A 12 L : 12 D cycle (lights off at 1830 h) was maintained throughout the study. Behavioral tests were conducted during the afternoon and early evening.

Apparatus and procedure

Drug administration. Old rats were randomly separated into three groups. Rats were injected IP every 2 days with

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TABLE 1

MEAN (SD) PLASMA FOLATE (ng/ml) AND VITAMIN B12 CONCENTRATIONS (pg/ml) OF YOUNG (10 MONTHS) AND OLD (21 MONTHS) RATS GIVEN EITHER PLACEBO, FOLINIC ACID (2.5 mg/kg), OR FOLIC ACID (5 mg/kg)

Group	*n*	Folate Concentrations (ng/ml)	Vitamin B12 Concentrations (pg/ml)
Young rats injected with placebo	10	72.1 (18.7)	850.0 (126.2)
Old rats injected with placebo	18	57.3 (10.9)*	976.3 (168.2)
Old rats injected with folinic acid	19	87.0 (18.9)*†	917.0 (144.9)
Old rats injected with folic acid	19	89.9 (14.7)†‡	974.0 (190.4)

*$p < 0.05$, ‡$p < 0.01$ compared to young rats.
†$p < 0.01$ compared to placebo (Dunnett's *t*-test).

folic acid (5 mg/kg, $n = 20$), folinic acid (2.5 mg/kg, $n = 19$), or placebo (0.9% saline, $n = 18$) for a period of 32 days (16 injections). Behavioral testing occurred every 2 days, on those days on which no injections were given, starting on day 2 and ending on day 32 (16 days). Young rats received saline injections during the same period.

Vitamin determinations. At the end of the study (on day 32), blood samples were taken after decapitation to determine the plasma folate levels of the three old groups and the young group as assessed by a radioassay kit (B12/folate, Combostat II, Micromedic, Horsham, PA) that also included vitamin B12 determinations because variable folate concentrations can alter B12 concentrations (3).

Behavioral Tasks

Four main types of behavioral tests were used. There were 16 days of behavioral testing: spontaneous alternation (days 1–9), motor activity and hole-poking (days 4–6 preceded by a habituation period on days 1–3), psychomotor performance (days 1–9), and spatial learning in a water maze (days 10–16).

Spontaneous alternation (18). A T-maze, made of transparent plastic (stem = 81.5 × 8.5 cm, arms = 30 × 8.5 cm, height of walls = 10 cm), was used for a two-trial spontaneous alternation test for 9 days (behavioral testing days 1–9). A single-day habituation period (1 day before the start of the experiment) was allowed so that rats could explore the apparatus (four rats at a time). The first trial was a forced trial in that the rat entered one arm of the T-maze, the other arm being blocked by a plastic barrier. In the second trial, the rat could either choose the same arm or alternate. The blocked arm changed every day, that is, the right side on day 1, the left on day 2, then the right again on day 3, etc. There was no intertrial interval [except for the brief time (about 15 s) necessary to wash the maze after every trial] on days 1, 4, and 7, an intertrial interval of 3 min on days 2, 5, and 8, and an intertrial interval of 10 min on days 3, 6, and 9. The T-maze was always installed in the same area in the experimental room.

Motor activity and hole-poking. Locomotor activity and hole-poking was evaluated in a 4 × 4 16-hole matrix divided into 25 14 × 14-cm squares. The overall floor surface was 70 × 70 cm (height of walls, 34 cm; distance between holes, 9 cm; diameter of holes, 4 cm; depth of holes, 2 cm). After preliminary adaptation to the wooden hole-board in groups of 8 (10 min per group on days 1–3), rats were placed in it one at a time for 3 consecutive behavioral testing days (days 4–6) in 4-min sessions. They were always placed at the same starting point, the square in the middle of the first row, facing the north wall. In addition to the total number of hole visits, the number of visits to one of the 4 center holes in the matrix was counted as distinguished from the number of visits to the 12 remaining holes in the periphery. The number of center squares and peripheral squares traversed (9 squares in the center and 16 in the periphery) was also counted. Moreover, the number of rears (vertical exploration) was observed. The 4-min session was divided into two 2-min sessions to test for intratrial habituation.

Psychomotor tests (16)

Grid test. A wire mesh screen (38 × 38 cm; 5 squares/cm, wooden frame), placed at a height of 89 cm from the ground (covered by a soft blanket), was used to test geotactic reflexes and grip strength. The rat was placed in the center of the

TABLE 2

SPONTANEOUS ALTERNATION RATE AT THREE RETENTION INTERVALS ABOVE (>) OR BELOW (<) THE MEDIAN

Group	0 Min (%)	3 Min (%)	10 Min (%)
Folinic acid ($n = 19$)			
<	73*	53	50
>	74*	74*	30
Total	74†	64	40
Folic acid ($n = 19$)			
<	77†	50	47
>	67	52	37
Total	72†	51	42
Placebo ($n = 18$)			
<	59	52	41
>	67	63	19
Total	63†	58	30

*$p < 0.02$.
†$p < 0.01$.

TABLE 3

MEAN (SD) NUMBER OF HOLE VISITS AND SQUARES TRAVERSED FOR FOLINIC ACID- (n = 19), FOLIC ACID (n = 20), AND PLACEBO (n = 18)-INJECTED RATS SUMMED OVER 3 DAYS IN A 16-HOLE MATRIX (THREE SESSIONS OF 4 MIN)

Group	Peripheral Holes	Center Holes	Peripheral Squares	Center Squares	Rears
First 2 min					
Folinic acid	3.3 (2.5)	0.8 (1.8)	15.0 (10.1)	2.3 (2.7)	1.5 (1.6)
Folic acid	2.9 (2.6)	0.6 (1.7)	12.9 (11.0)	2.8 (3.8)	1.5 (1.8)
Placebo	3.0 (2.7)	0.3 (0.6)	11.9 (10.6)	2.0 (2.4)	1.5 (1.5)
Last 2 min					
Folinic acid	0.4 (0.7)	0 (0)	1.8 (3.7)	0.1 (0.2)	0.3 (0.8)
Folic acid	0.5 (0.9)	0 (0)	2.1 (3.9)	0.3 (1.0)	0.5 (1.0)
Placebo	0.6 (1.1)	0 (0)	2.8 (5.0)	0.3 (0.6)	0.5 (1.0)

screen (inclined at 40°), facing downward. Four latencies were recorded: a) the latency until the rat turned to face upward; b) and c) the latency before reaching the top of the screen with either the snout or the front paws; d) the time spent on the grid (cut-off point = 120 s). Each rat had two trials a day with an intertrial interval of approximately 6 min during two blocks of 3 days (total = 12 trials: days 1-3 and 7-9).

Wire suspension. The front paws of rats were placed on a horizontal wire (2 mm in diameter) 96 cm above a soft blanket on a table. The latency before the rat fell from the wire was recorded (maximum time allowed = 60 s). There were two trials per day during two blocks of 3 days (total = 12 trials: days 1-3 and 7-9). The intertrial interval was approximately 1 min.

Round bridge. A metal bridge (length = 70 cm, diameter = 2.8 cm) was suspended between two platforms 70 cm above a foam cushion. The rat was placed in the middle of the bridge and the latency before falling was recorded (cut-off point = 60 s). Two trials per day were allowed (intertrial interval = approximately 2 min) for 3 days (total = six trials: days 7-9). None of the subjects was able to reach either platform.

Square bridge. Another metal bridge (length = 60 cm, 2.5 × 2.5 cm in cross-section, 53 cm above the foam cushion) was used with the same procedure as that for the round bridge. Here, again no animal could reach either platform.

Water maze spatial learning (14). Our water maze test was an adaptation of the Morris test (14). The apparatus was a large, rectangular pool made of stainless steel (108 × 66 cm, height = 51 cm) and filled with water (approximately 27°C) to a depth of 25 cm. A transparent escape platform (diameter = 4.5 cm) was placed in the pool either 3-4 cm below the water surface (place learning, invisible platform condition) or above water level (cue learning, visible platform condition). Many extramaze visual cues surrounding the maze were available, the experimenter being present in the same location (south).

During each trial, the rat was placed into the water close to and facing the wall of the pool at one of four equally spaced locations: north (N), east (E), south (S), and west (W). The pool was separated into four quadrants: NW, NE, SE, and SW. Rats were allowed to swim freely until they found the platform, on top of which they could climb. If a rat failed to locate the platform within 60 s, it was then placed on the platform by the experimenter, where it remained for 5 s. Each rat received eight trials per day and at each trial the starting position changed (the first one was always on the N side, followed by E, S, and W sides in that order). The intertrial interval was 3 min between trials 1-4 and 5-8 and 8 min between trials 4 and 5. For the first 3 days of maze testing (days 10-12 of behavioral testing), the submerged platform was placed in the NW quadrant and then in the SE quadrant for the following 3 days (days 13-15). On day 7 of water maze testing (day 16 of behavioral testing), the platform was visible, emerging from the water and situated in the SW quadrant.

RESULTS

Vitamin levels

The results of one of the rats in the folic acid group gave an aberrant value and was not included (n = 19 instead of

TABLE 4

MEAN (SD) LATENCY (SECONDS) BEFORE TURNING, REACHING THE TOP (SNOUT OR FRONT PAW CRITERIA), AND FALLING FROM AN INCLINED SCREEN FOR FOLINIC ACID- (n = 19), FOLIC ACID- (n = 20), AND PLACEBO (n = 18)-INJECTED RATS (12 TRIALS)

Group	Turning	Reach the Top With the Snout	Reach the Top With the Front Paws	Fall
Folinic acid	36.4 (38.6)	95.8 (42.6)	104.4 (34.5)	95.7 (34.7)
Folic acid	42.0 (42.4)	96.8 (40.5)	105.4 (32.0)	91.2 (32.5)
Placebo	42.8 (42.6)	94.6 (43.4)	103.0 (40.0)	94.1 (32.0)

TABLE 5

MEAN (SD) LATENCY (SECONDS) BEFORE FALLING OFF A WIRE (12 TRIALS), A ROUND BRIDGE (6 TRIALS), AND A SQUARE BRIDGE (6 TRIALS) FOR FOLINIC ACID- (n = 19), FOLIC ACID- (n = 20), AND PLACEBO (n = 18)-INJECTED RATS

Group	Wire Suspension	Round Bridge	Square Bridge
Folinic acid	10.2 (6.9)	8.0 (13.8)	30.1 (22.3)
Folic acid	10.7 (6.8)	6.2 (6.9)	30.1 (19.9)
Placebo	10.2 (6.8)	9.9 (14.2)	34.4 (21.8)

20). Analysis of variance (ANOVA) revealed a significant group difference in terms of folic acid levels $F(3, 62) = 16.42$, $p < 0.001$. Rats receiving no vitamin supplementation had lower plasma folate levels than young rats (Dunnett's $t = 2.37$, $p < 0.05$) (Table 1). Rats injected with either folic acid or folinic acid had higher plasma folate levels than those of old (folic: Dunnett's $t = 5.7$, $p < 0.01$; folinic: Dunnett's $t = 6.28$, $p < 0.01$) and young (folic: Dunnett's $t = 2.42$, $p < 0.05$; folinic: Dunnett's $t = 2.90$, $p < 0.05$) rats receiving placebo. There was no significant difference in vitamin B12 levels between any of the groups (Table 1).

Body weight

There was no difference in body weight between the three groups of rats (data not shown).

Spontaneous alternation

The placebo group did not alternate significantly above chance levels at any retention interval (Table 2). Rats receiving folic acid ($\chi^2(1) = 10.97$, $p < 0.01$) or folinic acid ($\chi^2(1) = 12.78$, $p < 0.01$) alternated at the 0- but not at the 3- or 10-min retention intervals (Table 2). When the folinic group was divided into two subgroups depending upon plasma folate concentrations above or below the median, it was found that the subgroup above the median had a 74% alternation rate at the 3-min retention interval ($\chi^2(1) = 6.2$, $p < 0.01$) whereas the subgroup below the median did not alternate (53%).

Motor activity and hole-poking

No differences were found in exploratory activities between the placebo group and either the folinic acid or the folic acid groups. (Table 3).

Psychomotor tests

There were no group differences in the grid (Table 4), wire suspension, round bridge, or square bridge tests (Table 5).

Water maze spatial learning

No differences were found between groups either in the number of quadrant entries or the latencies to escape in any of the three experimental procedures (Fig. 1).

DISCUSSION

Older rats had lower plasma concentrations of folates but not of vitamin B12. Previous studies had indicated that in aged rats there is a reduced absorption of folates in polyglutamate but not in monoglutamate forms (13,15). Thus, the rat is a good model to study possible folate-responsive behavioral disorders in old age, and this despite the many differences that exist between rat and human folate blood levels and requirements [see the monography by Botez and Reynolds (3)].

Previous experimentation has indicated that old rats have a lower spontaneous alternation rate than young rats, especially at longer intertrial intervals (17,18). This result may be due to perseverative responding or a defect in spatial memory. In the present study, nonvitamin-supplementated rats did not alternate at any retention interval (0, 3, or 10 min). In a previous study, rats alternated above chance levels at the 0- but not at the 4- or 10-min retention interval (18). In our apparatus, rats demonstrated perseverative responding, associated in part with a frontal lobe dysfunction (10). In the Zornetzer study (18), rats had a selective deficit in spatial memory. Either deficit may be present in old animals, depending possibly upon the age of the animal or the type of apparatus chosen.

While aged rats injected with placebo did not alternate, rats injected with either folic or folinic acid did. This result indicates that folate administration in rats may diminish perseverative responding. Vitamin supplementation was not accompanied by hyperactivity (Table 3) or a decrease in body weight. In addition, although the folinic acid group as a whole did not alternate at the 3-min retention interval the subgroup with plasma folate levels above the median for that group did

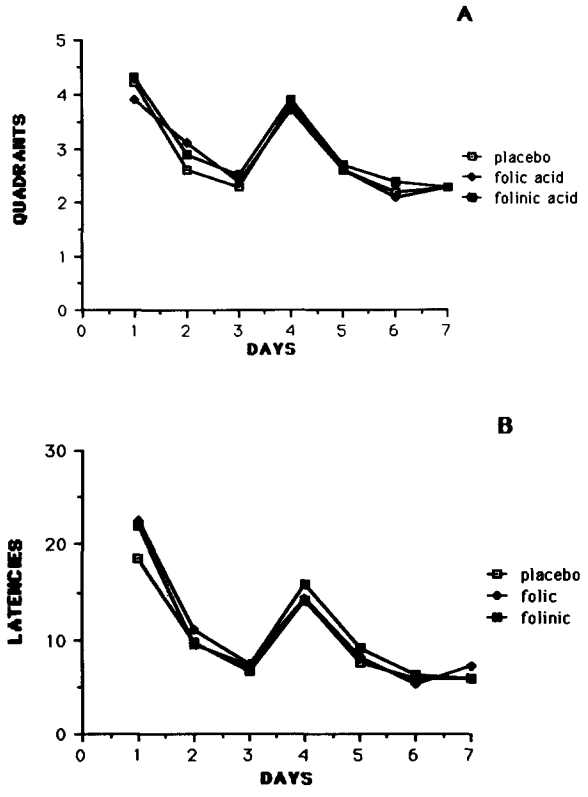

FIG. 1. Effects of folic acid and folinic acid on quadrant entries (A) and latencies (seconds) (B) in a spatial learning task in a water maze. On days 1-3, rats learned to reach an invisible platform in the NW position. On days 4-6, the position of the invisible platform was changed to the SE position. On day 7, the platform was visible at the SW position.

(Table 2). This result indicates that a pharmacological dose of folinic acid may improve spatial memory. However, there was no improvement in spatial learning in the water maze for either vitamin group, an indication that this amelioration is situation specific. Although showing limited improvement in these rats, vitamin supplementation should be attempted in older animals because they may be even more folate deficient. Thus, folate administration may produce a wider range of improvement because folate entry across the blood-brain barrier is facilitated in folate-deprived conditions (5).

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