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The String Test: An Early Behavioral Change in Thiamine Deficiency

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BARCLAY, L. L., G. E. GIBSON AND J. P. BLASS. *The string test: An early behavioral change in thiamine deficiency.* PHARMAC. BIOCHEM. BEHAV. 14(2) 153–157, 1981.—Thiamine deficiency is a useful animal model of the interaction between biochemistry and behavior. Although numerous biochemical changes have been detected in thiamine deficiency, studies of behavioral changes are relatively scarce. We have modified and quantitated the string test, originally described by Miquel and Blasco, for application to thiamine-deficient rats. The string test is reproducible with time, and control rats have a narrow range of scores. 50% of rats treated with thiamine-deficient diet and pyrithiamine, a centrally-acting thiamine antagonist, have persistently decreased string test scores. This decrease is already present on day 5 of treatment, long before the onset of weight loss or neurological symptoms. Rats treated with oxythiamine, a peripherally acting thiamine antagonist, do not have decreased string test scores, even when anorectic and moribund. These findings suggest that impaired string test performance is a central nervous system effect of thiamine deficiency, and that it may also be a useful behavioral parameter to follow in other animal models of metabolic encephalopathies.

Thiamine deficiency Pyrithiamine String test

EVER since Sir Rudolph Peters' discovery of the role of thiamine in pyruvic acid decarboxylation [11] and the description of neurological abnormalities in the Wernicke-Korsakoff syndrome [14], thiamine deficiency has been regarded as a potentially useful model of the interplay between biochemistry and behavior. In rats, treatment with thiamine-deficient diet in conjunction with the centrally acting thiamine antagonist pyrithiamine produces preterminal neurological symptoms which include ataxia, convulsions, and opisthotonus. Biochemical abnormalities in erythrocyte transketolase occur long before the onset of neurological symptoms [2], and there are also numerous biochemical changes in the central nervous system [3, 12, 17].

Although behavioral changes in thiamine deficiency have been reported previously [1, 4–7, 9, 10, 13, 15, 16, 18], these studies can be criticized on several grounds. Advanced thiamine deficiency is associated with decreased maze learning when food is used as reinforcement, and with impaired development of a conditioned eyelid response [1]. However, the use of food as reinforcement is of dubious value in anorectic animals, and neither study used pair-fed animals to control for the effects of caloric deprivation. Khairy *et al.* [5,6] used pair-fed controls, but found no significant effects of advanced thiamine deficiency on open-field behavior, on discrimination situations, or on instrumental conditioning situations. Water maze performance was decreased only when the rats developed polyneuritis. Rats with advanced thiamine deficiency did react differently from controls in 3 different measures of reaction to conflict [6]. However, this effect was only significant as the number of conflict trials increased; the initial responses of thiamine-deficient rats closely resembled those of pair-fed controls. Vorhees *et al.* found no impairment in 2-way shuttle box avoidance acquisition until ataxia occurred, but did note thiamine-deficiency induced mouse-killing just before neurological symptoms [15,16]. Since oxythiamine, a peripheral thiamine antagonist, caused this phenomenon as well as pyrithiamine, a central thiamine antagonist, muricide does not seem to be related to a central nervous system lesion.

Another approach to studying the effects of thiamine deficiency on behavior is to reverse advanced thiamine deficiency with thiamine, and to then look for persistent behavioral defects. When infant rats were made thiamine-deficient from gestation to 8 weeks of life and were then given thiamine-containing diets to regain normal weight, they performed poorly on escape-from-water mazes [9,13]. However, pair-fed animals were not used to control for the effects of early malnutrition. Rats which were given thiamine-deficient diet until neurological symptoms developed and which were then reversed with thiamine showed decreased learning in a Y-maze avoidance-discrimination apparatus [16].

Studies of behavioral effects of early stages of thiamine deficiency have been relatively scarce. Although increased

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running is an early symptom of thiamine deficiency [4], it is also seen non-specifically in food or water deprivation [18]. Rats given thiamine-deficient diet for 4 days show increased startle response to electric shock, and this effect progressively increases on day 7, 10, and 13 [10]. However, this effect may be a peripheral rather than a central nervous system effect.

In hopes of finding a simple, objective behavioral parameter to follow in thiamine deficiency, we modified and quantitated the "string test" for use in pyrithiamine-treated or oxythiamine-treated rats. The string test, first described by Miquel and Blasco for studies of aged mice [8], measures the ability of animals to travel along a string. Our findings indicate that the string test is a reliable and reproducible measure of neurological competence in normal animals, and that it can detect early behavioral changes in thiamine deficiency long before the onset of gross neurological symptoms.

The string test may be a useful behavioral parameter to follow in other experimental models of central nervous system dysfunction, including aging, hypoxia and other toxic or metabolic encephalopathies. Since the string test is reproducible and can be easily repeated without affecting the result, it can be measured in a single animal before and after treatment. Therefore, it might serve as a useful index of experimentally induced central nervous system dysfunction.

TABLE 1
SCORING PROCEDURE FOR STRING TEST

Scoring:
1 point for each paw the rat keeps on the twine for at least 5 seconds.
1 point if the rat keeps its tail on the twine for at least 5 seconds.
3 points if the rat travels along the twine for at least 5 seconds.
2 points if the rat reaches one of the vertical poles in less than 25 seconds.
−3 points if the rat falls in 0–15 seconds.
−2 points if the rat falls in 16–30 seconds.
−1 point if the rat falls in 31–60 seconds.
Minimum score = −3 Maximum score = 10

The testing apparatus consisted of a piece of twine 50 cm long tied tightly between 2 vertical poles and suspended over a landing pad. The rat was held by the tail and suspended over the twine near its midpoint until it fell off the string, traversed the string completely, or until 1 minute elapsed.

METHOD

Animals

Male Wistar rats weight 50–70 g were obtained from Charles Rivers Breeding Laboratories, Wilmington, Massachusetts. The rats were individually housed in stainless steel cages measuring 17.5×20.5×25.0 cm with wire mesh bottoms to help reduce coprophagia. The animal room was routinely controlled for temperature (68–72°F) and humidity (30–55%), and had 12-hour light-dark cycles (lights on 0700–1900). Upon arrival to the laboratory, rats were given distilled water and Vitamin B Complex Diet, Complete (ICN Nutritional Biochemicals, Cleveland, OH) ad lib. This diet was identical to the thiamine-deficient diet used in our thiamine-deficiency experiments, except that it contained thiamine. Rats were allowed to acclimate to the laboratory and to the diet for at least 2 days before testing began. Rats with respiratory or eye infections or with other evidence of illness were eliminated from the experiment. At the beginning of testing, rats weighed 55–85 g.

String Test Technique

We modified and quantitated the string test initially described by Miquel and Blasco [8]. The testing apparatus consisted of a piece of twine 2 mm in diameter and 50 cm long tied tightly between two vertical poles and suspended 1.5 meters over a lightly cushioned landing pad. The rat was held by the tail and suspended over the twine near its midpoint, with the rat facing the investigator. When the rat grasped the twine with its forepaws, the investigator released the tail and began timing. Observation and timing continued until the rat fell off the string or reached one of the vertical poles, or until one minute elapsed. The rat was scored from −3 to +10 based on the use of paws and tail, traveling behavior, and falling. Detailed scoring criteria are given in Table 1. String tests were performed between 0800–1200 on acclimated, healthy rats.

If the rat's initial score was 5 or less, the rat was retested after a rest period of approximately 2–3 min, and the second score was recorded. On the following day, string tests were repeated, and retests were done on those animals with scores of 5 or less. Rats which had low string test scores on the second trial of the second day were eliminated from thiamine deficiency experiments. In general, fewer than 10% of rats were eliminated for poor string tests scores. A total of 11 experiments were performed; each contained 9–66 rats.

Factors which must be carefully controlled in performing the string test include string tautness, landing pad, and noise level. Since rats do not perform as well when the string is slack, the twine must be tightened between trials by moving the poles as far apart as possible. The landing pad must not be so thin as to traumatize the rat, or so thick that the rat soon learns that falling causes no discomfort. In this laboratory, we use a plastic wastebasket lined with a disposable diaper folded double thickness. Sudden noises may frighten the rat, causing it to fall off the string prematurely, or they may cause a stationary rat to begin traveling. Other types of stress which appear to alter string test performance include respiratory or eye infections, food or water deprivation, prior surgery, and rough handling. Therefore, only healthy, regularly-fed animals should be used, and they should be handled gently, preferably by the same investigator.

String Test Studies in Pyrithiamine- and Oxythiamine-Treated Rats

Thiamine deficiency was induced with thiamine antagonists and thiamine-deficient diet according to the model of Plaitakis *et al.* [12]. This model was chosen for its reproducibility and constancy of symptom onset, with neurological signs occurring at day 12–13 and death at day 13–14 of treatment with pyrithiamine. Oxythiamine, which acts only peripherally, does not produce neurological signs, and causes death after 6–8 days of treatment. Rats with string test scores of 6 or greater on the second day of initial testing were divided into treatment groups by weight. Pyrithiamine-

TABLE 2

REPRODUCIBILITY OF STRING TEST SCORES

	1	2	3	4	5	6	7	8	10	11	12	15	17	Mean±S.E.M.
1	8	8	4	7	7	8	8	8	6	6	7	8	8	7.2±0.4
2	8	8	8	8	7	8	8	6	8	5	5	5	5	6.8±0.4
3	9	7	8	8	8	8	8	5	8	5	8	5	8	7.3±0.3
4	8	8	8	8	8	10	10	10	10	10	8	8	8	8.8±0.3
5	8	8	7	7	7	8	8	8	8	8	8	8	8	7.8±0.1
Mean ± S.E.M.	8.2 ± 0.2	7.8 ± 0.1	7.0 ± 0.9	7.6 ± 0.3	7.4 ± 0.3	8.4 ± 0.4	8.4 ± 0.4	7.4 ± 1.0	8.0 ± 0.7	6.8 ± 1.1	7.2 ± 0.7	6.8 ± 0.8	7.4 ± 0.7	

String tests were performed on 5 ad lib control rats over a 17-day period. Despite day-to-day variation in each rat, mean string test scores remained relatively constant in each rat over the 17-day period, and in the group of 5 rats on each day. If the score was 5 or less on the first two days, the animal was retested, and the second score was recorded. On the following days, only one string test was performed.

treated rats, oxythiamine-treated rats, and ad lib controls received thiamine-deficient diet ad lib. Pair-fed controls received thiamine-deficient diet in amounts equivalent to that consumed by pyrithiamine-treated rats. Pyrithiamine-treated rats were injected daily with pyrithiamine hydrobromide 0.5 mg/kg, and oxythiamine-treated rats were injected daily with oxythiamine hydrochloride 40 mg/kg intraperitoneally. Ad lib controls and pair-fed controls were injected daily with thiamine hydrochloride 0.1 mg/kg. All drugs were obtained from Sigma (St. Louis, MO). All injections were in 0.9% sodium chloride in a final volume of 0.5 cc/100 g body weight. Weights and string test scores (only 1 trial per day, even if low) were recorded daily.

RESULTS

Reproducibility of String Test

Table 2 illustrates the string test scores of five ad lib controls recorded over a 17-day period. Although the score of each rat may vary somewhat from trial to trial, the standard error for the thirteen trials was always less than 6%. The mean score of the five rats did not vary significantly from day to day, indicating that the string test score is stable with time in control rats. Although the present study only examined the string test score after 5 days of pyrithiamine or oxythiamine treatment, Table 2 validates the string test for use over longer periods.

Distribution of String Test Scores in Normal Rats

Figure 1 describes the distribution of initial string test scores in 359 untreated rats before elimination of rats with low string test scores. Most scores fall within a very narrow range (upper quartile 9, median 8, lower quartile 7). Only 7% of rats had initial scores of 5 or less. It should be noted that in one of eleven experiments, rats had much lower initial string test scores; post-mortem studies disclosed occult viral infections in these rats.

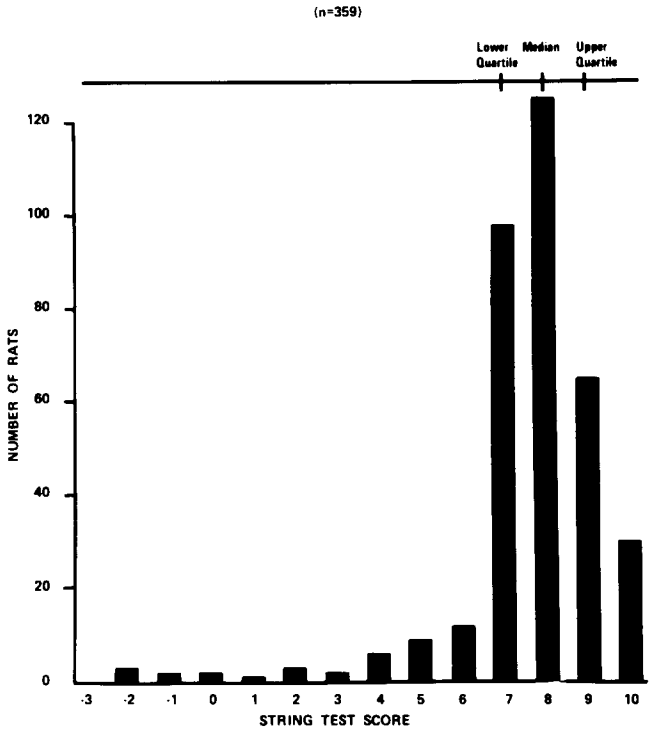

FIG. 1. Initial string test scores of 359 untreated rats. After 2 days of acclimatization to the laboratory, rats were given string tests. If the score on the initial trial was 5 or less, the rat was retested after a 2–3 min rest period, and the second score was recorded.

Distribution of String Test Scores in Pyrithiamine-Treated Rats

Figure 2 depicts the distribution of string test scores after five days of pyrithiamine treatment in 233 rats. The spread is much broader than in untreated rats (upper quartile 7, median 3, lower quartile 1). The distribution appears to be bimodal, with one peak at 7 representing rats whose string test scores were unaffected by treatment; and with a second peak around 2 representing rats whose string test scores decreased markedly with treatment. Despite the discrepancy in string test scores in these two groups of rats, they were otherwise indistinguishable from each other and from con-

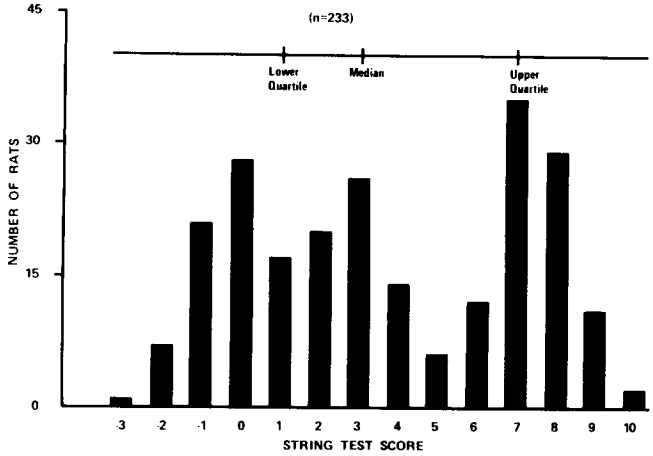

FIG. 2. String test scores of 233 rats after 5 days of pyrithiamine treatment. Rats with string test scores were made thiamine-deficient with a combination of thiamine-deficient diet and daily pyrithiamine injections, as described in Method. String test scores were recorded on the 5th day of treatment.

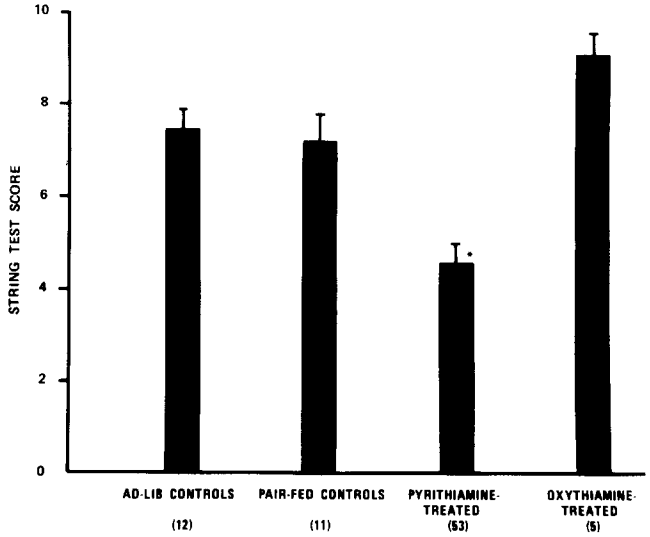

FIG. 3. Effect of thiamine deficiency on mean string test score. Rats were made thiamine-deficient with a combination of thiamine-deficient diet and daily pyrithiamine or oxythiamine injections. Ad lib controls received thiamine-deficient diet ad lib, and pair-fed controls received the same diet in amounts equal to that consumed by pyrithiamine-treated rats. Both ad lib and pair-fed controls were injected daily with maintenance doses of thiamine (see Method). The mean string test score of each group was determined after 5 days of treatment. Data are the mean score of 3 experiments in which string test scores were measured on the fifth day of treatment. *Denotes significantly different from control ($p<0.01$) by the least significant difference test.

trols. No gross neurological symptoms appeared in any rats until day 12 of pyrithiamine treatment. In 11 experiments with 233 pyrithiamine-treated rats, approximately 50% of rats developed low string test scores long before the onset of weight loss or neurological symptoms. In one of the eleven experiments, however, only 25% of rats had decreased string test scores. Rats in this experiment had a higher starting weight (85–100 g) and had 3 days of string testing before treatment began.

Effect of Pyrithiamine and Oxythiamine on Mean String Test Score

Although the range of string test scores in pyrithiamine-treated rats is broad, the mean string test score after 5 days of pyrithiamine treatment is significantly lower ($p<0.01$ by analysis of variance and the least significant difference test) than that of controls (Fig. 3). This figure represents the mean scores of 3 experiments in which the string test scores were measured on the fifth day of treatment. In contrast, the mean string test score of oxythiamine-treated rats did not decrease, despite the anorexia and weight loss that was already evident in these rats after five days of treatment. Pair-fed controls were not significantly different from ad lib controls in terms of string test performance.

DISCUSSION

The string test is a simple, easily performed, objectively quantitated behavioral test which is reproducible in a given animal from day to day and in populations of animals on a given day. However, the investigator must be careful to control the variables described in Method, especially with regard to the use of healthy animals only. Even when these precautions are observed, a small percentage (less than 10%) of control animals show persistent decreases in string test score, or an animal with persistently high string test scores may occasionally score poorly.

Despite these problems, the string test can detect abnormalities in pyrithiamine-treated rats long before weight loss or classical neurological signs occur. The mean string test score of pyrithiamine-treated rats is significantly lower than that of controls after only 5 days of treatment. Other results indicate that low string test scores in pyrithiamine-treated rats can be at least partially reversed with thiamine (Barclay, Gibson, Blass, in preparation). Since oxythiamine-treated rats do not have decreased string test scores, impaired string test performance seems to be a central nervous system effect rather than a peripheral effect of thiamine deficiency. The lack of change in string test scores of oxythiamine-treated rats relative to controls is surprising, as these animals were already markedly anorectic and died after 6–8 days of treatment. An increasing percentage of the pyrithiamine animals had decreased string test scores as they approached death after 13–14 days of treatment (Barclay, Gibson, Blass, in preparation).

The bimodal distribution of string test scores in pyrithiamine-treated rats is striking, and shows that apparently similar rats respond differently to pyrithiamine treatment. Only 50% of rats respond with persistently decreased string test scores before ataxia occurs. Treated rats with low scores and with high scores are otherwise indistinguishable from each other and from controls. This difference in behavioral response to pyrithiamine treatment invites further studies of biochemical or genetic differences between responders and non-responders. Other data indicate that string test score in pyrithiamine-treated rats does not correlate with erythrocyte transketolase activity (Barclay, Gibson, Blass, in preparation). Correlation of string test score with early biochemical changes may help determine the critical "biochemical lesion" responsible for the development of thiamine deficiency encephalopathy.

Using the string test as a behavioral marker in other animal models of metabolic encephalopathies may also shed some light on the interrelationship between biochemistry and behavior. The reliability, reproducibility and ease of measurement of the string test make it a useful index of central nervous system dysfunction. Specifically, string test scores may be an ideal behavioral variable to follow in studies of behavioral toxicology, behavioral teratology, hypoxia, and aging.

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