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Effects of Central Nervous System Accumulation of Tellurium on Behavior in Rats[1]

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WALBRAN, B. B. AND ROBINS, E. *Effects of central nervous system accumulation of tellurium on behavior in rats.* PHYSIOL. BEHAV. 9(3) 297–300, 1978.—Rats were treated for 112 days with daily injections of 2 mg/kg potassium tellurite in Sorensen's phosphate buffer or with the buffer vehicle only. At sacrifice, the cerebral gray matter of the animals treated with tellurite was grossly darkened. The presence of tellurium in cerebellum was confirmed by atomic absorption spectrophotometry. Growth of the tellurite-treated animals was significantly impaired when compared with control animals. However, in a T-maze the activity level of the tellurite-treated animals was increased. On a simple delayed response task, the performance of the tellurite-treated animals was more consistent than that of the buffer-treated animals.

Tellurium Cerebral lipofuscinosis Central nervous system Heavy metals

NEUROPATHOLOGICALLY, the accumulation of lipofuscin pigment with advancing age is a consistent observation. Lal *et al.* [11] induced cerebral lipofuscinosis and reduced brain weight in male rats by feeding them a diet deficient in vitamin E for 14 months. The treatment had no effect on locomotor activity as measured in an activity wheel. However, both the vitamin E deficient rats and the age-matched control animals were found to be impaired on delayed alternation tasks when compared with young rats. The rats on the vitamin E deficient diets showed greater impairment than the age-matched control animals. The authors postulated that the deficits in memory resulted from the accumulation of lipofuscin pigments and the reduced brain weight.

Recently, the potential for inducing cerebral lipofuscinosis through systemic administration of tellurium has been reported [7]. Pilot work by the authors demonstrated that the chronic intraperitoneal (IP) injection of 2 mg/kg potassium tellurite (K_2TeO_3) would produce cerebral lipofuscinosis without reducing absolute brain weight. The present study was undertaken, therefore, to assess in rats the effects of cerebral lipofuscinosis induced by chronic in vivo treatment with K_2TeO_3 on the learning of a simple delayed alternation task.

METHOD

Twenty-three male Long-Evans (pigmented) rats (Charles River Labs, Wilmington, MA) were assigned at random to receive daily IP injections of either 2 mg/kg K_2TeO_3 in Sorensen's phosphate buffer, pH 7.6, or the buffer only. The animals were housed in 30.5×36.6×17.7 cm plastic cages under standard laboratory conditions. Fifteen rats, having a mean weight of 98.3 ± 3.2 g at the start of the experiment, made up the K_2TeO_3 group. The 8 rats in the buffer-only group had a mean weight of 101.2 ± 2.9 g. During the 112 days of treatment, 5 rats (33.3%) from the K_2TeO_3 group died. No control rat died.

On Day 113, 4 rats from the K_2TeO_3 group and 3 from the buffer-only group were decapitated. The brains were removed, dissected into 10 regions and stored at −90°C for future biochemical studies. Rats were selected for decapitation such that the mean weight of the decapitated animals approximated the mean weight of the animals not sacrificed.

The remaining rats, 6 from the K_2TeO_3 group and 5 from the buffer-only group, were placed on food deprivation sufficient to reduce them to 80% of their body weight. Rations were manipulated on a weekly basis in order to maintain the animals at this level. During the last week of the experiment, improved performance by the group treated with K_2TeO_3 resulted in weight gain sufficient to return the animals to 98% of their body weight at the start of the experiment.

All testing was done in a T-maze made from 1.9 cm plywood. The start box of the maze measured 35.56 cm and the wings were 57.15 cm in length. The walls of the maze were 21.59 cm high and were spaced 10.16 cm apart. A black Plexiglas guillotine door separated the start box from the two wings of the maze and the wings from each other.

On Day 114, each rat was placed in the start box of the maze from which the guillotine door had been removed. Each wing contained a clear Plexiglas food cup holding a small amount of powdered Purina rat chow mixed with water. Each rat was allowed to explore the maze for 5 min. The total number of wings visited during that time was recorded. A wing visit was defined as the crossing of the midline of the maze or an excursion back into the start box.

The same procedure was followed for 3 days. On the 4th maze day (Day 117 of the study), the guillotine door was inserted before the training session was begun. The rats were trained to visit alternate wings of the maze for food reward.

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Each rat was given 5 consecutive trials per day. If an animal went to the right on the first trial, he was not again rewarded until he went left. The first trial of the day was correct by definition, i.e., food was available in both wings. On the 4 successive trials, access to the food cup in the incorrect wing was prevented by a clear Plexiglas cover. Criterion was 100% correct choices for 3 consecutive days. The guillotine door was opened for one min on each trial. If a rat did not leave the start box during that time, the door was closed and the trial scored as "did not run". A correct choice was rewarded by one min of feeding in the maze.

When the initial alternation was mastered, the rat was delayed in the start box for 5 sec between each trial. Criterion for mastery again was 100% correct choices for 3 consecutive days. As each delay was mastered, the interval between trials was lengthened by 5 sec. Training was continued for 45 days. At the end of the training period, all rats were decapitated, the brains were removed, dissected into 10 regions and stored at -90°C in the same manner as those taken prior to the learning experiment.

Previous pilot work done by the authors indicated that the tellurium levels in the cerebellum approximated those of whole brain homogenates. Therefore, tellurium levels were determined in cerebellum removed from animals sacrificed both before and after the learning experiment. Samples were run on an IL 251 Atomic Absorption Spectrophotometer, using a hollow cathode lamp specific for tellurium (IL-62940). Whole rat brain homogenates to which known amounts of K_2TeO_3 had been added were used as standards.

RESULTS

Behavior

Treatment of rats with 2 mg/kg K_2TeO_3 resulted in garlic breath which persisted for almost 3 weeks after treatment was discontinued. Growth was significantly impaired in K_2TeO_3-treated animals, but activity level in the T-maze was heightened. Performance on a simple delayed response task was more consistent for the K_2TeO_3-treated animals than for the buffer-treated animals.

Figure 1 illustrates the extent to which growth was impaired. Mean weights of each group were compared at weekly intervals by means of two-tailed T-tests. All differences between the groups after Day 1 were significant (all p's<0.01).

Figure 2 presents the mean number of wings visited per 5 min experience during the free exploration phase of the experiment. A repeated-measures analysis of variance showed that the mean number of wings visited declined significantly as a function of time, $F(2,18)=6.25$, $p<0.01$, but did not differ as a function of treatment conditions, $F(1,9)=1.06$, $p>0.05$. Nor was there a significant interaction between treatment conditions and experience, $F(2,18)=0.78$, $p>0.05$, although the animals treated with buffer only tended to change locations less frequently on successive days than the animals treated with K_2TeO_3.

This tendency toward lessened activity became significant when the guillotine door was put into place on Day 4. On that day, all 5 of the animals treated with buffer only refused to leave the start box on 2 or more trials. In contrast, only 1 of the 6 rats treated with K_2TeO_3 refused to run on 2 or more trials (Fisher's Exact Probability, two-tailed test, $p<0.05$).

The effects of treatment with K_2TeO_3 on willingness to leave the start box were analyzed by dividing the experiment into 6 blocks of 35 trials each. Comparisons of the differences in the overall performance of the animals in the 2 experimental groups and evaluation of the changes in performance shown by the animals during the experimental session showed that treatment with K_2TeO_3 did not change significantly the willingness of the animals to leave the start box, $F(1,9)=4.67$, $p>0.05$. Over time, however, both groups showed a significant decline in the number of refusals to leave the start box, $F(5,45)=18.65$, $p<0.001$. In addition, there was a significant interaction between refusal to leave the start box and treatment conditions. No animal treated with K_2TeO_3 refused to run on any trial after the first 35.

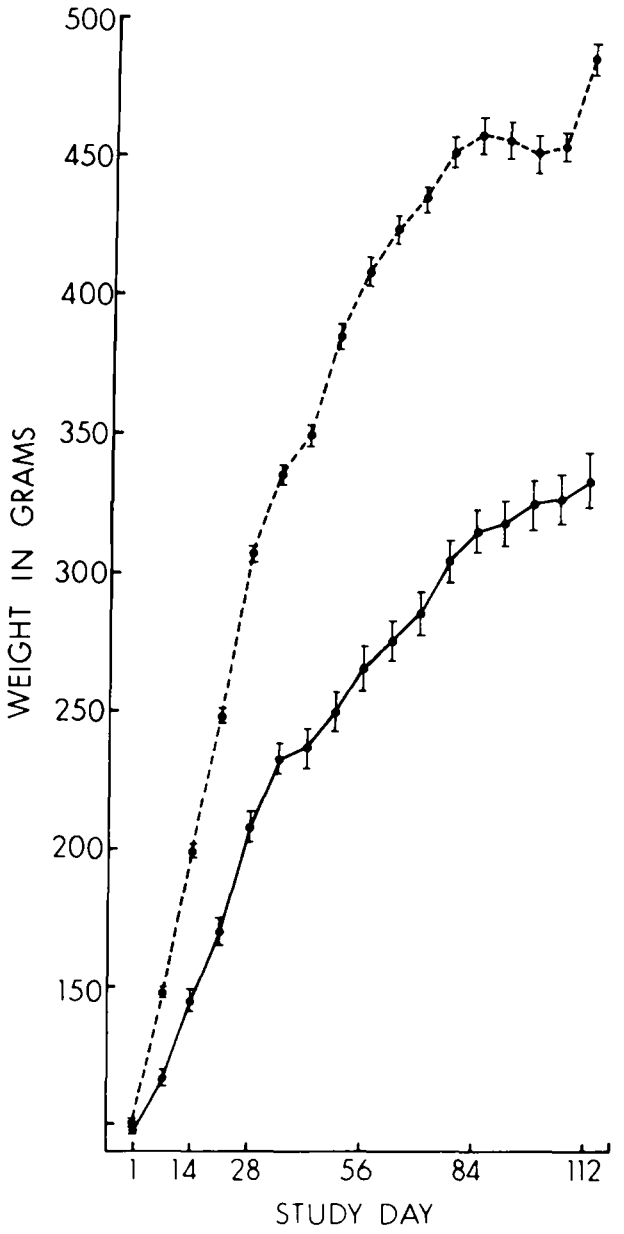

FIG. 1. Growth curves for groups treated daily with 2 mg/kg K_2TeO_3 (solid line) or buffer only (dotted line). After Day 1, all differences between groups are significant (all p's<0.01).

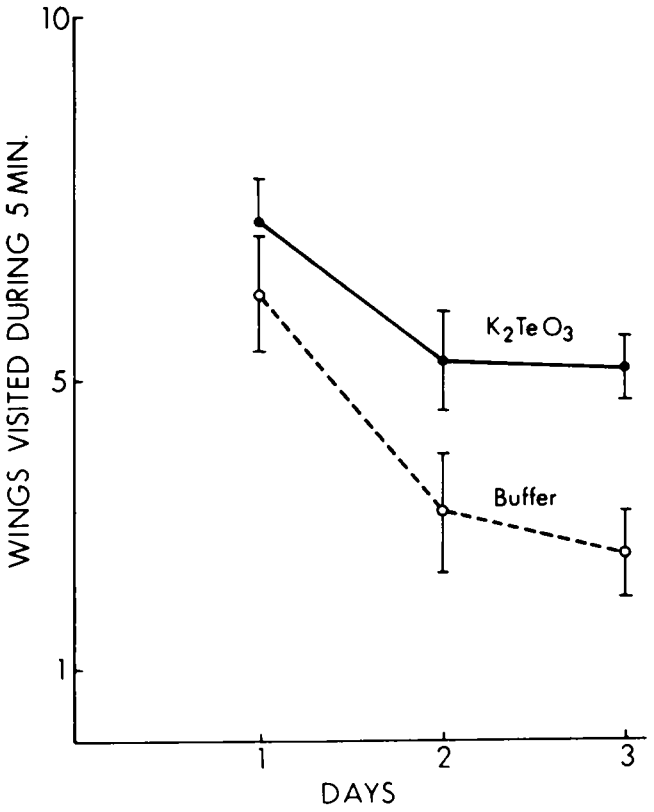

FIG. 2. Mean number of wings visited per day during 3-day free exploration period. (See text for definition of visits to wings.) Number of wings visited each day declined significantly as a function of time ($p<0.01$), but not as a function of treatment conditions.

During the remaining 175 trials, however, animals treated with buffer continued to refuse to run a total of 24 trials spaced at random intervals, $F(5,45)=3.05$, $p<0.02$.

Table 1 presents an overall picture of the learning achieved by both groups in 45 days. The mean number of days to criterion on the initial discrimination for the buffer group (24.6, S.D. 10.1) was 1 1/2 times as long as the mean number of days required by the tellurite-treated group (16.3, S.D. 3.7). This difference did not reach statistical significance. However, there was a significant difference in the variance of the two groups, $F(4,5)=7.52$, $p<0.03$, indicating more consistent performance among animals treated with K_2TeO_3. Four of the K_2TeO_3 group mastered delays of 20 sec or longer between trials. None of the buffer-only group were able to attain criterion with a delay longer than 15 sec ($p=0.10$, two-tailed test, Fisher's Exact Probability).

Histochemistry. The gray matter of brains of animals decapitated after treatment with 2 mg/kg K_2TeO_3 for 112 days was a deep grayish-blue or black color on gross examination. In contrast, myelinated fiber tracts remained white. Other organs, for example, kidney and testes, also showed a gross blackening at autopsy.

Brains of animals decapitated 47 days after cessation of K_2TeO_3 treatment were not distinguisable on gross examination from those removed prior to the learning experiment. Kidney and testes of those animals seemed perhaps to have lightened somewhat.

Table 2 presents mean absolute and relative weights of

TABLE 1

DELAY INTERVAL MASTERED

Group	Seconds Between Trials 0	5	10	15	20	25	30	35
K_2TeO_3 (6)								
N	6	6	4	4	4	1	1	1
Percent	100	100	67	67	67	17	17	17
$\bar{X}$ Days	16.3							
SD	3.7*							
Buffer Only (5)								
N	5	4	4	3	0	0	0	0
Percent	100	80	80	60	0	0	0	0
$\bar{X}$ Days	24.6							
SD	10.1*							

* $F(4,5)=7.52$, two-tailed test, $p<0.05$

cerebellum removed from animals both before and after the learning experiment. There was no difference in the absolute weights of cerebellum between groups or at the 2 points in time. However, at the end of the treatment period, the group treated with K_2TeO_3 had 0.959 ± 0.094 mg cerebellum per g of body weight in contrast to 0.598 ± 0.040 mg cerebellum per g of body weight in the group treated with buffer only, $t(3)=3.84$, $p<0.05$, two-tailed test. This difference obviously is dependent on body weight. The unanticipated weight gain of the K_2TeO_3 animals during the last week of the learning experiment combined with the experimentally reduced weight of the buffer-only animals so that the difference was no longer statistically significant.

Analysis of tellurium concentrations by atomic absorption showed the amount of tellurium in the brains of animals to be the same both before and after the learning experiment. Cerebellum taken from the animals treated with K_2TeO_3 for 112 days had a mean concentration of 0.065 ± 0.012 mM tellurium as compared with 0.067 ± 0.004 mM in cerebellum from animals sacrificed 47 days later. These concentrations approximate 8 parts per million. Cerebellum taken from animals treated with buffer did not contain tellurium in any detectable concentration.

DISCUSSION

The results of the present study with rats confirm the reports that systemic administration of tellurium results in garlic breath [2, 4, 8, 14, 17], impaired growth [7] and the so-called "black-brain syndrome" [1, 4, 7, 8, 16]. The excess mortality (33.3% of the K_2TeO_3 group) suggests that even very small amounts of tellurite compounds can be quite toxic.

The significantly greater relative weight of the cerebellum in animals treated with K_2TeO_3 is in accord with Scharer's [13] statement that the brain develops according to age and not in parallel with body weight. Since pilot work by the authors had shown that treatment with K_2TeO_3 did not reduce absolute brain weight, there is some possibility that tellurium may not exert its primary toxic effect on the central nervous system (CNS). However, since the brains were dissected prior to weighing, it is possible that other areas of the brain did not grow at the same rate as the cerebellum.

There are at least 2 possible explanations for the more

TABLE 2

MEAN CEREBELLUM WEIGHT ± SEM-ABSOLUTE AND RELATIVE

	Mean Cerebellum Wt. (gms.)	Mean Body Wt. (gms.)	Mg Cerebellum/ gm Body Wt.
Pre-Learning (Animals sacrificed after 112 days of treatment)			
K_2TeO_3 (N=4)	0.3021 ± .0086	325.0 ± 36.57	0.959 ± 0.95*
Buffer (N=3)	0.297 ± .0057	494.0 ± 37.16	0.598 ± .040
Post-Learning (Animals sacrificed after 112 days of treatment and 45 days of maze experience)			
K_2TeO_3 (N=6)	0.2834 ± 0.146	336.3 ± 15.46	0.8476 ± .042
Buffer (N=5)	0.2947 ± .0089	417.6 ± 13.40	0.7086 ± .029

*Significantly greater than group treated with buffer, (t (3)=3.85, two-tailed test, $p<0.05$)

consistent performance on the delayed alternation task of the rats treated with K_2TeO_3. It may be that the animals treated with K_2TeO_3, having an impaired growth curve, were more motivated to learn the task. Consistent with this interpretation is the fact that the introduction of the guillotine door into the maze had a significantly greater effect on animals treated with buffer than on those treated with K_2TeO_3. Furthermore, there was a significant interaction between treatment condition and number of trials on which animals refused to leave the start box.

The significantly reduced variability in the performance of the K_2TeO_3 animals also might be explained as an alteration in normal shift-of-attention patterns. It has been reported that animals with hippocampal destruction are less distractable from goal-oriented behavior when hungry [12,15], show greater persistance in activity in approach situations [9] and are less inclined to shifts of attention [10] than normal animals.

Heavy metals are known to be associated in normal rat brain both with lysosomes in general [3] and with the hippocampus in particular [6]. Further, recent investigations have shown that tellurium does accummulate in lysosomes of mammalian brain parenchymal tissue [5,16]. Although there is no evidence that CNS accumulation of tellurium results in structural damage, pilot work with electron microscopy did note the accumulation of tellurium crystals in choroid plexus and hippocampus. However, all areas of brain were not examined. Nonetheless, the possibility exists that the presence of tellurium in brain could contribute to some biochemical dysfunction the symptoms of which might approximate those of selective lesions.

Although there is a possible motivational confounding in the present study, the results tend to suggest that cerebral lipofuscinosis, in the absence of reduced brain weight, may not have deleterious effects on simple memory tasks.

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