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Fenfluramine-Induced Modification of Palatability: Analysis by the Taste Reactivity Test

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BARNFIELD, A., L. A. PARKER, A. M. DAVIES AND C. MILES. *Fenfluramine-induced modification of palatability: Analysis by the taste reactivity test.* PHARMACOL BIOCHEM BEHAV **48**(4) 875-879, 1994.—The ability of various doses (0, 1.25, 2.5, and 5.0 mg/kg) of fenfluramine to modify the palatability of sucrose and quinine solutions was assessed by means of the taste reactivity test. Although fenfluramine did not modify the positive hedonic ingestive reactions elicited by sucrose solution, it consistently enhanced the negatively hedonic aversive reactions elicited by unpalatable 0.05% quinine solution and moderately palatable 2% sucrose solution. The results suggest that fenfluramine enhances the aversive properties of tastants without suppressing the positive hedonic properties of tastants. The results support a two-dimensional model of palatability.

Palatability Taste reactivity Fenfluramine Serotonin 5-HT Feeding Ingestive behavior Taste aversion Sucrose Quinine

FENFLURAMINE has long been shown to reduce food intake in animal species and in man. Although a substituted amphetamine, it does not induce the stimulatory (euphoric) effects characteristic of amphetamine and amphetamine-like agents (12,15). The anorectic action of fenfluramine is thought to be via increasing availability of 5-hydroxytryptamine (5-HT; serotonin). It is believed to facilitate release and, to some extent, inhibit reuptake of 5-HT [e.g., (4,12)]. Fenfluramine administration, therefore, leads to increased availability of 5-HT at the synapse.

While it has been proposed that fenfluramine anorexia may be due to earlier onset of satiety, possibly related to slowing of gut clearance (4,6,10), Fletcher (11) questions this hypothesis. The marked reduction in eating rate caused by fenfluramine is difficult to reconcile with a satiety effect, and is also seen with quinine adulteration of food. Fletcher, thus, investigated the possibility that fenfluramine anorexia may be due to a reduction in palatability of food. The results of his research implied that the decrease in eating rate was not influenced by shifts in palatability. The two-bottle choice tests used by Fletcher led to a general reduction of fluid intake, leading him to the broad conclusion that fenfluramine exerted multiple effects on ingestive behavior.

That palatability may be influenced by fenfluramine was concluded by Cabanac and Lafrance (7). Experiments with rats foraging in cold (−15°C) for palatable bait, while chow was freely available in a shelter (25°C), showed that: "Even at doses too small to reduce consumption of basic food [0.6 or 1.25 mg/kg] *d*-fenfluramine decreased the drive to get palatable food."

Leander (14) showed that fluoxetine, the 5-HT reuptake blocker, also caused dose-dependent reduction of palatability-induced ingestion. Nondeprived rats were allowed 1 h access to various concentrations of saccharin solution (0.001 to 0.1 M sodium saccharin), after injection of fluoxetine. The rats selectively suppressed their consumption of the palatable saccharin solutions. Leander (14) concluded that palatability-induced intake appeared to be more sensitive to 5-HT uptake inhibition than deprivation-induced feeding. A similar dose-related suppression of palatable food consumption postfluoxetine was reported by Cooper et al. (8), and Morris and Cooper (17) showed that 1.8 and 3.0 mg/kg fenfluramine caused a nonsatiety related decrease in sucrose consumption.

The effects of fenfluramine on sucrose palatability have exclusively been assessed by means of consumption tests. However, consumption tests only indirectly assess the palat-

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ability of a taste. A more direct measure of palatability is the Taste Reactivity (TR) test devised by Grill and Norgren (13). When a rat is intraorally infused with a tastant, it displays a characteristic set of taste reactions that are dependent upon the palatability of the solution. Sucrose elicits characteristic ingestive reactions (tongue protrusions, mouth movements, and paw licks) and quinine elicits characteristic rejection reactions (chin rubs, gapes, paw treads). The following experiment employed the TR test to determine the effect of pretreatment with various doses (0, 1.25, 2.5, and 5.0 mg/kg) of fenfluramine on the palatability of sucrose (2%) and quinine (0.05%) solutions. If the suppressant effects of fenfluramine on food intake are mediated by a palatability shift, then fenfluramine would be expected to reduce the positive palatability and enhance the aversive taste properties of both solutions.

METHOD

Subjects

Forty male Sprague–Dawley rats, weighing between 298–318 on the first test day, served as subjects. They were maintained in individual stainless steel cages on a 12 L : 12 D schedule. They were maintained on ad lib rat chow and water, except as indicated.

Procedure

All rats were surgically implanted with intraoral cannulae 1 week after arriving in the laboratory. The surgical procedure has previously been described by Parker (18). Briefly, all rats were given an intraperitoneal (IP) injection of atropine (0.25 mg/kg), 5 min prior to receiving an IP injection of a mixture of ketamine (100 mg/kg) and rompun (3 mg/kg). Once the rats were anaesthetized, a 15 ga thin-walled stainless steel needle was inserted in the midneck region and brought subcutaneously (sc) around the ear and out on the inside of the rat's cheek behind the first molar. Then, PE 90 (i.d. 0.86 mm; o.d. 1.27 mm) intramedic tubing was inserted through the shaft of the needle and the needle was removed. The tubing was held in place with a rubber washer on the inside of the cheek and a 20 ga adaptor in the back of the neck. The rats were allowed 1 week to recover from surgery before they were given adaptation and test trials.

All rats received three adaptation trials on consecutive days, each separated by 24 h. On each adaptation trial, a rat was placed in the glass test chamber (22.5 × 26 × 20 cm) with the 30 cm infusion hose attached to the adapter cap of its cannula. The mirror beneath the chamber was illuminated by two 25 W lights on either side of the chamber. One minute later, an infusion pump (Harvard Apparatus, Model 22) began to deliver water through the cannula at the rate of 1 ml/min for a 10-min test period.

Twenty-four hours after the final adaptation trial, the rats received the first of two TR test trials. Two hours prior to each TR test trial, the rats were injected subcutaneously (SC) with 0.0 (saline, $n = 11$), 1.25 ($n = 10$), 2.5 ($n = 9$), or 5.0 ($n = 10$) mg/kg of Fenfluramine (FEN) HCl in solution with saline. The interval between fenfluramine pretreatment and the TR test trial was 2 h to prevent the acquisition of conditioned taste avoidance [e.g., (2)]. This interval is well within the time course of the reported anorexic effects of fenfluramine (1,23). All injections were administered in a volume of 1 ml/kg. The test trials were conducted in the same manner as the adaptation trials for each rat except that the test flavor was infused through the cannula instead of water. The test flavor was either 0.05% quinine solution or 2.0% sucrose solution. Half of the rats received the quinine solution on the first day and the other half received sucrose solution on the first day. The second test day occurred 3 days after the first test day. On the second test day, the rat received the solution that it did not receive on the first test day. The rats were videotaped from a mirror located at an angle beneath the test chamber on both test days.

The videotapes of the TR test trials were later scored by raters blind to the experimental conditions. The behaviors measured included the aversive reactions of chin rubbing (mouth in direct contact with the floor or a wall and projecting the body forward), gaping (large-amplitude, rapid opening of the mandible with concomitant retraction of the corners of the mouth), and paw treading (sequential extensions of one forelimb forward against the floor while the other forelimb is being retracted). These scores were combined to produce a composite aversive reaction category. Additionally, the mildly aversive, neutral reaction category of passive drips (frequency of drips of the solution from the rat's mouth when the rat is not actively rejecting the solution) were measured. The ingestive reactions of tongue protrusions (protrusions of the tongue on the midline or on either side of the mouth), mouth movements (low-amplitude, rhythmic openings of the mandible), and paw licking (licking the forelimb paws while they are held close to the mouth) were combined to produce a composite ingestive reaction category. Finally, the activity measures of occurrences of vertical rears and horizontal active locomotion were combined to produce the composite activity measure.

RESULTS

The TR test trial data for each test flavor were analyzed and depicted separately combined across test days. Figure 1 presents the mean frequency or duration of each TR category for each group tested with 2% sucrose solution at each min of testing. The data for each TR category were analyzed as a 4 by 10 mixed factor ANOVA with the between group factor of pretreatment dose (0.0, 1.25, 2.5, 5.0 mg/kg FEN) and the within-group factor of minutes of testing (min 1–10). The only TR category that revealed evidence of a pretreatment dose effect was that of aversive reactions. For this TR category, the analysis revealed a significant pretreatment dose effect, $F(3, 36) = 3.5$, $p < 0.05$, and a pretreatment dose by minute interaction that approached significance, $F(27, 324) = 1.4$, $p < 0.10$. Subsequent Newman-Keuls tests revealed that group 5.0 displayed more aversive reactions than did all other groups (all $p < 0.05$). Subsequent single-factor ANOVAs for each minute of testing revealed that during min 1 only, the groups differed significantly, $F(3, 36) = 2.8$, $p < 0.05$; a Newman-Keuls analysis of the group differences for min 1 revealed that group 5.0 Fen displayed more aversive reactions than any other group (all $p < 0.05$).

Although the analysis of the duration of ingestive reactions revealed no significant pretreatment or pretreatment by minutes interaction, inspection of Fig. 1 suggests that the fenfluramine-pretreated rats displayed fewer ingestive reactions during the later minutes of testing than the saline-pretreated rats. In fact, during the final minute of testing only (min 10), a single-factor ANOVA revealed a significant effect, $F(3, 36) = 3.1$, $p < 0.05$; group 5 Fen demonstrated less ingestive responding than group saline ($p < 0.05$). This finding suggests that a suppressant effect of fenfluramine on ingestive responding may have been revealed with a longer TR test period.

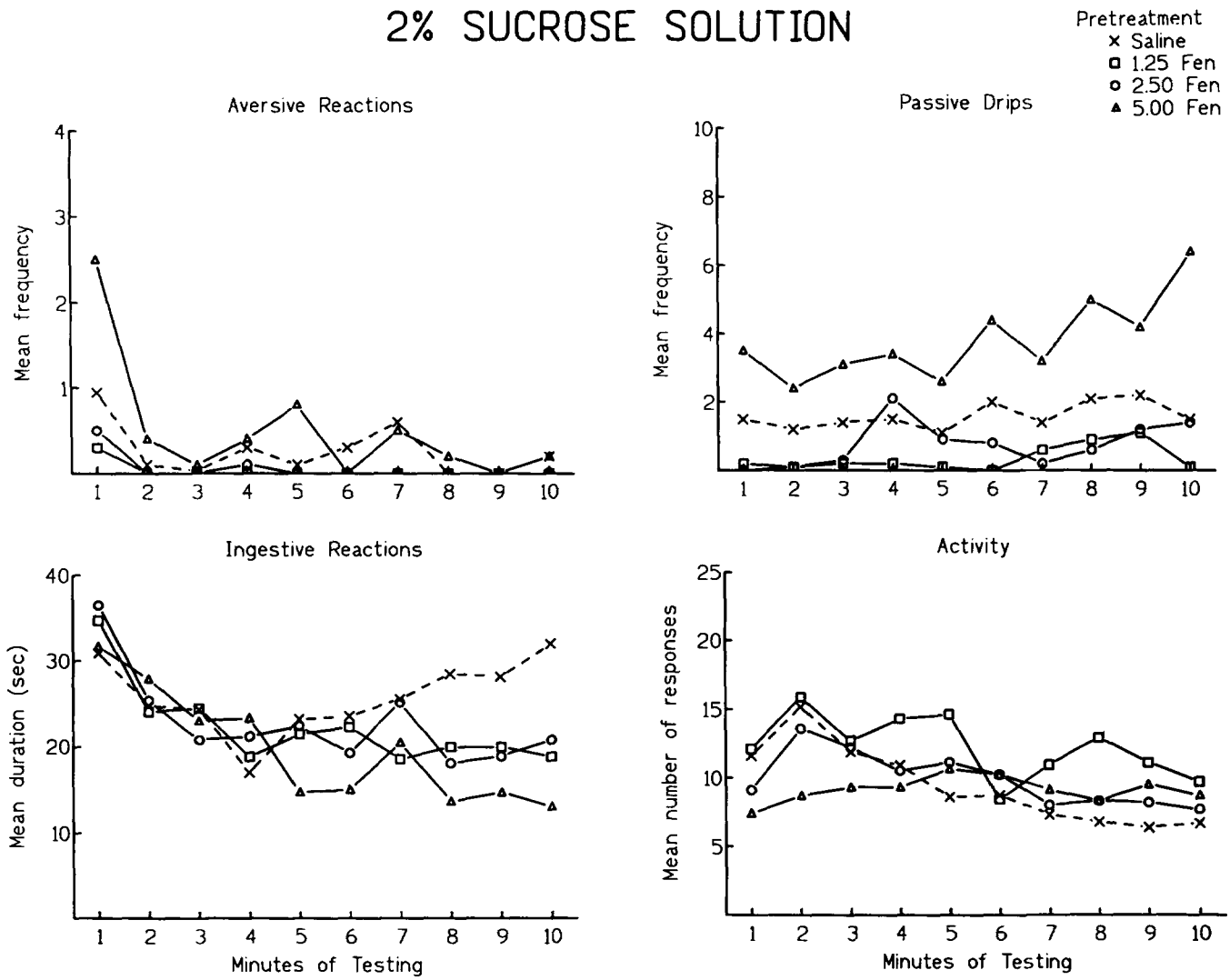

FIG. 1. Mean frequency or duration (s) of taste reactions elicited by 2% sucrose solution in rats pretreated with various doses of fenfluramine.

Figure 2 presents the mean frequency or duration of the various TR categories elicited by exposure to 0.05% quinine solution. The only TR category that revealed a pretreatment effect was that of aversive reactions. The 2 by 10 mixed-factor ANOVA revealed only a significant pretreatment dose effect, $F(3, 36) = 3.8$, $p < 0.025$. Subsequent Newman-Keuls tests revealed that groups 5.0 and 2.5 displayed significantly more aversive reactions than groups saline or 1.25 (all $p < 0.05$). Pretreatment with fenfluramine did not modify any of the other TR categories depicted in Fig. 2 that were elicited by quinine solution.

DISCUSSION

Pretreatment with 2.5–5.0 mg/kg of fenfluramine enhanced the frequency of aversive taste reactions displayed during an intraoral infusion of an unpalatable 0.05% quinine solution. This finding supports the hypothesis that the anorexic properties of fenfluramine are mediated by a palatability shift. Additionally, when a high dose of fenfluramine (5 mg/kg) was administered, rats also demonstrated enhanced aversion to a mildly palatable 2% sucrose solution. The finding that activity was not modified by pretreatment with fenfluramine regardless of whether the test solution was quinine or sucrose, suggests that the enhancement of aversive properties of these tastes was not the result of nonspecific motoric effects of fenfluramine.

On the other hand, if fenfluramine does produce a palatability shift, then one would also expect that fenfluramine-pretreated rats should display suppressed ingestive responding throughout an intraoral infusion of sucrose solution. This effect did not occur. However, the pattern of results suggests that during the last minute of testing, fenfluramine produced a suppressant effect on ingestive reactions elicited by sucrose. Therefore, a longer TR test might reveal suppressed ingestive responding during the latter period of the test. It is not clear, however, that suppressed ingestive responding during the later min of testing would reflect fenfluramine-induced modification of palatability or postingestive processes.

If fenfluramine produces anorexia by modification of the

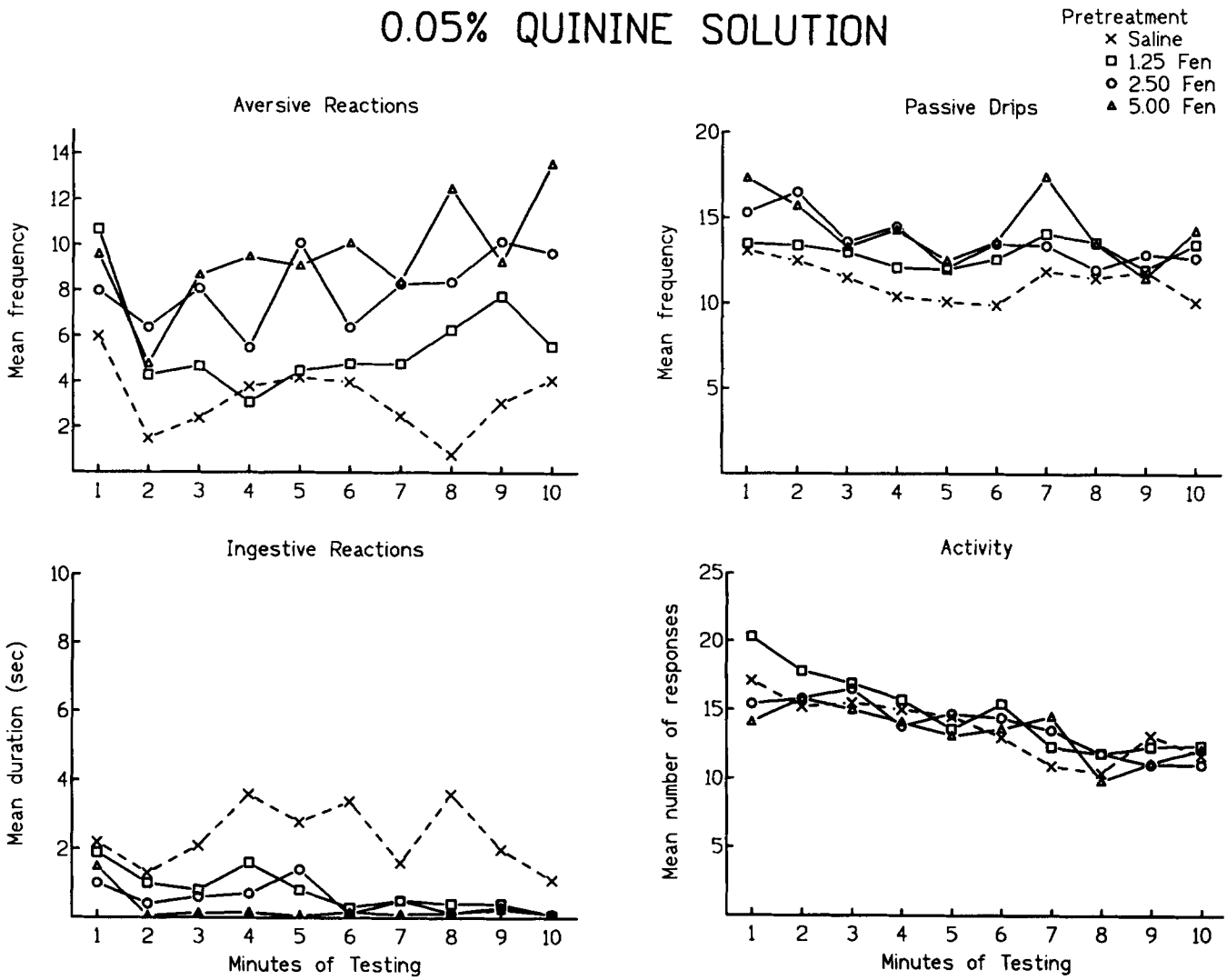

FIG. 2. Mean frequency or duration (s) of taste reactions elicited by 0.05% quinine solution in rats pretreated with various doses of fenfluramine.

palatability of a tastant, then our data suggest that the anorexia is the result of increased aversiveness of the tastant, not suppression of its positive hedonic properties. Berridge and Grill (3) have proposed a two-dimensional model of palatability that suggests that the positive hedonic properties of a tastant can be modified independently of its negative hedonic properties. The above effects may support such a model of palatability.

GENERAL DISCUSSION

Fenfluramine enhanced aversive reactions displayed during intraoral infusions of both sucrose and quinine solutions. This finding supports the hypothesis that fenfluramine modifies the palatability of tastants. The doses necessary to enhance aversive reactions elicited by 0.05% quinine solution were 2.5–5.0 mg/kg, but only a dose as high as 5.0 mg/kg enhanced aversive reactions elicited by a mildly palatable 2% sucrose solution. On the other hand, the failure of fenfluramine to suppress ingestive reactions elicited by sucrose solution does not support the hypothesis that fenfluramine-induced anorexia is mediated by a shift in the palatability of the tastant. These findings suggest that the modification of feeding produced by fenfluramine may involve an increase in the aversiveness of the tastant without necessarily reducing the positive palatability of the tastant. This finding supports the two-dimensional model of palatability proposed by Berridge and Grill (3). They reported that with the addition of quinine to sucrose solutions, the aversive properties of tastants may be modified without modification of the ingestive properties of tastants. Similarly, the above experiment demonstrated that fenfluramine pretreatment enhanced aversive taste reactivity without modifying ingestive taste reactivity.

Parker and Lopez (20) have also reported that pimozide, a dopaminergic antagonist, enhances aversive reactions elicited by quinine solution, although it does not modify the frequency of aversive reactions elicited by sucrose. Although fenfluramine serves as a serotonergic agonist and pimozide serves as a

dopaminergic antagonist, these agents both appear to enhance the aversive reactions elicited by unpalatable tastants. Fenfluramine has also been reported to be capable of producing a conditioned place aversion at the doses that enhanced aversive TR reactions above (9), suggesting that it has aversive hedonic properties. Interestingly, morphine and amphetamine, both possessing positive hedonic properties as assessed in both drug self-administration (24) and place conditioning paradigms (22), also both have the capacity to suppress aversive reactions elicited by quinine solution (19,21), an action opposite in direction to that of fenfluramine.

During none of the tests did fenfluramine modify general activity level as assessed by a composite rearing and active locomotion score. Therefore, the enhancement of aversive reactions does not merely reflect an indirect motoric effect but, instead, is more likely to reflect the direct effects of fenfluramine on the palatability of sucrose and quinine solutions.

Because our results suggest that fenfluramine directly modifies the aversive palatability of substances, it is conceivable that the modification of lab chow intake [e.g., (4)] produced by fenfluramine may be the result of the taste of the chow becoming more aversive rather than the chow becoming less palatable. This suggestion is supported by our finding that fenfluramine pretreatment not only increased the aversiveness of naturally aversive quinine solution, but also increased the aversiveness of naturally palatable sucrose solution. Because the rats were nondeprived during testing, it is unlikely that the fenfluramine-induced increase in aversive taste reactions elicited by quinine and sucrose were the result of a modification in the need state of the animal rather than a modification in the taste properties of the solutions. Furthermore, if the increased aversiveness of the tastants was the result of enhanced satiety, then one might expect that the greater the intake of the solution, the greater the frequency of aversive reactions. Yet, during intraoral infusions of sucrose solution, which the rats ingested during the 10-min infusion, the fenfluramine-induced enhancement of aversive reactions only occurred during the first minute of testing. The present findings suggest that the fenfluramine-induced enhanced aversiveness of quinine and sucrose taste reactivity is the result of a modification of palatability, rather than a modification of the animal's need state; however, such a distinction is worthy of further investigation.

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