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Intravenous Cocaine Self-Administration in Mice Lacking 5-HT1B Receptors

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ROCHA, B. A., R. ATOR, M. W. EMMETT-OGLESBY AND R. HEN. *Intravenous cocaine self-administration in mice lacking 5-HT1B receptors.* PHARMACOL BIOCHEM BEHAV **57**(3) 407–412, 1997.—The present experiment tested the hypothesis that 5-HT1B receptors are involved in the reinforcing effects of cocaine. Transgenic mice lacking 5-HT1B receptors were used as subjects and compared with wild-type mice for the acquisition and maintenance of intravenous (IV) cocaine self-administration. Male 129/Sv-ter and 5-HT1B-minus 129/Sv-ter inbred mice (Columbia University, New York) were initially trained to press a lever under a fixed-ratio schedule 2, first for sweetened condensed milk as reinforcer and subsequently for cocaine (2.0 mg/kg/infusion). When a stable baseline of responding was obtained, each subject was tested under different doses of cocaine (1.0, 2.0, and 4.0 mg/kg), with the number of reinforcers per hour used as the dependent variable. Both strains successfully acquired food-shaping and cocaine self-administration, but the mutant mice presented a significantly shorter latency to meet IV cocaine self-administration acquisition criteria ($p < 0.05$). However, both wild-type and mutant mice had similar dose–response to cocaine. These results suggest that the 5-HT1B receptors may be implicated in the propensity to self-administer cocaine, but other mechanisms might be involved in the maintenance of cocaine self-administration. © 1997 Elsevier Science Inc.

Cocaine Reinforcing effects IV self-administration 5-HT1B receptors Transgenic mice

A large body of evidence supports the hypothesis that the reinforcing effects of cocaine are dependent on the ability of this drug to augment dopamine (DA)-mediated neurotransmission by inhibiting DA uptake at terminals of the mesocorticolimbic system (22,24,26,42,57,58). However, some agents that activate the dopaminergic system, but are devoid of strong serotonergic activity, are neither intensively abused by humans nor self-administered by animals (14,29). Thus, it is possible that actions of cocaine in non-dopaminergic systems are critical for the abuse potential of this drug. The fact that cocaine also augments serotonin (5-HT)-mediated neurotransmission by binding to the 5-HT transporter and inhibiting the reuptake of 5-HT in vitro (37–39), together with evidence of a functional interaction between 5-HT and DA in the mesolimbic system (1,11,30–32,51,56), suggests that 5-HT processes may play a modulatory role in the reinforcing effects of cocaine. Enhancing 5-HT levels via dietary tryptophan (8) or systemic injection of the 5-HT uptake inhibitors fluoxetine or citalopram (9,33,49), decreased the amount of cocaine that was self-administered in rats or squirrel monkeys. Depletion of 5-HT levels due to 5,7-DHT lesions appears to increase the reinforcing efficacy of cocaine in a progressive ratio schedule of reinforcement (27).

To understand how 5-HT can modulate the reinforcing effects of cocaine, it is important to dissect the contributions of individual receptor subtypes. Molecular biology studies reveal the existence of at least 13 subtypes of serotonin receptors (19,34). Although very few selective agonists or antagonists are available for 5-HT receptor subtypes (19,34), a recent study has pointed toward a role of 5-HT1B receptors, which are the rodent homolog of 5-HT1Dβ receptors (17), in modulating the subjective effects of cocaine. Callahan and Cunningham (5) showed that the 5-HT1A/1B receptor agonist RU24969 partially substitutes for and enhances the discriminative stimulus effects of cocaine, whereas 5-HT1A agonists, such as 8-OH-DPAT or NAN 190, do not substitute for or alter the cocaine discriminative stimulus. Also, *in vitro* experiments showed that injection of 5-HT or the mixed 5-HT1B/2C agonist TFMPP into the ventral tegmental area (VTA) resulted in an increase in DA release in the nucleus accumbens, whereas the 5-HT1A agonist 8-OH-DPAT had no effect (16). In addition, Cameron and Williams (6) reported that the 5-HT1B agonist sumatriptan, as well as cocaine, caused an indirect modulation of VTA DA neuron activity, via 5-HT acting through 5-HT1B receptors to modify GABA neuronal disin-

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hibition. Taken together, these data suggest that an interaction between 5-HT and DA, through 5-HT1B receptors, may be implicated in the reinforcing effects of cocaine.

The purpose of the present study was to test the role of 5-HT1B receptors in the reinforcing effects of cocaine. Mice obtained through gene targeting technology such that they lacked specifically 5-HT1B receptors (45) were used as subjects. These mice were compared to the wild-type mice in their acquisition of operant behavior for food and for intravenous (IV) cocaine self-administration, as well as for their rate of responding to different doses of cocaine used as a reinforcer.

METHODS

Subjects

Male wild-type 129/Sv-ter and homozygous 5-HT1B-minus 129/Sv-ter inbred mice (Columbia University, New York) were used as subjects. The animals were housed individually in a room of constant temperature (21 ± 1°C) in a 12 L:12 D cycle. They had access to water *ad lib*, but were maintained at 28 g of weight (±5 g) by restricting their access to food to 3–4 g/day. They were received at approximately 6 months of age, and experiments were performed over the next 3 months.

Apparatus

Food-shaping and IV cocaine self-administration experiments took place in mouse operant chambers (model ENV-300; Med Associates, Inc., Georgia, VT, USA). The chambers contained a top loading door, side panels of 0.25-inch (6.4-mm) clear acrylic, and a stainless steel back panel. The front panel contained a liquid dipper (model ENV-202A) situated between two ultra-sensitive mouse levers (model ENV-310A). A single stimulus light (model ENV-221, 3 W) was placed in the chamber ceiling. The chambers were in sound- and light-attenuating enclosures. Two pump/syringe sets were used for each self-administration chamber: one syringe contained cocaine solution, and the other syringe contained a saline vehicle solution (0.9% saline with 4 U/ml heparin). The two syringes were connected through a series of Tygon tubing (0.06-inch o.d.; Tygon Microbore Tubing; Norton Performance Plastics, Akron, OH, USA) and microvolume T-connectors (model N-06365-70; Cole-Parmer, Chicago, IL, USA) to a single-channel fluid swivel (model 375/22; Instech Laboratories, Inc., Plymouth Meeting, PA, USA). A Tygon line, enclosed in a stainless steel spring, exited the swivel and immediately entered the chamber ceiling. The swivel was mounted on a counterbalanced arm. The total dead space from the T-connectors to the tip of the catheters was approximately 1 ml. Scheduling of response contingencies and recording of data were accomplished via MS-DOS-compatible microcomputers using OPN software (50).

Procedure

Food-shaping. Initially animals were trained to press a lever under a fixed ratio 1 (FR1) schedule, and subsequently under a FR2 schedule, using food as a reinforcer (sweetened condensed milk solution: 2 parts water/1 part milk). Sessions lasted for either 1 h or for up to 20 reinforcers. Stable responding was defined as having occurred when the animals obtained at least 75% of the total number of reinforcers within the 1-h daily session. The food-shaping program was completed when stable responding occurred on either lever, under first the FR1 and then the FR2 schedules.

Catheter implantation. Following the completion of the food-shaping program, mice were implanted with indwelling IV catheters in a modification of the method originally described by Weeks (52) and used successfully for rats (12). Under anesthesia (20 mg/kg xylazine and 65 mg/kg ketamine, IP), a silastic catheter (0.009-inch i.d., 0.027-inch o.d.; Dow Corning, Midland, MI, USA) was inserted into the right external jugular and its tip advanced into the right atrium. The catheter was secured to the vein using 5-0 Prolene sutures (Ethicon, Somerville, NJ, USA), and its free end ran subcutaneously to an incision in the skin at the top of the skull, where it was then connected to a modified C313G cannula assembly (Plastic One, Roanoke, VA, USA). The entire unit consisting of catheter and guide cannula was then embedded in dental acrylic cement (Geristore Dual Cure; DenMat, Santa Maria, CA, USA) and fixed to the skull. The total dead space from the guide cannula to the end of the catheter was approximately 5 μl.

Catheter patency. A detachable catheter system allowed us to remove the mice from the chambers between sessions. The basic procedure was similar to that described before by this laboratory (12). A heparin regimen on a daily basis, which is the key to maintaining catheter patency over several weeks in rats, was adapted for use in mice. At the end of each self-administration session, subjects received an injection of saline solution (25 μl) containing heparin (30 U/ml).

Cocaine self-administration. The acquisition phase of cocaine self-administration started 2 days after catheter implantation. Mice were allowed to self-administer cocaine (2.0 mg/kg/injection) under a FR1 schedule with a maximum of 20 cocaine injections within a 180-min session. At the start of each session, a priming injection of cocaine (2.0 mg/kg/injection) was given through the catheter. Each injection was accompanied by a flashing of the stimulus light followed by a 30-s timeout in the dark when lever-pressing had no consequences. The timeout period was included to prevent overdose. Acquisition criteria were defined as intake of at least 16 injections within the session (approximately 5 reinforcers/hour) and a 3:1 ratio of active to inactive lever presses occurring over 3 consecutive days. Once cocaine self-administration was acquired, the mice went to a training phase under a FR2 schedule. Each FR2 training session lasted for 90-min and consisted of an unlimited number of reinforcers; a stable baseline was defined as a rate of cocaine intake (measured as number of reinforcers per hour) that did not vary by more than 20% across 3 consecutive training sessions. Each subject presenting a stable baseline was submitted to dose–response tests in which each dose of cocaine (1.0, 2.0, and 4.0 mg/kg/injection) was tested independently over 3 consecutive days. Testing sessions were identical to training sessions, with the exception that the priming dose was the same as the dose available to test. Following dose–response tests, saline substituted for cocaine over 3 consecutive days.

Data analysis. Data collected under the FR2 schedule were scored as the number of reinforcers per hour and were analyzed for dose–response testing by repeated measures analysis of variance (ANOVA) performed with Systat software (53), in a between-groups and within-groups design.

RESULTS

All of the 129/Sv-ter wild-type ($n = 34$) and the 129/Sv-ter mutant mice ($n = 20$) successfully completed the food-shaping program. Twenty days were necessary for all of the wild-type mice to meet the food-shaping criteria, whereas 13 days

were necessary for the mutant mice (Fig. 1A). Means ± SEM for wild-type and 5-HT1B knockouts were 12.35 ± 8.1 and 8.9 ± 2.1 sessions, respectively, which just missed being significantly different [$F(1, 51) = 3.4$; $p = 0.07$]. Among the 20 wild-type and 15 mutant mice that survived surgery, 6 wild-type and 9 mutant mice did not present any technical problems within the first 3 days of self-administration, and thus were tested for acquisition of cocaine self-administration. Subsequently, 5 wild-type and 7 mutant mice successfully completed the dose–response curve for cocaine. The main cause for unsuccessful surgery was bleeding; the causes for losing animals during the experiments were leakage from the catheters and loss of the head mounts.

Figure 1B shows the number of sessions required to meet cocaine self-administration acquisition criteria for each strain. Eight days were necessary for 100% of the wild-type mice tested to meet the acquisition criteria, while only 4 days were necessary for the mutant mice. One-way ANOVA confirmed a significant difference in the number of sessions required to meet cocaine self-administration acquisition criteria between the wild-type and the mutant mice [$F(1, 13) = 9.75$; $p < 0.05$]. However, once they met the acquisition criteria, both strains presented a very similar and regular pattern of responding within the session. Figure 2 shows the pattern of responding of one representative subject from each strain under the FR1 schedule, during the last day of the self-administration acquisition phase. Both mice received all 20 cocaine injections (2.0 mg/kg injection) within 90-min, which corresponds to a cumulative intake of approximately 26.6 mg/kg of cocaine per hour. Under the FR2 schedule with an unlimited number of reinforcers available, the average total hourly intake of cocaine was maintained between 16 and 20 mg/kg, dependent of the dose available (1.0–4.0 mg/kg/injection; see Fig. 3). Both the wild-type and the mutant mice showed a dose-dependent response to cocaine, with the number of reinforcers taken decreasing as the dose of cocaine increased (Fig. 3). A multivariate repeated measures ANOVA confirmed a significant effect of dose of cocaine within subjects [$F(2, 20) = 49.03$; $p < 0.0005$], whereas it did not show a significant effect between strains [$F(1, 10) = 0.33$; $p > 0.05$]. Data in Fig. 3 also show responding when saline was substituted for cocaine (data shown are from the third day under saline). Over the 3 days with saline access, animals showed typical extinction burst phenomena (on the first day, numbers of saline injections were 18.8 ± 1.8 and 17.22 ± 1.6 for the wild-type and the mutant mice, respectively, declining to 1.33 ± 0.4 and 3.9 ± 1.5). The number of saline injections in the third day did not differ between strains ($t = -1.7$; $p > 0.05$).

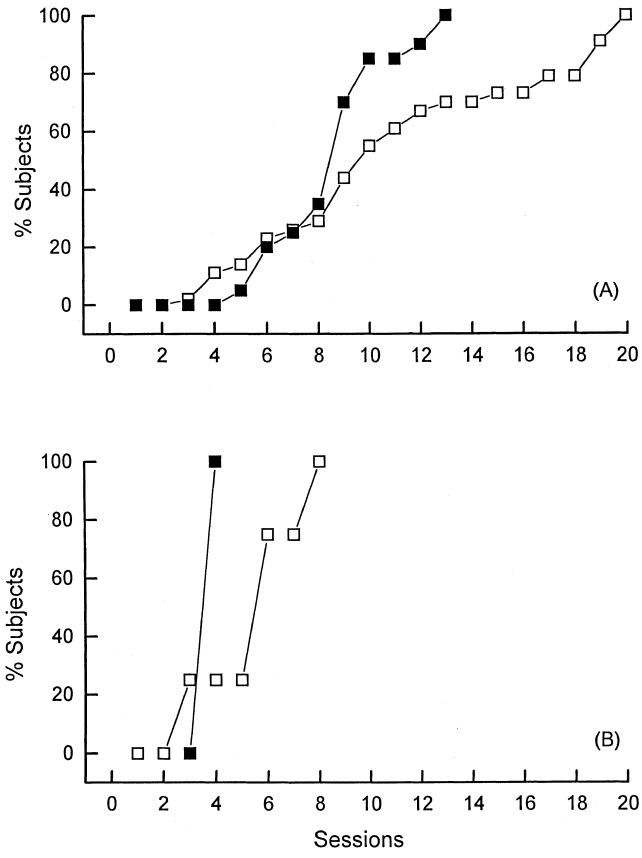

FIG. 1. Cumulative percentage of 129/Sv-ter wild-type (□: $n = 6$) and 129/Sv-ter 5-HT1B knockout mice (■: $n = 9$) meeting criteria for (A) acquisition of food-shaping, and (B) acquisition of IV cocaine self-administration (2.0 mg/kg/injection). The *y*-axis shows the percentage of subjects tested; the *x*-axis shows the number of sessions.

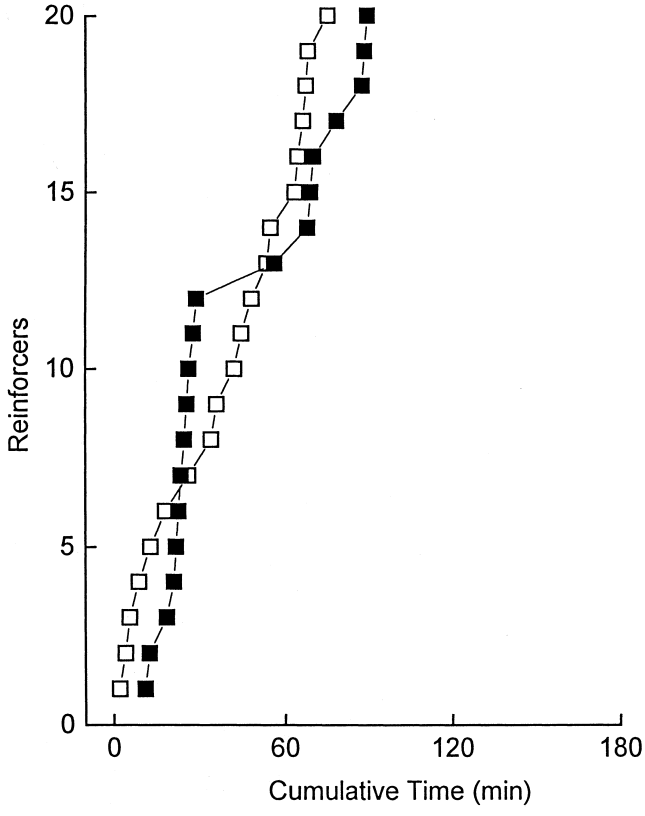

FIG. 2. Rate of cocaine self-administration (2.0 mg/kg/injection) of one representative subject from each strain (□: 129/Sv-ter wild-type mouse; ■: 129/Sv-ter 5-HT1B knockout mouse) under the FR1 schedule, with a maximum of 20 cocaine injections within 180 min. The *y*-axis shows the number of reinforcers taken during the session; the *x*-axis shows the duration of the session. Values represent the time when each reinforcer was taken during the 180-min session.

DISCUSSION

In previous studies characterizing 129/Sv-ter wild-type and 5-HT1B knockout mice, Buhot et al. (3) reported that the mutant mice had a higher level of object exploratory activity. In the present study, the mutant mice acquired cocaine self-administration behavior more quickly than their wild-type counterparts, and they nearly were significantly faster in their rate of acquisition of the operant task with food as a reinforcer. Anecdotally, our laboratory has also noted that the wild-type and knockouts are discernible in that the knockouts are noticeably more active in the experimenter's hands at the end of operant sessions. Thus, our data support those of Buhot et al. (3) and suggest that loss of the 5-HT1B receptor leads to greater levels of motor activity.

Dopamine neurotransmission has been proposed to be a critical determinant of locomotor activity as well as of the abuse potential of drugs (58). Preliminary evidence suggests that stimulation of 5-HT1B receptors may modulate neurotransmission in the mesolimbic system by disinhibiting dopaminergic neurons in the ventral tegmental area (6). In support of this hypothesis, the relatively selective 5-HT1B agonist RU24969 has been found to increase locomotor activity (54), and this same drug enhances the discriminative stimulus effects of cocaine (5). Consequently, it was expected that the loss of 5-HT1B receptors through gene targeting knockout produces animals with lower levels of cocaine-induced hyperlocomotion. However, it is surprising that these animals present higher spontaneous levels of activity in the open field (46), as well as an increased propensity for aggressive behavior (45), and for object exploration (3). These effects may confound the analysis of the psychostimulant effects of cocaine (46) and may have contributed to the more rapid acquisition of cocaine self-administration behavior. It may be the case that knockout of the 5-HT1B receptor engenders changes in the mouse during development, inducing compensatory up- or down-regulation of other 5-HT receptors or neurotransmitter systems, which may account for the present data. The appearance of a 5-HT1B antagonist and/or the use of "inducible knockout" techniques (13) should resolve this question.

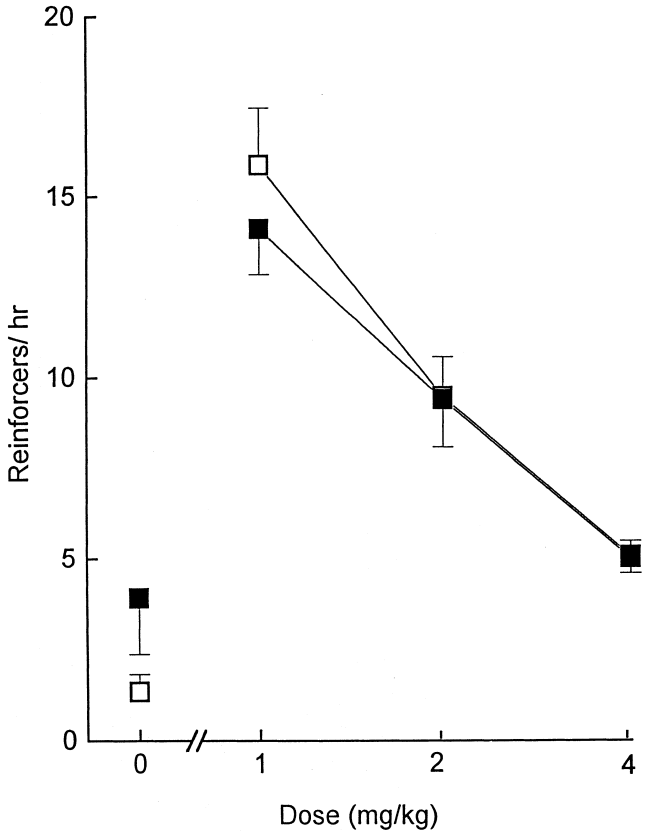

FIG. 3. Dose–response curve for cocaine IV self-administration for the 129/Sv-ter wild-type mice (n = 5) and the 129/Sv-ter 5-HT1B knockout mice (n = 7). The x-axis shows the doses of cocaine tested (1.0, 2.0, and 4.0 mg/kg/injection); the y-axis shows the number of reinforcers taken per hour during a 90-min period. Each dose was tested independently on 3 consecutive days; subsequently, saline was substituted for cocaine (dose 0). Values represent the mean ± SEM of reinforcers taken per hour during the third day of each dose for the wild-type mice (□) and for the mutants (■). For both strains, as the dose of cocaine increases, the number of reinforcers taken decreases. When saline was substituted for cocaine, both strains showed extinction behavior.

Higher levels of motor activity and object exploratory activity could be expected to lead to more rapid acquisition of operant behaviors through more frequent encounters with the manipulanda in the environment. Alternately, it may also be the case that these animals acquire self-administration more rapidly because the reinforcing efficacy of cocaine may be greater. Schenk et al. (48) have shown that, in rats, the latency to acquisition of cocaine self-administration is faster with larger doses of cocaine, and they have argued that this reflects the greater motivational properties of higher doses. In choice tests, animals prefer larger doses of drug to smaller doses (28), suggesting that animals do indeed respond differentially to drug dose. In the present experiment, both strains of mice were trained under the same dose of cocaine (2.0 mg/kg/injection); thus, it may be the case that an increased rate of acquisition of cocaine self-administration reflects the greater reinforcing efficacy of this dose in the knockout mice.

Environmental variables are well known to influence the rate of acquisition of CNS stimulant self-administration. For example, the propensity to continue self-administration of psychostimulants has been attributed to differential reactions of humans to their initial cocaine exposure (10); in animal experiments, variables such as social housing (2,47), stress (36), and prior drug exposure (18,35,59) are critical determinants of the rate of acquisition of self-administration of drugs. Changes in DA neurotransmission in the mesolimbic system have been hypothesized to underlie these environmental influences on drug self-administration (21,44), and because of the potential modulation of the mesolimbic DA pathway by 5-HT1B receptors, it may not be surprising that knockout of these receptors alters efficacy to psychostimulant drugs.

If efficacy of the reinforcing effects of cocaine is indeed greater in the knockout mice, it was not reflected in their rate of cocaine consumption once stable responding was achieved: the two strains were essentially interchangeable for their cocaine dose–effect curves under the FR2 schedule of reinforcement, and showed comparable extinction behavior under saline. However, the maintenance of responding for drug self-administration in low value ratio paradigms may not reflect the animals' motivation to self-administer the drug (23). Perhaps a better choice of schedule of reinforcement for studying the decrease or increase of this motivation is the progressive ratio (PR) (40–43). Thus, in the present study, the FR schedule of reinforcement used may account for the comparable responding between the wild-type and the mutant mice. Other studies testing 5-HT1B

minus mice and wild-type mice in a PR schedule for cocaine self-administration are under way to evaluate the role of 5-HT1B receptors in the reinforcing efficacy of cocaine.

In conclusion, the present study shows preliminary data suggesting a role of 5-HT1B receptors in the propensity to self-administer cocaine, but further studies are still necessary to understand the role of these receptors in the maintenance of cocaine self-administration. For a variety of technical reasons, mice are likely to be the predominant species used in transgenic studies. The present results demonstrate that knockout mice may be used to study the role of specific gene products in the reinforcing effects of drugs of abuse, and support previous studies showing the utility of this species in self-administration experiments (4,7,15). Previous studies with mice have used nose-poking operants, and the present study extends this technology to behavior requiring operation of manipulanda. The pattern of behavior engendered by this technique in the wild-type and knockout mice corresponds well to the pattern of behavior maintained by cocaine self-administration across a wide variety of species [(25,55); for a review, see (20)]. In addition, the fact that mice lacking 5-H1B receptors acquired cocaine self-administration faster than wild-type mice suggests that 5-HT1B ligands might be useful in the treatment of addictive behaviors.

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