

## Repeated 2 Hz peripheral electrical stimulations suppress morphine-induced CPP and improve spatial memory ability in rats

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### Abstract

Our previous studies have shown that 2 Hz peripheral electrical stimulation (PES) can suppress morphine-induced conditioned place preference (CPP) in the rat, although the mechanisms remain unclear. Since CPP involves the mechanism of learning and memory, it is rational to ask whether the suppressive effect of repeated 2 Hz PES on morphine-induced CPP is due to an impairment of the function of spatial learning and memory. Rats were trained with 4 mg/kg morphine, i.p. for 4 days to establish the CPP. Twenty-four hours after the CPP testing, they were given PES at 2 Hz once a day for 1, 3 or 5 days, followed by another CPP testing. The results showed that (1) the morphine-induced CPP was significantly inhibited by 3 or 5 consecutive sessions, but not by single session of 2 Hz PES. (2) A test of spatial learning and memory ability using the Morris water maze task revealed that 2 Hz PES per se exhibited a promoting, rather than a deteriorating effect on the ability of spatial memory. (3) 2 Hz PES by itself produced a moderate yet significant CPP. The results imply that (a) a low frequency PES can produce a rewarding effect as revealed by the CPP testing, which may account, at least in part, for its suppressive effect on morphine induced CPP, (b) the suppressive effect of PES on morphine induced CPP is not due to a deteriorating effect on the ability of spatial memory.

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*Keywords:* Peripheral electrical stimulation (PES); Conditioned place preference (CPP); Rewarding effect; Spatial learning and memory

### Introduction

Classical manual acupuncture has been used in China for thousands of years. One of the recent technical development was to use peripheral electrical stimulation (PES) applied via the acupuncture needles inserted into the acu-points, so that electrical stimulation is used to replace the mechanical stimulation. The PES has been demonstrated to be a safe, gentle, low-cost and effective self-healing approach for treating various kinds of chronic and stubborn diseases such as pain of various causes (Ahmed et al., 2000; Carlsson, 2002; Chen and Han, 1992; Hamza et al., 2000; Humaidan and Stener-Victorin, 2004; Huang et al., 2002; Stener-Victorin et al., 2003a, 2004), neurodegenerative disorders

(Gao et al., 2002; Lin and Lin, 2000; Pei et al., 2001; Scherder and Bouma, 1999; Scherder et al., 1995, 1998, 2000), coronary heart diseases (Meng, 2004), polycystic ovaries (Stener-Victorin et al., 2003b), and climacteric symptoms (Sandberg et al., 2002). PES has also been reported for the treatment of drug dependence, especially of opiate withdrawal syndrome (Fung et al., 1980; Malin et al., 1988; Shuaib, 1976; Ho et al., 1978; Wen et al., 1979). We have reported the efficacy of PES in treating heroin dependence in animals and humans, including physical and psychic dependence (Han and Zhang, 1993; Wu et al., 1999).

Conditioned place preference (CPP) is presumed to be a useful tool to test the efficacy of pharmacological or nonpharmacological interventions on the rewarding or reinforcing effects of drugs of addictive potency (Tzschentke, 1998). Recent work in our laboratory have shown that pretreatment with single-session PES of 2 Hz

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prior to the testing task could block or prevent the expression of morphine-induced CPP in rats (Wang et al., 2000). This finding provided the first evidence that the PES might possess an anti-craving activity. In the present study, experiments were designed to test whether single- or multiple-session of 2 Hz PES can block or abolish the already existing morphine-induced CPP in rats. Considering the possibility that the inhibitory effects of PES on morphine-induced CPP might be resulted from its deteriorating effects on the ability of learning and memory of the rats, experiments were performed to assess whether 2 Hz PES would affect the behavior on Morris water maze (MWM) task. Finally, experiments were designed to test whether 2 Hz PES per se has a rewarding effect.

## Materials and methods

### *Subjects*

Male Sprague–Dawley rats of 3 months old were obtained from the Beijing Experimental Animals Center, Beijing. They were housed four in a cage on a 12:12 hour light–dark cycle (lights on at 7:00 P.M.). All experiments were performed during the dark phase. Food and water were provided ad libitum. The experimental procedures were approved by the Committee on Animal Care and Use of the Peking University.

### *Drugs*

Morphine hydrochloride (the First Pharmaceutical Factory of Shenyang, China) was dissolved in 0.9% saline to the final concentration of 4 mg/ml.

### *Conditioned place preference*

Place conditioning was conducted in a three-compartment apparatus with an unbiased design. The apparatus was a black rectangular PVC box (75 × 22 × 30 cm) divided into three chambers separated by guillotine doors. The two end chambers (30 × 22 × 30 cm) used for conditioning were connected by a smaller center chamber (15 × 22 × 30 cm). The two end chambers were distinguished from each other in two ways. One had a group of 4 lights arranged in a square pattern on the end wall and a stainless steel mesh floor (1.3 × 1.3 cm<sup>2</sup>), whereas the other had the lights arranged in a triangle form on the wall and a rod floor (1.3 cm apart) (Shi et al., 2003). The center chamber had gray walls and a smooth floor. Fifteen infrared beams spaced 5 cm apart were monitoring the motion of the rat. The Infrared sensors communicated to a computer every 100 ms through an interface. All experimental events were controlled and recorded automatically by the computer and the interface located in the same room. The computer also provided continuous white noise served to mask external sounds.

The CPP procedure consisted of three phases including pretest, conditioning and test. Prior to the start of experiment, the subjects were handled twice daily (at 8:00 A.M. and 2:00 P.M.) for 5 days. On the pretest day (Day 0), rats were placed individually in the center chamber with the guillotine doors removed. They were allowed to freely explore the entire apparatus for a 15-min session. The amount of time spent in each compartment was recorded automatically. Rats that spent more time (over 100 s) in one of the end chambers than the other were excluded from the experiment. Over the next 8 sessions (2 sessions per day) subjects received a double-alternating sequence of differential conditioning. In the morning, rats were injected with saline (1 ml/kg) and immediately placed in the compartment assigned as “non-drug” for 45 min. In the afternoon (6 h later), rats were injected with morphine at the dose of 4 mg/kg and placed in the compartment assigned as “drug”. The schedule was counter balanced in the next day, that is, morphine in the morning and saline in the afternoon. After each conditioning session, the rats were returned to their home cages and the entire apparatus was cleaned with alcohol wipes to minimize trapped odors. On the test day (Day 5), rats were tested under the conditions used for pretest without morphine or saline injection. The amount of time spent in each compartment was recorded to assess individual preference.

### *Morris water maze*

The Morris water maze task was assessed in a water tank consisted of a circular black pool (diameter 120 cm, depth 60 cm), filled with 29 cm of water kept at a temperature of 24 ± 1°C. The pool was divided into four quadrants with an escape platform placed in one of the quadrants (the target quadrant). The escape platform was made of clear Plexiglas of 10 cm diameter, submerged 1 cm below the surface of the water, 25 cm apart from the pool-side. A video camera was mounted in the center above the circular pool and the movement of animals was pictured and the signals transmitted to a computer. Rats received two trials every day during seven daily acquisition sessions. Trial A was started by placing a rat into the pool, facing the wall of the tank. Each of the four starting positions (north, east, south and west) was used once in two consecutive trials; their order was randomized. A trial was terminated as soon as the rat had climbed onto the escape platform or when 60 s had elapsed. The rat was allowed to stay on the platform for 15 s. It was then taken from the platform and gently dried with a towel and returned to its home cage. Trial B was started approximately 2 hours later. The escape latency of each rat on each trial was automatically recorded by the computerized system. On the seventh day of training, the platform was removed from the pool and the rat was given one 60-s probe trial test. The time spent in each quadrant was recorded and expressed as a percentage of the total swimming time (60 s), respectively.

### Electro-acupuncture

Rats were kept in special holders with their hind legs and tails exposed (Han et al., 1991). Two stainless steel needles of 0.3 mm diameter were inserted into each hind leg in the acupoints ST36 (5 mm lateral to the anterior tubercle of the tibia) and SP6 (3 mm proximal to the superior border of the medial malleolus, at the posterior border of the tibia). Constant current square-wave electric stimulation was produced by a HANS LH-800 programmed pulse generator (Beijing Astronautics and Aeronautics Aviation University Beijing). The frequency of PES was set at 2 Hz. The intensity was increased stepwise from 0.5 mA to 1 mA and ended at 1.5 mA, with each step lasting for 10 min. To control the unavoidable effects of restraint stress from PES treatment, the subjects of the restraint group were simply restrained in the holder for 30 min.

### Evaluation of the effect of PES on the expression of morphine-induced CPP

The morphine CPP rats were randomly divided into 3 groups administered with single or multiple sessions of PES (once a day for 3 or 5 days), each of which was subdivided into 3 subgroups of 10: Control (no treatment), Restraint (kept in the special holders only) and PES. PES was administered 24 h after the morphine-CPP procedure and the effects of PES (or Restraint) on the expression of morphine-CPP were assessed 24 h after the final PES administration.

### Evaluation of the effects of PES on the spatial learning and memory

Seventy-two rats were divided randomly into 6 groups of 12 each. Three groups were used for the experiment in which the PES (or restraint) treatment was given once daily for 5 days before the water maze learning trials. The other 3 groups were given 2 Hz PES after the place (escape) tasks, with the same protocol of 30-min PES daily for 5 days. The rats were tested again for the escape tasks 24 h after the final PES administration. The probe trial test was also performed immediately following the place test.

### Evaluation on the possible rewarding effect of the PES

The CPP paradigm was employed in these experiments. The procedure was similar to the morphine-induced CPP as described in Conditioned place preference in a counter balanced manner. Briefly, after the pretest, a 4-day conditioning was carried out. In the morning, rats ( $n = 16$ ) were administered with the PES in a neighbor room. At the ending of the PES session they were immediately carried to the CPP room, and placed in the “PES” compartment for 45 min. In the afternoon (6 h later), rats were directly placed in the “non-PES” compartment. The rats were then tested

under the same condition as that of the pretest. In the control experiment 12 rats were conditioned in the same environment except that the rats were put into the holder for restraint without electrical stimulation.

### Data analysis

In the CPP studies, the preference scores were expressed as a ratio of the time spent in the drug (or PES)-paired compartment to the total time spent in both compartments (Shi et al., 2003). The preference scores were presented as mean  $\pm$  SEM and analyzed by ANOVA. The changes in the latencies to find the platform after PES treatment in the water maze task were analyzed by repeated measures ANOVA. One-way ANOVA was used to analyze the percentage of time spent in the target quadrant in the probe trial test. When significant differences were found, post hoc analyses were conducted using LSD test. The accepted level of statistical significance is  $P < 0.05$ .

## Results

### Effects of PES on the expression of morphine-induced CPP

#### Induction of morphine-induced CPP

In the pretest, 102 rats spent almost equal time in the drug and non-drug paired compartments with a CPP score  $0.499 \pm 0.007$  (mean  $\pm$  SEM). The rats were then divided randomly into 2 groups to be trained with morphine ( $n = 90$ ) or saline ( $n = 12$ ). Fig. 1 showed that after conditioning the preference scores of rats trained with 4 mg/kg morphine ( $0.618 \pm 0.012$ ) were significantly higher than that in pretest level ( $0.498 \pm 0.008$ ) ( $P < 0.001$ ). In the rats conditioned with saline, there were no significant changes in the preference scores after conditioning ( $P > 0.05$ ). Taking post-conditioning preference score for comparison, the morphine group was also significantly higher than the saline control group ( $P < 0.001$ ).

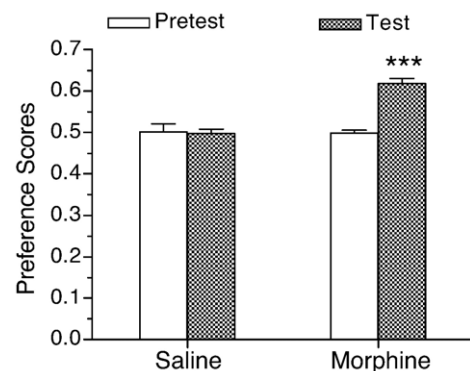


Fig. 1. Conditioned place preference induced by 4 mg/kg morphine, i.p. ( $n = 90$ ). Morphine training was administered for 4 days, and tested in the 5th day as stated in Materials and methods. \*\*\* $P < 0.001$  compared with the saline control test group ( $n = 12$ ) and the pretest value.

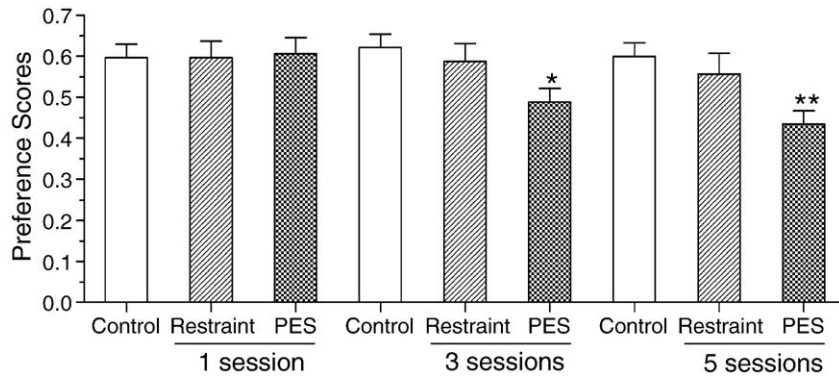


Fig. 2. Effects of PES on the expression of morphine-induced CPP ( $n = 10$ ). After the development of morphine induced CPP, PES was given once a day for 1, 3 or 5 days. The expression of CPP was tested again 24 h after the final PES. \* $P < 0.05$ ; \*\* $P < 0.01$  compared to their corresponding control groups.

*Effects of PES on the expression of morphine-induced CPP*

Fig. 2 showed the effects of single or multiple sessions of PES on the expression of morphine-induced CPP. There were no significant differences in the preference scores among the groups when the PES (or restraint) was given only once after the test trial ( $F(2,27) = 0.023, P > 0.05$ ). When the PES treatment was administered up to 3 or 5 times, there were significant differences in the preference scores among the groups ( $F(2,27) = 3.526, P = 0.044$ ; or  $F(2,27) = 4.239, P = 0.026$ ); LSD post hoc test revealed that the score in the PES group was significantly lower than that in the Control group ( $P = 0.017$ ; or  $P = 0.008$ ). No significant differences were found between the restraint group and the other two groups ( $P > 0.05$ ).

*Effects of PES on the spatial learning and memory*

Firstly, the rats were administered with five sessions of PES (or restraint) before the water maze learning sessions. Results of the water maze training are shown in Fig. 3. Repeated measures ANOVA on the data of escape latencies

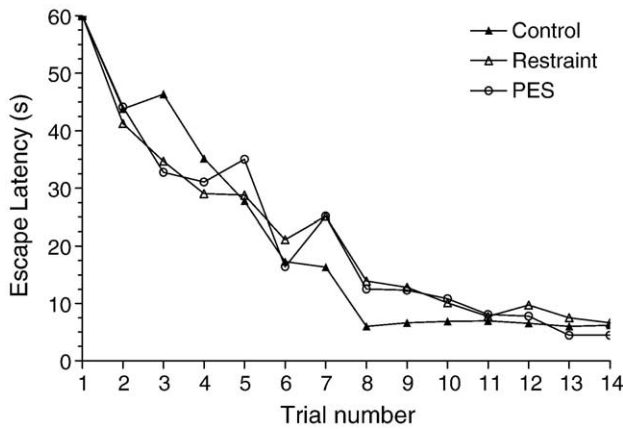


Fig. 3. Effects of PES on the acquisition of spatial learning and memory. Shown are the mean escape latencies of the rats pretreated with PES before the learning sessions. No significant differences were found among the groups in each session.

for all 14 trials revealed significant effect of the trial number ( $F(13,408) = 45.676, P < 0.0001$ ), but no significant effects of the treatment (control, restraint or PES) ( $F(2,408) = 0.921, P > 0.05$ ) and their interaction ( $F(26,408) = 1.267, P > 0.05$ ). In the probe trial test, rats in each group spent more time in the target quadrant than the other three quadrants ( $P < 0.001$ ), and no significant difference in percentage of time spent in the target quadrant was found among the groups (data not shown). The results seem to suggest that the PES has no significant effect on the spatial learning.

In another experiment, rats were given water maze training, followed by five daily sessions of PES (or restraint), and then tested again 24 h after the final PES session. As depicted in Fig. 4, the rats in the control and restraint group showed a marked prolongation of the escape latency, implying a loss of memory with time. In contrast, the rats in the PES group showed significantly shorter escape latencies than that of the restraint group ( $P = 0.046$ ) and the control group ( $P = 0.017$ ).

No significant differences were found among the groups in the probe trial test, no matter the PES was given prior to or after the MWM training (one-way ANOVA,  $P > 0.05$ ) (data not shown).

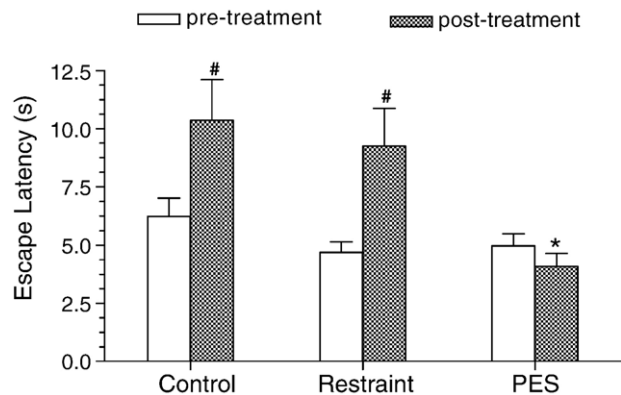


Fig. 4. Effects of PES on the retention of spatial learning and memory ( $n = 12$ ). Shown are the mean escape latencies obtained before and after the PES treatment. \* $P < 0.05$  compared to the post-treatment value of the control and restraint group; # $P < 0.05$  compared to the corresponding pretreatment values.

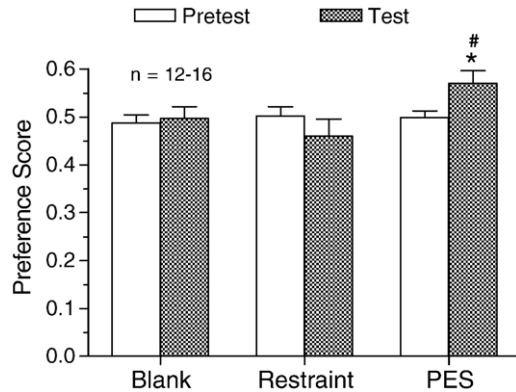


Fig. 5. Conditioned place preference induced by PES ( $n = 12-16$ ). Rats trained with PES produced a significant preference toward the PES-paired compartment. \* $P < 0.05$  compared to the corresponding pretest value; # $P < 0.05$  compared to the blank and restraint test value, respectively.

#### Rewarding effect of the PES per se

Rats trained with the PES displayed a preference for the PES-paired compartment. Fig. 5 illustrated that the preference scores of PES group were significantly higher than that of before conditioning ( $P = 0.039$ ). It is also significantly higher than that of the restraint group ( $P = 0.015$ ) and the blank control group ( $P = 0.044$ ) after the conditioning.

#### Discussion

The present study demonstrates that the expression of morphine-induced CPP can be suppressed by repeated administration of 2 Hz PES. We have reported previously that a single-trial of 2 Hz EA administered 12 h before the test task prevented the expression of morphine-induced CPP (Wang et al., 2000), whereas 100 Hz EA prevented the expression of cocaine-induced CPP (Ren et al., 2002;). The most recent study published from our group demonstrated that daily PES of either 2 Hz or 100 Hz for 3 days produced a complete blockade of the morphine-induced CPP (Shi et al., 2004). In the present study, 2 Hz PES was administered daily for once, 3 times or 5 times after the establishment of morphine-induced CPP, using restraint group and blank control group for comparison. Results shown in Fig. 2 indicate that one session of PES was not enough to suppress the already existing morphine CPP ( $P > 0.05$ ). Three sessions of 2 Hz PES produced a partial but significant blockade ( $P < 0.05$ ), whereas 5 sessions produced a complete blockade ( $P < 0.01$ ). This was not due to the extinction of CPP, since no significant decay was observed in the parallel run control group and the restraint group. The results are in favor of the hypothesis that 2 Hz PES may have the ability to antagonize the existing morphine CPP, an index related with craving for opiates.

The mechanisms underlying the suppression of morphine-induced CPP by 2 Hz PES are not clear. Two possibilities can be proposed. Firstly, since the induction

of CPP involves the mechanisms of learning and memory, if PES does have a deteriorating effect on learning and memory, it would naturally affect the induction or expression of CPP. Secondly, PES per se might have a rewarding effect through the release of endogenous opioid peptides in the CNS (Han, 2003; Han et al., 1999), which may compensate the rewarding effect offered by morphine.

To test the first hypothesis, rats were trained in Morris water maze (MWM) for spatial learning and memory. The speed of learning is reflected by the sessions of training needed to reach the plateau of shortest escape latency. Fig. 3 shows that no significant difference was observed between the 3 groups, suggesting that pretreatment with PES produced no acceleration, nor retardation of learning and memory process.

In order to observe the effect of PES on the consolidation of spatial memory, PES was administered after the completion of MWM training (Days 1–7). On Days 8–12, the rats were given PES or restraint once a day for 5 days, with the blank control group leaving in the home cage in the same time period. Results shown in Fig. 4 reveal that in the control group and restraint group, there was a marked prolongation of the escape latency, implying a fading of memory with time. However, in the 2-Hz PES group, no significant change was observed as compared to the pre-PES value, suggesting that PES not only produces no dysfunction or worsening of memory rather causes a promotion/strengthening of memory maintenance or consolidation.

Several lines of evidence have suggested the beneficial effects of PES in treating senile patients with memory deficit and patients in the early stage of Alzheimer's disease (AD) (Cameron et al., 2003; Guo et al., 2002, 1995; Scherder and Bouma, 1999; Scherder et al., 1992, 1995, 1998, 2000). Whether the results obtained in the present study will have some implication for the understanding of the above mentioned phenomenon deserves further investigation.

However, it is notable that while data from place test are statistically highly significant, those from probe test seem not supportive. The hypothesis mentioned above, therefore, needs further verification.

To test whether PES (or restraint) per se would produce certain degree of preference, rats were given PES (or simple restraint) once a day for 5 days, and the place preference was tested. Results shown in Fig. 5 indicate that in contrast to the blank control and restraint control group, where no preference was observed, the 2-Hz PES group produced a significant, although mild preference, suggesting that 2 Hz PES per se did produce a significant rewarding effect.

The endogenous opioid peptides in the CNS are implicated in the rewarding mechanisms by interactive modulation with the dopaminergic system (Kent et al., 1998; Narayanan et al., 2004). It is well known that there are three major subtypes of opioid receptors, the mu, delta

and kappa receptors, that have been studied extensively for their rewarding and motivational effects (Di et al., 1992; Narayanan et al., 2004; Van et al., 1999). Activation of mu and delta receptors by relevant agonists can produce euphoria in humans and function as positive reinforcers in animals, whereas the kappa agonists and receptors induce dysphoria and aversive effects (Bodnar and Hadjimarkou, 2002; Narita et al., 2001). It has been demonstrated that the 2-Hz PES could stimulate the release of endogenous opioid peptide enkephalins and endomorphin in CNS which interact with mu and delta opioid receptors (Guo et al., 1996; Han, 2003). The mu and delta opioid receptors are also involved in the antinociceptive effects of 2 Hz PES as well as its inhibitory effects on morphine-induced CPP (Han, 2003; Han et al., 1999; Shi et al., 2003; Wang et al., 2000). Our recent results showed that PES at 2 Hz increased preproenkephalin (PPE) mRNA levels in the nucleus accumbens of morphine CPP rats when the morphine-induced CPP was attenuated by 2 Hz PES (Shi et al., 2004). Hence, it is likely that the endogenous opioids and their interaction with mu and delta receptors might be involved in the rewarding effect of 2 Hz PES. On the other hand, there is evidence showing that electroacupuncture can modulate the production of dopamine in the CNS, which might result in a rewarding effect (Ma et al., 1993; Zhu et al., 1996). This may also lessen the expression of morphine-induced CPP.

In conclusion, 2 Hz PES can suppress the morphine-induced CPP in the rat which may help in reducing the craving for opiates in drug addicts. A rewarding effect induced by PES itself may play a role in the underlying mechanisms. 2 Hz PES improves the consolidation of spatial memory in Morris water maze task, which may be useful for the treatment of memory deficit.

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