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Swimming reduces the severity of physical and psychological dependence and voluntary morphine consumption in morphine dependent rats

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A B S T R A C T

Previous studies have indicated that voluntary exercise decreases the severity of the anxiogenic-like behaviors in both morphine-dependent and withdrawn rats. This study examined the effects of regular swimming exercise during the development of dependency and spontaneous morphine withdrawal on the anxiety-depression profile and voluntary morphine consumption in morphine dependent rats. The rats were chronically treated with bi-daily doses (10 mg/kg, at 12 h intervals) of morphine over a period of 14 days. The exercising rats were allowed to swim (45 min/d, five days per a week, for 14 or 21 days) during the development of morphine dependence and withdrawal. Then, rats were tested for the severity of morphine dependence, the elevated plus-maze (EPM), sucrose preference test (SPT) and voluntary morphine consumption using a two-bottle choice paradigm in animal models of craving. The results showed that withdrawal signs were decreased in swimmer morphine dependent rats than sedentary rats \((P<0.05)\). Also, the swimmer morphine-dependent and withdrawn rats exhibited an increase in EPM open arm time and entries \((P<0.05)\), higher levels of sucrose preference \((P<0.001)\) than sedentary rats. Voluntary consumption of oral morphine was less in the swimmer morphine-withdrawn rats than the sedentary groups during four periods of the intake of drug \((P<0.01)\). We conclude that regular swimming exercise reduces the severity of morphine dependence and voluntary morphine consumption with reducing anxiety and depression in morphine-dependent and withdrawn rats. Thus, swimming exercise may be a potential method to ameliorate some of the deleterious behavioral consequences of morphine dependence.

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1. Introduction

Morphine dependence and withdrawal from chronic opiates is associated with an increase in physical and psychological dependence signs including autonomic-somatic symptoms, anxiety and depression (Janiri et al., 2005; Miladi-Gorji et al., 2012; Miladi-Gorji et al., 2011; Schürks et al., 2005). Exposure to morphine produces plastic changes in neuronal circuitry, most importantly in the reward processing system of brain (Bao et al., 2007; Kauer and Malenka, 2007), which in turn leads to drug-seeking behavior and craving years after cessation of drug (Hyman et al., 2006). It has been shown that stressful situations or anxiety are potent stimulator of drug-seeking behaviors and relapse of drug in addicted individuals (Ferguson et al., 2004; Weiss, 2005). Thus, prevention of anxiety and depression may be useful in the treatment of relapse.

Recent studies have shown that exercise was associated with a reduction in anxiety and depression in humans (Blumenthal et al., 1999; Greenwood and Fleshner, 2008) and rodents (Duman et al., 2008; Fox et al., 2008). We have previously shown that the voluntary exercise diminished the severity of physical dependence and anxiety behavior in both morphine-dependent and withdrawn rats (Miladi-Gorji et al., 2012; Miladi-Gorji et al., 2011). While, the role of regular swimming exercise as forced and stressful training in development of morphine dependence is unknown. Given that stress may modify the susceptibility to the reinforcing effects of morphine and a later vulnerability to relapse (Goodman, 2008; Shaham et al., 1992). Thus, we have extended our previous study to investigate whether regular swimming exercise as forced exercise during induction of morphine

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dependence and spontaneous withdrawal would attenuate the severity of physical and psychological dependence on morphine and also, with an emphasis on voluntary consumption of morphine in animal models of craving in rat.

2. Materials and methods

2.1. Animals and induction of morphine dependence

Adult male Wistar rats (230 ± 10 g) were housed in cages a 12-h light/dark cycle at 22–24 °C and food and water were available ad libitum. All of the experimental procedures were conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Additionally, the number of animals used per each group was the minimum, as far as possible.

Morphine sulfate (Temad Company, Iran) was injected subcutaneously at a dose of 10 mg/kg, twice per day at 12 h intervals, as described previously (Miladi-Gorji et al., 2012; Miladi-Gorji et al., 2011) for 14 days in the presence or absence of regular swimming exercise (see timeline) in Experiment 1 and 2. The control rats were treated similarly, with injections of saline.

2.2. Regular swimming exercise

The exercise protocol was performed, as described previously (Sigwalt et al., 2011) with slight modifications. Swimming pool was a blue circular pool (140 cm in diameter and 50 cm high) filled to a 25 cm depth with 31 ± 1 °C water. To acclimate to the new environment and also, to reduce stress, all rats were adapted to water before beginning the experiment, which took four days. First, rats were placed for 5 min into the swimming pool with shallow water only for standing. At the second day, rats spent 5 min in head-high water in order to start of the swim. At the third day, the water was deep enough, so they had to swim for 5 min. At the fourth day of adaptation, the animals had to swim for 15 min. During the initial adaptation period of Exp 1, morphine was not injected to rats. On the first and second days of morphine dependence, the rats swam for 30 min. Then, exercising rats were submitted to swimming sessions (45 min/d, five days per a week, for 14 days). The exercise protocol used in this study was a moderate-intensity exercise (Mazzardo-Martins et al., 2010; Sigwalt et al., 2011). The adaptation period of Exp 2 was started two days before the end of morphine dependence.

2.3. Withdrawal rating scale

Immediately after naloxone hydrochloride (Sigma–Aldrich, Germany) injection (1 mg/kg, intraperitoneally), withdrawal signs were recorded and scored according to a modified version of the Gellet–Holtzman scale as described previously (Miladi-Gorji et al., 2012; Miladi-Gorji et al., 2011) for 30 min. Graded signs including jumps, wet dog shakes, and abdominal contractions were counted as the number of events occurring during the total test time. Body weights were recorded immediately before and 60 min after naloxone injection, and the percentage of body weight changes was calculated. Checked signs including diarrhea, ptosis, erection or genital grooming, teeth chattering, writhing, and irritability were counted as positive if the sign occurred at any time during the observation period.

2.4. Anxiety measurement in the EPM (elevated plus maze test)

In the test of anxiety, the rats were individually placed in the center of the EPM with two open (50 × 10 cm) and closed (50 × 10 × 40 cm) arms, and a central platform (10 × 10 cm) facing an open arm, and allowed to explore the apparatus for 5 min. The apparatus was placed at a height of 70 cm from the floor (Miladi-Gorji et al., 2012). The following variables were measured during each 5 min test: (1) time spent in open and closed arms as a percentage of the total time spent exploring both the open and closed arms; (2) the number of entries into the open and closed arms. In addition, the total number of arm entries was used as relative index of general activity. The apparatus was cleaned after each trial with water. It should be noted that anxious rats entered the open arms less frequently and spent less time in the open arms compared to the closed arms when allowed to freely explore the EPM (Pellow et al., 1985). The maze was dimly illuminated by a single lamp of 100 W in a soundproof room.

2.5. Sucrose preference test (SPT)

The SPT investigate the anhedonic state as a depressive-like behavior in rats (Casarotto and Andreatti, 2007). All rats were maintained in individual cages for 24 h before testing. Modified model of the SPT as previously described (Casarotto and Andreatti, 2007; Pisu et al., 2011) was carried out as follows. Rats were allowed access for 48 h to two bottles in each cage, one with 200 ml of 32% sucrose (w/v) and the other also with 200 ml of tap water. The positions of the bottles were changed every 12 h to avoid learning. Fluid intake and sucrose were measured every day. At the end of 48 h, the bottles were removed and sucrose preference was calculated as: 100 × sucrose solution consumption (ml)/total fluid consumption (ml).

2.6. Two-bottle choice (TBC) paradigm

Voluntary morphine consumption and preference was quantified using modified model of two-bottle choice as described previously (Belknap, 1990; Berrettini et al., 1994; Ferraro et al., 2005; Haydari et al., 2014) in animal models of craving in rats. Each rat was housed individually in cages after testing of SPT with two bottles for a period of 16 days of testing. In one bottle, morphine sulfate was dissolved in 3% sucrose solution and also 3% sucrose solution was in control bottle as follow, respectively: On days 1–4 of test, (0.3 mg/ml morphine); On days 5–8 of test, (0.5 mg/ml morphine); On days 9–12 of test, (0.7 mg/ml morphine). On day 13–16 (0.7 mg/ml morphine, without sucrose), Rats were allowed continuous access to both bottles. To minimize effects related to learning, the position of the bottles in the cage was changed at the time of daily bottle weighing. Fluid intake was measured by weighing the bottles between 9:00 and 10:00 am daily. Body weights of the rats were measured in the start of each period. The average morphine consumption was evaluated during a 4-day period.

2.7. Statistical analysis

The data expressed as the mean ± standard error of the mean (S.E.M.). These data were analyzed using one-way or two-way analyses of variance (ANOVA), with repeated measures as required. Post-hoc analyses included Tukey’s test. Graded somatic signs of opiate withdrawal were analyzed by Student’s t-test, and expressed as the mean ± S.E.M. Checked somatic signs of opiate withdrawal were analyzed by the Mann-Whitney U-test, and expressed as the percentage of rats in a particular experimental group which exhibited that sign during the observation period. Statistical differences were considered significant at P < 0.05.
2.8. Experimental protocol

2.8.1. Experiment 1

This experiment examined the effects of regular swimming exercise on the severity of morphine dependence. Sixteen naive rats were divided into two groups (n=8 rats per group): dependent-sedentary (D/No Swim), dependent-swimming exercise (D/Swim). On day 15, the global severity of morphine withdrawal scores induced by naloxone (1 mg/kg, IP) was measured in both groups 2 h after a morphine injection (Fig. 1).

In a pilot study, we tested the effects of swimming exercise on the severity of morphine withdrawal responses after an injection of naloxone (1 mg/kg, IP) (n=4–5 per group). The results of our pilot study showed that there were no difference between the two groups of Sal/No swim (1.43 ± 0.25) and Sal/Swim (1.96 ± 0.16) in the total Gellert–Holtzman scale score (t=−1.86, P=0.104). Here, we then examined the two groups of morphine dependent rats.

2.8.2. Experiment 2

This experiment examined the effects of swimming exercise on the anxiety-like behaviors of morphine dependent rats. Thirty rats were divided into four groups (n=7–8 rats per group): saline-sedentary (Sal/No Swim), saline swimming exercise (Sal/Swim), dependent-sedentary (D/No Swim) and dependent-swimming exercise (D/Swim). The exercising group was allowed swim for 14 days. In days 15, 2 h after a morphine injection all animals were tested in the EPM and followed by SPT in days 16–17. In the two-day sucrose preference test, morphine injection was continued (Fig. 1).

2.8.3. Experiment 3

This experiment examined the effects of swimming exercise on anxiety and depressive-like behaviors during spontaneous morphine withdrawal which lasted 21 days. Thirty two rats were divided into four groups (n=8 rats per group): saline-sedentary (Sal/No Swim), saline swimming exercise (Sal/Swim), dependent-sedentary (D/No Swim) and dependent-swimming exercise (D/Swim). In four groups, saline or morphine injection was performed for 14 days. Then, groups were maintained in their home cages without any morphine injection (drug abstinence) for 21 days. However, the exercising rats were simultaneously exposed to a regular swimming exercise for 21 days. On day 37, rats were rested. In days 38, all animals were tested in the EPM and followed by SPT in days 39–40. Morphine-withdrawn rats was housed individually in cages after testing of SPT with two bottles for a period of 16 days to evaluate the voluntary consumption of morphine (in days 41–57). From day 37 to 57 swim training was discontinued (Fig. 1). To control for differences in swimming performance, we also recorded each animal’s swimming velocity as index of motor activity for 1 min during the last day of Fig. 1. Timeline of experiments.
swimming in Exp 3, using a tracking system (EthoVision, Noldus, The Netherlands) as described previously (Miladi-Gorji et al., 2011).

3. Results

3.1. Experiment 1: regular swimming exercise reduces the severity of withdrawal signs in morphine-dependent rats

The overall Gellert–Holtzman scores were significantly lower in the swimmer dependent rats than in the sedentary dependent rats (T14 = 6.042, P < 0.0001, Fig. 2). Among the graded signs in swimmer morphine-dependent rats, such as abdominal contractions (T14 = 5.88, P < 0.0001), wet dog shakes (T14 = 3.347, P < 0.005), loss weight (T14 = 4.07, P < 0.001) and jumping (T14 = 3.86, P < 0.002) were lower compared to the sedentary dependent rats (Fig. 2).

Among the checked signs (Table 1), the number of rats per group with diarrhea (U = 16, P < 0.025) and writhing (U = 16, P < 0.025) and genital grooming (U = 20, P < 0.05) were decreased compared to the sedentary dependent rats. There were no statistically significant changes in irritability, teeth chattering, and ptosis between two groups.

3.2. Experiment 2: regular swimming exercise decreases the anxiety and depressive-like behaviors in morphine-dependent rats

Fig. 3. A, B shows results of the EPM testing for the sedentary and swimmer dependent rats. ANOVA showed a significant difference between the groups in the number of open arm entries (F3, 26 = 8.98, P < 0.0001). Comparisons between groups showed that the number of open arm entries in the swimmer dependent group was more than the sedentary dependent rats (P < 0.0001) (Fig. 3A). There was no significant difference in the number of closed and total arm entries among the groups.

Fig. 3B shows a significant difference between the groups in the percentage of time spent in open (F3, 26 = 23.25, P < 0.0001) and closed arms (F3, 26 = 18.1, P < 0.0001). Comparisons between groups indicated that the percentage of time spent in the open and closed arms in swimmer morphine-dependent rats was more and less than sedentary dependent rats (P < 0.0001, both), respectively. Also, sedentary morphine-dependent rats had a less and more time spent in the open and closed arms than the sedentary saline rats (P < 0.001, both), respectively. Swimmer saline rats spent significantly more and less time in open and closed arms than the sedentary saline group (P < 0.021, P < 0.05, respectively).

Results in sucrose preference test (SPT) in Fig. 3C shows a significant difference between the groups in the percentage of sucrose preference (F3, 26 = 9.57, P < 0.0001). Comparisons between groups showed that sucrose preference in sedentary morphine-dependent rats was less than those control (P < 0.006). The swimmer morphine-dependent group had a higher preference for sucrose than sedentary morphine-dependent group (P < 0.0001).

3.3. Experiment 3: regular swimming exercise decreases the anxiety and depressive-like behaviors and voluntary morphine consumption in morphine withdrawn rats

Results in the EPM (Fig. 4A) showed significant difference between groups in the number of open (F3, 26 = 31.28, P < 0.0001) and closed (F3, 26 = 10.29, P < 0.0001) arm entries. Comparisons between groups showed that the number of open and closed arm entries in swimmer morphine withdrawn rats were more (P < 0.0001) and less (P < 0.003) than those control. Also, the number of open and closed arm entries in swimmer saline group were more and less (P < 0.0001, P < 0.008) than those control, respectively.

Fig. 4B showed a significant difference between groups in the percentage of time spent in open (F3, 26 = 39.92, P < 0.0001) and closed (F3, 26 = 29.88, P < 0.0001) arms. Comparison between groups indicated that the percentage of time spent in the open and closed arms in swimmer morphine withdrawn (Both, P < 0.0001) or saline groups (P < 0.0001, P < 0.001, respectively) were more and less than sedentary groups. Also, sedentary morphine withdrawn rats spent significantly less and more time in open and closed arms, respectively than the sedentary saline group (Both, P < 0.0001).

Results SPT in Fig. 4C shows a significant difference between the groups in the percentage of sucrose preference (F3, 26 = 25.83, P < 0.0001). Comparisons between groups showed that sucrose preference in sedentary morphine withdrawn group was less than control (P < 0.0001). While, swimmer morphine withdrawn rats had a higher percentage of sucrose preference than control group (P < 0.0001).

Two-way ANOVA with repeated measure (day) for the voluntary consumption of morphine during four period of intake revealed a significant effects of days (F3, 42 = 8.82, P < 0.0001), a significant effect of groups (F3, 14 = 3763, P < 0.0001) and significant interaction between day × group (F3, 42 = 3.15, P < 0.05) (Fig. 5). In general, voluntary consumption of morphine during four period of TBC test is decreased significantly in the swimmer morphine-withdrawn rats compared to the control group (Respectively, P < 0.001, P < 0.004, P < 0.017, and P < 0.001vs. D/No Swim).

Lastly, we found no difference in the swimming speeds between groups (F3, 26 = 2.8, P > 0.098): Sal/No Swim (19.5 ± 0.24 cm/s), Sal/
Swim (20.06 ± 0.97 cm/s), D/No Swim (21.16 ± 0.68 cm/s), and D/Swim (22.4 ± 0.46 cm/s).

4. Discussion

4.1. Regular swimming exercise reduces naloxone-precipitated withdrawal signs

The results of our study indicated that a period of 14 days of swimming exercise during the development of dependency significantly decreases the severity of naloxone-precipitated morphine withdrawal signs, which are consistent with our previous results (Miladi-Gorji et al., 2011). Presently, the neurobiological mechanisms underlying the decreased morphine withdrawal signs after swimming are still unclear. Previous findings indicate that access to chronic exercise decreases the rewarding effects of cocaine (Smith et al., 2008), and the potency of morphine (Smith and Lyle, 2006). It may also be due to activation of the reward pathway and neuroplastic changes following exercise (Greenwood et al., 2003), which reduces the sensitivity to morphine (Hosseini et al., 2009; Smith et al., 2004). Alleviating in some observed withdrawal signs might be due to different reasons, for example; the number of abdominal contractions due to interaction of opioid and serotonergic receptors (Mazzardo-Martins et al., 2010), jumping by releasing of brain GABA (De Groote and Linthorst, 2007; Zarrindast and Mousa-Ahmadi, 1999), diarrhea by releasing of endogenous opioid peptides and reduction in abdominal pain (Mazzardo-Martins et al., 2010) and the number of wet dog shakes through releasing of brain noradrenaline (Park, 2009) followed swimming. Taken together, these findings indicate that the rewarding properties of swimming exercise, and modulation of neurotransmitter release could contribute to reduce some morphine withdrawal signs.

4.2. Regular swimming exercise decreases the anxiety and depressive-like behaviors in morphine-dependent and withdrawn rats

We found that morphine-withdrawn rats showed anxiogenic responses 21 days after morphine withdrawal in the EPM test. Our finding is in agreement with previous studies showing that morphine
withdrawal is associated with an increase in anxiogenic-like behavior in rats (Miladi-Gorji et al., 2012; Schulteis et al., 1998). However, there was no significant difference in the number of the total arm entries and swimming velocity between groups, suggesting that reduced open arm activity in the sedentary morphine-withdrawn group was due to increased anxiety and not hypo-activity or motor impairment.

Also, the lower levels of sucrose preference in sedentary morphine-withdrawn rats can be interpreted as an indicator of anhedonia. This finding is in accordance with a previous study showing that rats exhibited a reduced preference to sweet solutions for six days after morphine withdrawal (Lieblich et al., 1991). However, in our study, morphine withdrawal lasted 21 days showing that depression and anxiety are persistent behaviors in morphine-dependent rats. A study has shown that there is a correlation between reduced BDNF and increased CRF with anxiety and depressive-like behaviors following four weeks of protracted abstinence from repeated morphine administration (Lee et al., 2014).

Also, the results of our study indicated that a period of 14 and 21 days of swimming exercise respectively during the development of dependency and spontaneous morphine withdrawal blunted anxious and depressive behaviors using the EPM and SPT tests. In consistent with our findings, clinical (Barbour and Blumenthal, 2005; Dunn et al., 2005; Strawbridge et al., 2002) and animal (Lapmanee et al., 2013; Liu et al., 2013; Salim et al., 2010; Teixeira et al., 2011) studies have documented that voluntary or forced physical activity decreases anxious or depressive behaviors. Our previous study has also shown that voluntary exercise reduces anxiety levels in both morphine-dependent and withdrawn rats (Miladi-Gorji et al., 2012). Presently, the neurobiological mechanisms to reduce anxiety or depression levels following the physical training are still not known. It may be due to increase in serotonin (Greenwood et al., 2003), noradrenalin

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**Fig. 4.** Effect of regular swimming exercise on the anxiety and depressive-like behaviors in morphine-withdrawn rats. A) The number of entries into the open, closed and the total arms of the EPM. B) The percentage of time spent in the open and closed arms of the EPM. C) The percentage of sucrose preference using the SPT. Swimmer rats spent significantly respectively more and less time in the open and closed arms and made significantly respectively more and less entries into the open and closed arms than the sedentary control rats. The swimmer morphine-withdrawn rats had a much higher percentage of sucrose preference. The D/No Swim group spent significantly respectively less and more time in the open and closed arms and also had a lower percentage of sucroso preference than the sedentary control rats. In A) \(* \* \* p < 0.0001\) and \(* \* \* p < 0.0001\) vs. Sal/No Swim, \(\widehat{\times} p < 0.0001\), \(\widehat{\times} p < 0.003\) vs. D/No Swim. In B) \(* \* \* p < 0.0001\) and \(* \* \* p < 0.001\) vs. Sal/No Swim, \(\widehat{\times} p < 0.0001\) vs. Sal/No Swim, \(\widehat{\times} p < 0.0001\) vs. D/No Swim. In C) \(* \* \* p < 0.0001\) vs. Sal/No Swim, \(\widehat{\times} p < 0.0001\) vs. D/No Swim.
(Stranahan et al., 2009), dopamine (Meeuse and De Meirleir, 1995) and brain-derived neurotrophic factor (BDNF) (Duman and Monteggia, 2006; Pietropaolo et al., 2008), a decrease of oxidative stress (Salim et al., 2010), serum corticosterone (Liu et al., 2013) and the hypothalamic corticotropin-releasing factor (CRF) (Stavropoulos-Kalinoglou et al., 2013), and also compensatory changes in monoaminergic receptor populations (MacRae et al., 1987) following the voluntary or forced exercise. Therefore, these findings indicate that regular swimming exercise probably with adjustment of brain neurotransmitters and by reducing oxidative stress could contribute to reduce the anxiety and depressive-like behaviors in morphine-dependent and withdrawn rats.

### 4.3. Regular swimming exercise during spontaneous morphine withdrawal decreased the voluntary consumption of morphine in morphine-withdrawn rats

In this study, the rats had free access to 3% sucrose solution and morphine dissolved in 3% sucrose solution which sedentary morphine-withdrawn rats preferred morphine to 3% sucrose solution, reflecting an incentive demand for the drug in the TBC model. It seems that morphine-induced neurochemical changes of dopamine and serotonin turnover (Wu et al., 2009), less dopaminergic activity in the striatum (Tjon et al., 1994), a decrease in the expression of hippocampal BDNF, an increase in the CRF expression (Lee et al., 2014), stressful events (Ferguson et al., 2004; Weiss, 2005), negative emotional states such as anxiety, depression (Aston-Jones and Harris, 2004; Self and Nestler, 1998) may be involved to the higher susceptibility to relapse following protracted abstinence from repeated morphine administration.

Our findings have also shown that swimmer morphine-withdrawn rats had a lower voluntary consumption of morphine during four periods of the intake of drug using a TBC paradigm as an animal model of relapse. This finding is in accordance with previous studies showing that access to a running wheel reduces self-administration and the craving of morphine (Hosseini et al., 2013), the rewarding effects of cocaine (Lett et al., 2002) in rats. Thus, we conclude that regular swimming exercise decreases the rewarding effects of morphine which can reduce the risk of relapse and drug seeking after protracted abstinence. It may be due to an increase in serotonin (Greenwood et al., 2003), noradrenalin (Stranahan et al., 2009), and BDNF (Li et al., 2008; Miladi-Gorji et al., 2011), dopamine (Meeuse and De Meirleir, 1995) and a decrease in CRF (Droste et al., 2003; Stavropoulos-Kalinoglou et al., 2013) following the voluntary and forced exercise. It is also assumed that swimming exercise-induced antidepressant and anxiolytic effects can be attributed to the attenuation of voluntary morphine consumption in the swimmer morphine withdrawn rats. Future studies need to examine the neurobiological mechanisms induced by swimming.

### 5. Conclusions

This study provides novel evidence that regular swimming exercise during the development of dependency and spontaneous morphine withdrawal can decrease the severity of physical and psychological dependence and also the voluntary consumption of morphine after protracted periods of abstinence. Thus, swimming exercise may be of benefit in the treatment of the anxiety, depression and associated disorders frequently observed in addicts.

### Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the article.

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