

STUDY OF EMOTIONAL AND EXPLORATORY BEHAVIORS IN THE FIRST GENERATION OF MORPHINE ADDICTED MICE USING OPEN FIELD AND ELEVATED PLUS MAZE

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Summary

Introduction: morphine addiction changes parental endocrine secretions in pregnancy, that affects behavior. If the next generation is susceptible was our question.

Materials and Methods: Emotional and exploratory behaviors of first generation of morphine addicted male and female mice were studied by Elevated Plus Maze(EPM) and Open Field(OF) procedures.

Results: EMP test showed that male offspring of addicted parents are less enthusiastic than control to enter to open arm and also showed that the female offspring of addicted parents spend more time in close arm to decrease the stress and increase the security ratio.

Female children of none-addicted parents and female children of addicted mothers are different in two important factors; the number of standing and washing time; it means that some children had been delivered from some kinds of addicted mothers that in OF condition spent more numbers for standing but this group of children spent less time in washing also the children of addicted fathers spent more time in washing in compare with the two other groups.

Conclusion: Many researches had been done and still doing about the effects of addicted parents on physiological and psychological system of their children both in animals and human.

But the main goal of this research is the examination about the effects of morphine addicted male parents on their first generation children. What became clear in OF test result is that the wide behavioral changes happen in male and female children of addicted parents in compare with the other groups. But the most important thing is the effects of addicted parents on female children; for analyzing we refer to the OF test result again that female children of addicted fathers showed more seeking reactions

Keywords: Addiction, Morphine, Offsprings behavior

Introduction

Opioid addiction has been one of the human being problems all of the times . Personal knowledge of side effects of opioids on the body can decrease consumption of these drugs . Expanded studies have done on side effects of opioids on several systems of the body.

Collected informations from several sources display that mean age of opioid consumer are approximately 33 ± 10 and of first time consumer are 22.2 ± 7.1 years in Iran .Addiction to opium is more than other opioids .

Informations show that 90% of addicted persons are male and others are female . $\frac{2}{3}$ are married and $\frac{1}{3}$ are singles (1) .

Morphine is one of the most important and abundant alkaloids of opium . This drug has several known pharmacologic effects on organs of the body . In the previous study that we evaluated the effects of addiction on tissue of testis and different stages of spermatogenesis, we conclude that morphine decreases the Glycoconjugates different cells such as spermatogonia and spermatozooids (2) . One of these glycoconjugates is sialic acid that has important protective role on spermatozooids during the transferring through the female genital tubes (3). In another study, different side effects of morphine consumption on newborns of mice were assessed . Findings of this study obviously revealed increasing Of newborns mortality. Decrease in weight of offsprings and increase in numbers of newborns and decrease in duration of pregnancy were obvious in relation to there were meaningful differences between case and control groups(4). Other wise, effects of natural and chemical materials which are consumed by parents in reproductive systemes and fetal periods can change the behaviors of offsprings even in next generations (5). Differences between addicted and nonaddicted parents even in sex cells induced us to study this process in Balb/c mice. Some of excitatory and exploratory behaviors of rodents have resemblance to human . So we selected the mouse for this study . Elevated Plus Maze (EPM) and open field (OF) tests are used for exploratory behaviors in animal (6 ,7) .

These tests have been used for behaviors related to anxiety (8) . These procedures have been used for study the effect of environmental factors and also for pharmacologic effects of animal behaviors (9,10) . Some excitatory behaviors such as startle and freezing responses are related to amygdaloid nucleus . If sympathetic and parasympathetic nerve fibers are the way of these reactions ? If behaviors of addicted parents separately or together change the behaviors of offsprings ? These were the questions for our study . We used the of and EPM tests for finding the answers .

Materials and Methods

Experimental interventional procedure was used in this study .

1. Laboratory animals

100 female and male Balb/c mice selected and divided in four groups (25 each) randomly. Female and male mice in each group were divided in two separated groups. One of these groups received morphine.

2. Morphine sulfate amples, each of which was contained 10mg/ml, were prepared from darupakhsh , They were injected for three periods and each period was six days. So in first period 10mg/kg in second period 15mg/kg and in third stage 20mg/kg morphine was injected. Injection was one time in each day at injection of one dose Naloxan was tacked place and seeing the addiction syndrome for confidency of

production of addiction. Standard condition of animal house was prepared and after confidency of addiction. Coupling of male and female tacked placed as below.

1. In first experimental group , males were addicted and females non-addicted (M+F-)
2. In second experimental group, males were non-addicted and females were addicted (M-F+).
3. In third experimental group, both male and female were addicted (M+F+).
4. In control group , both male and female were non-addicted (M-F-). After mating and vision of vaginal plaque we recorded the occurrences such as pregnancy and birthes newborns of all groups were cared in laboratory conditions all of individuals were studied simultaneously. behaviors of groups were studied as :
 1. Emotional by elevated plus maze (EPM)
 2. Exploratory by open field (OF) types of behavioral studies are several as mentioned previously. We selected elevated plus maze and open field procedures

Of procedure is a classic test for determining the range of exploratory reaction of mice. This procedure is very different from others because of physical specialty of system and the kind and environment of dest it has short-time (2-3 minutes) and long-time (8-15 minutes) tests .

In this study we used a fiberglass bot with 40 ×100×100 dimension a fiberglass green plate with 100×100 centimeter dimension was embedded 8^{cm} above the floor of the box . This plate was divided to 25 equal square . punctures with 3 centimeter diameter were made in centers, corners and midde parts of each side of these squares . each labeled mouse was entered in open field from a definite location for 5 minntes-and behavioral factors were measured. These factors were as below .

1. number of entrance to side squares .
2. number of entrance to middle squares
3. numbers that animal enters its head to holes .
4. numbers of animal defecations .
5. numbers of standing on feet
6. duration of grooming
7. duration of silence

Other factors which were measured are as below

1. number of passage through side squares
2. number of passage through midde squares
3. number of standing through on feet
4. number of entrance to holes by heed .
5. duration of grooming .

Results

Male newborns on of procedure :

Offspring in all groups compared with each others . data showed notable differences (P<0.05)

1. In comparing of M⁺F⁺ , M⁻F⁻ and , M⁻F⁺ offsprings, we found notable differences .
2. Findings in female offsprings in of procedure, as chart 1 showed notable differences . Female offsprings of , M⁻F⁻ had notable difference, with M⁺F⁺ in standing on feet and grooming factors . that is offsprings of addicted mothers stands on feet rather than others. These offsprings spend fewer time for than others. Offsprings of addicted fathers spend higher times for groomis . B Elevated plus maze procedure E MP is a standard procedure for defining the level of anxiety and emotion in animals . this maze is used for recognition of

emotional behaviors in mice . EPM has two open and two shut arms . shut arms have high walls . open and shut arms are located vertically in relation to each other so they form a crescent .

Shut arms are to security and open arms are determinant of exploratory behaviors. So if one mouse spends fewer time on open arms , will be more anxious . If it spends higher time , will have lower fear .

Measured factors are

1. duration of silence
2. duration of grooming
3. duration of spending time on open arm
4. duration of spending time on shut arm

Cleaning the maze is necessary between two subsequent tests . data analysis has been done by spss software as chart z , studied factors are as below :

1. times of entrance to shut arm
2. times of entrance to open arm
3. times of standing of feet

A. Findings for male offsprings on EPM test : charts 4-6 and chart 2 are designed for these data male offsprings of all groups have been compared with each others times of entrance to open arm of maze was only factor that has notable difference between M^+F^+ and M^-F^- offsprings,

B. Findings for female offsprings in EPM test :

M^+F^- in comparison with M^-F^+ offsprings there were notable reduction on times of entrance to shut arms of maze (chart 5) M^+F^+ in comparison to M^+F^- offsprings showed notable reduction on times of entrance to open arms (chart 6) .

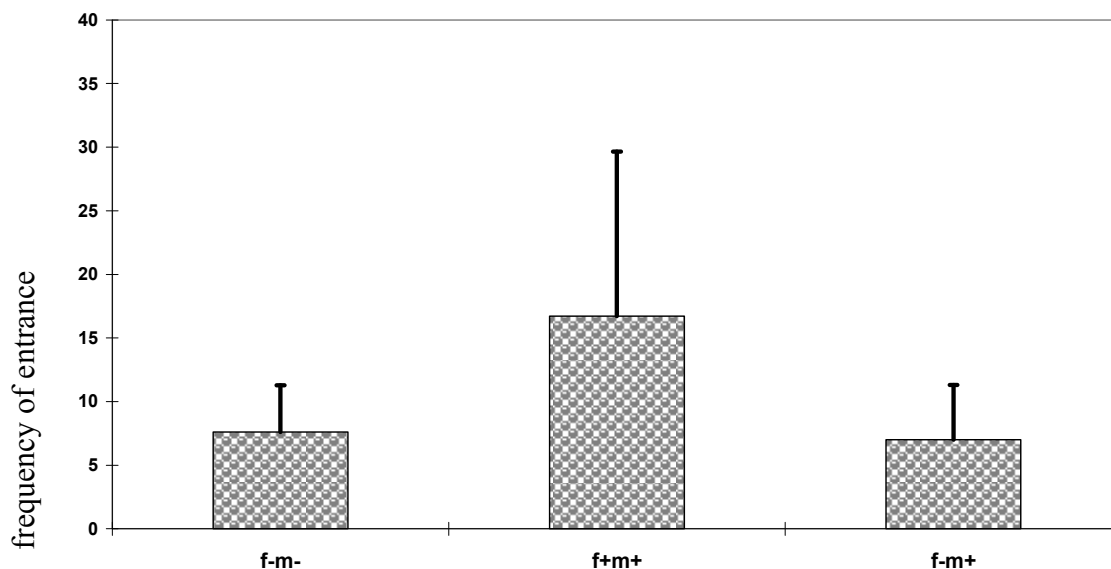


Fig. 1. Comparison of mean of fifth factor (frequency of standing) on Open Field behavioral procedure of male newborns

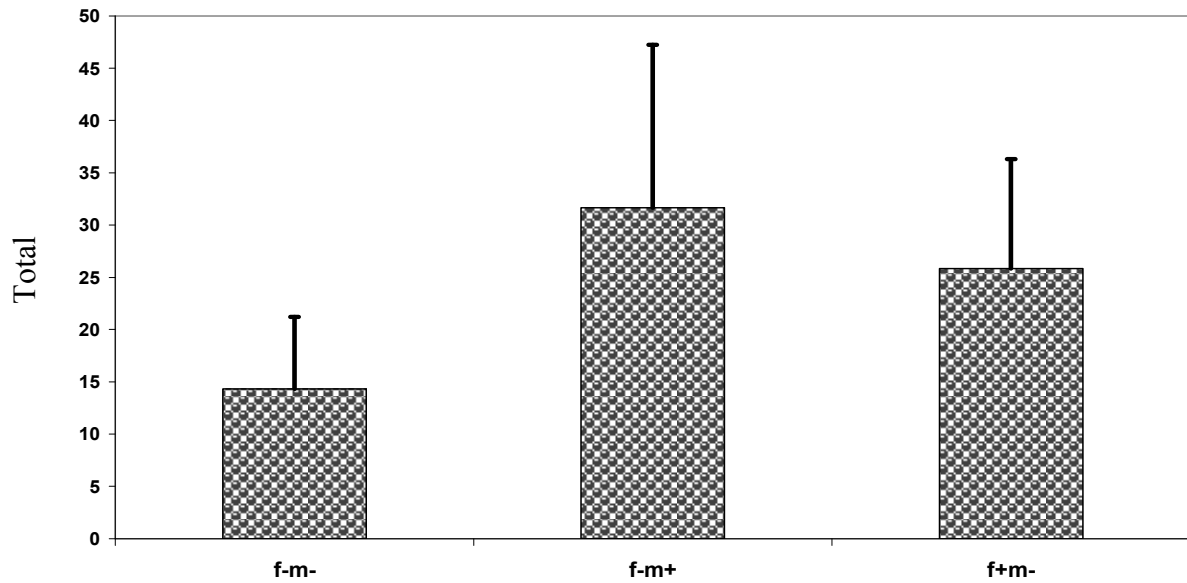


Fig. 2. Comparison of mean of third factor (frequency of head entrance to hole)on Open Field behavioral procedure of female newborns

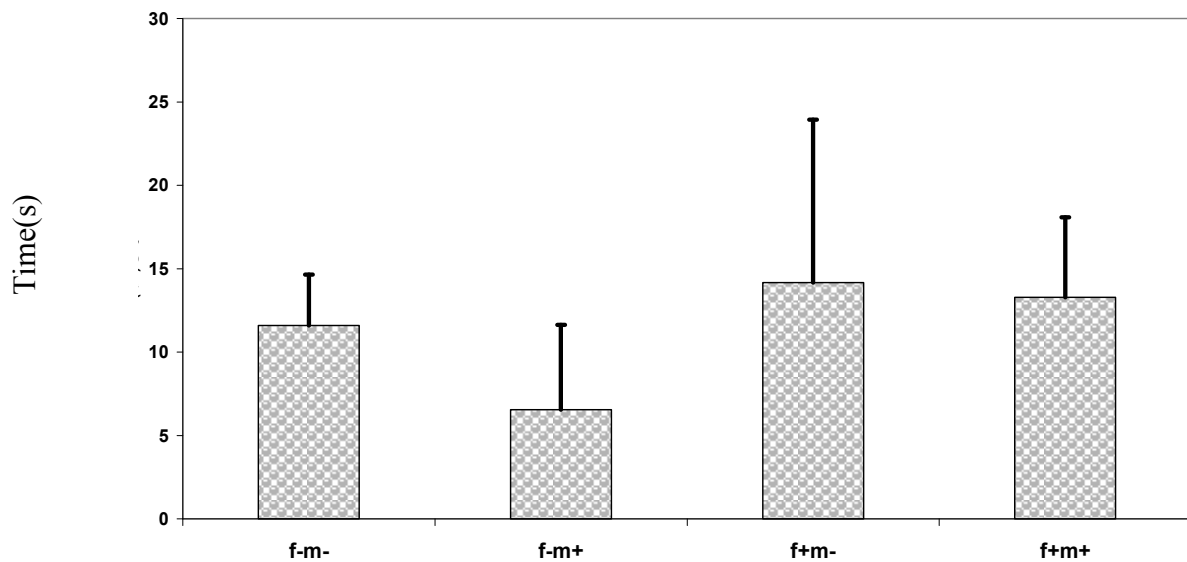


Fig. 3 Comparison of mean of fifth factor (frequency of standing)on Open Field behavioral procedure of female newborns

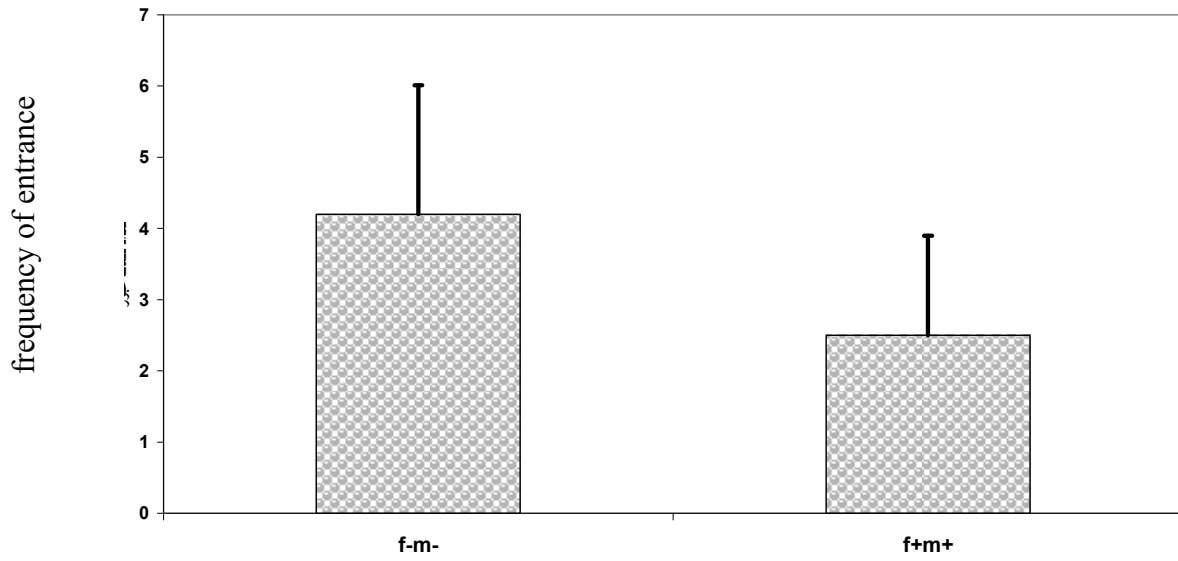


Fig. 4. Comparison of mean of second factor (frequency of entrance to open arm) on Open Field behavioral procedure of male newborns

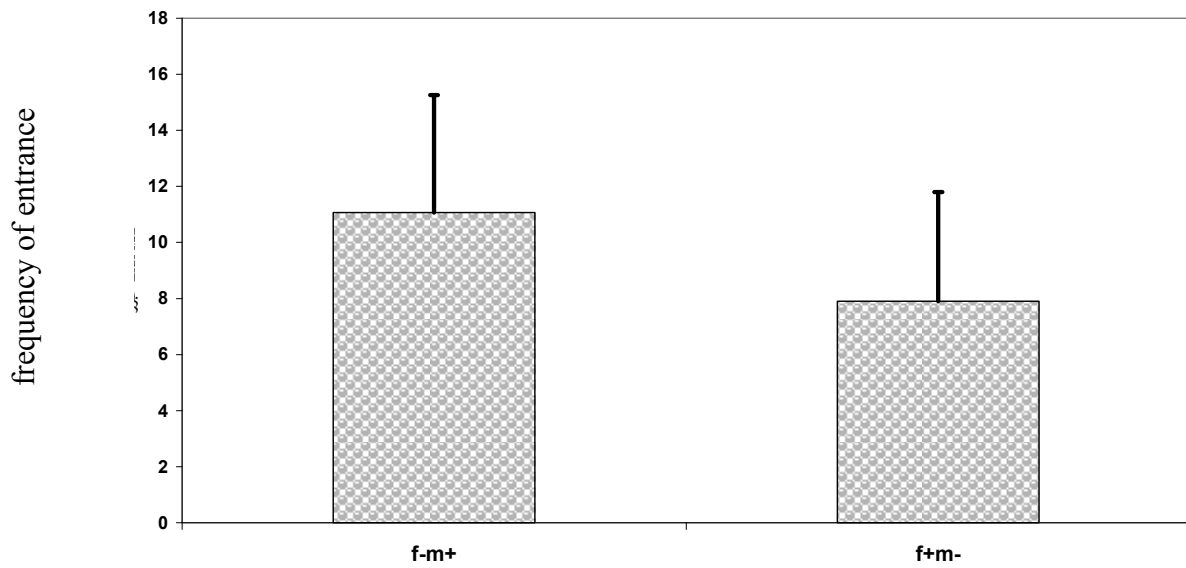


Fig. 5. Comparison of mean of first factor (frequency of entrance to closed arm) on Open Field behavioral procedure of female newborns

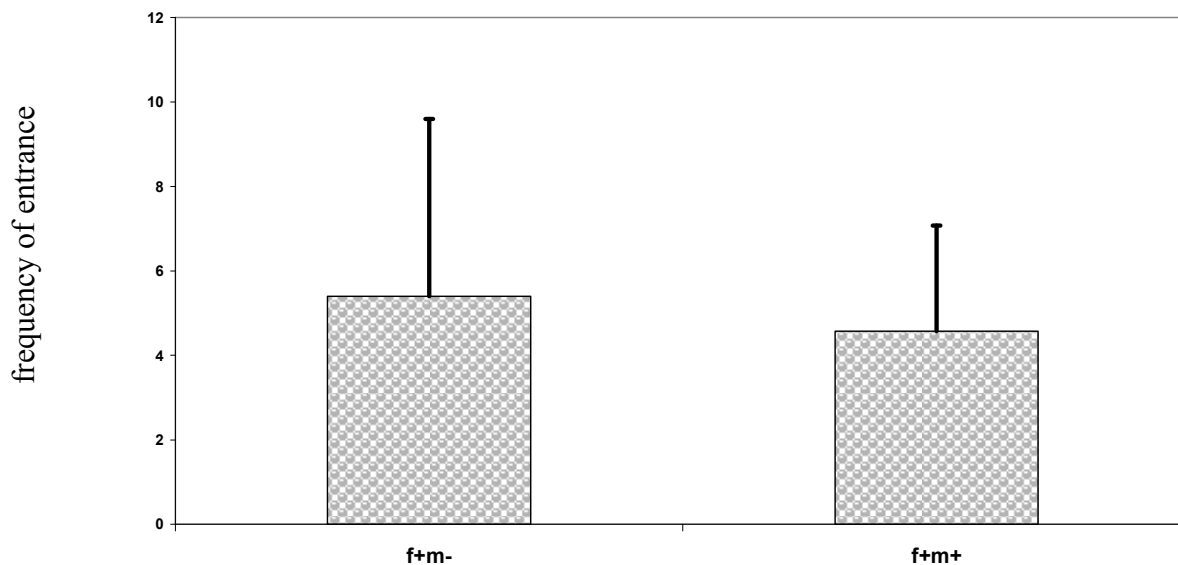


Fig. 6. Comparison of mean of second factor (frequency of entrance to open arm) on Open Field behavioral procedure of female newborns

Discussion

As a biologic view very extensive receptors and opioidergic synapses in the brain on one side and behavioral difficulties which are produced by minimal changes at the level of neuromediator and neuromodulator on the other side, confront us with a complex phenomenon. In addition, existence of natural opioid synapses for normal functions related to reward and punish system is obvious. Complexity of opioids role increases by interference of other neurotransmitters and catecholamines. Extensive searches have been done or in the way for study of effects of parental addiction on physiologic and psychologist behaviors of offsprings. In this search, our main goal have been to study the possible effects of male addiction on behaviors of first generation.

So the male have not any direct role on self offsprings after fertilization, finding analysis will be difficult and need to extensive molecular and biochemical studies.

This study is the continuation of other searches such as molecular studies. As mentioned above, continuation of animals with a stressful environment will produce emotional behaviors. C.S. Hall suggested that measuring the behavior of mice in a closed and unfamiliar environment will produce emotional behaviors. Specially he believes that high defecation and low activity show fear and reduction of exploratory behaviors of animals. So it is a good procedure for measuring of emotion. Findings of test showed the significant behavioral changes on male and female offsprings of both addicted parents in comparison to other groups. Both sexes offsprings of addicted mothers showed behavioral changes rather than nonaddicted mothers group too. But in chart 2-3 related to of tests meaningful behavioral changes of female offspring of addicted fathers are seen in comparison to control group.

That is female offsprings of addicted fathers, spent more time for grooming and many times for standing on feet. Analysis of these behaviors show that in unfamiliar environment, offspring, have more motor actions and more capability of risk acceptance.

EPM test is a procedure for behavioral study on very stressful condition in this tests not only unfamiliar conditions or exploratory reactions, but produced elevation and scrutiny are other

criteria in this test open arms are related to security and fear reduction (11-12) EPM have cleared that male newborns of F⁺M⁺ in comparison to F⁻M⁻ have fewer entrance to open arm (chart 4 EPM) .

These findings show that they spend more times on shut arms . chart 6 of this test shows that female offsprings of F⁺M⁺ spend more time on shut arm for decreasing the anxiety and increasing the security . these findings confirm the findings of other researchers about the role of addicted females on behaviors of offsprings . But which is notable in the findings of this study is the effect of addicted parents on female offsprings .

If female offsprings have more tolerance than males . It is no understood yet . sympathetic and parasympathetic systems are responsible for emotional behaviors this traditional opinion but sexual dimorphism is a phenomenon that can affect the behaviors . so role of addicted male on genetic differences will be prominent . so we can suggest that morphine may affect the Genes of offsprings . in addition , wall et al , believe that functions of D₁, and D₂ receptors on infralimbic structures affect on emotional reactions in EPM test (13). Aliana et al also believed that 5HT_{2C} receptors affect the emotional behavior of animal in EPM tests. so because of above opinions , possibility of morphine affection on gene will be a prominent suggestion. in addition morphine consumption in wistar rat increase the melatonin density (14) . This increase will change the sexual dysfunction in parents and also affect the sex cells specialty in the first or second generations . prinik et al suggested that even one dose 10 $\frac{mg}{kg}$ morphine consumption can affect the Genes. Susanne et al also suggested that Gene in some animals as Rat will be more active after morphine consumption (15) . So we can define that behavioral changes in offsprings will occur after morphine consumption by parents . this is the result of data analysis from our findings and others which mentioned . more researches are necessary for finding the exact mechanism of the effects of parental addiction on behaviors of offsprings .

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