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Chronic opiate exposure in the male rat adversely affects fertility

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Abstract

We examined whether morphine administration to adult male rats adversely affected pregnancy outcome after mating with drug-naive females and at what point in the complex series of steps leading to viable offspring it exerted its actions. The results indicate that chronic paternal morphine exposure markedly influenced fertility measures in a number of important ways. There was a pronounced increase in pseudopregnancies in females mated with males treated chronically with morphine (40%) when compared to controls (< 6%), indicating that vaginal penetration occurred, but successful impregnation failed; only 33% of matings between drug-naive females and morphine-treated males resulted in pregnancies, as compared to 74.5% in controls. In addition, there were fewer implantation sites in gravid females mated with morphine-treated males than in controls. Taken together, these observations suggest that morphine-exposed male rats were apparently able to copulate, but there was a failure in successful impregnation of the females. These findings suggest a primary defect in either the quality of male sexual behavior or a complete failure of the fertilization or conception processes in females mated with morphine-exposed males. This potentially important effect of paternal morphine administration on conception and/or preimplementation loss of embryos has not been previously noted and deserves more systematic study.

Keywords: Reproductive function; Morphine and reproduction; Paternal opiate administration; Opiates; Paternal influences; Opiates and reproduction

1. Introduction

It is well established that the administration of opiates and other abused substances to pregnant females produces adverse physiological and behavioral effects on their offspring (Allebeck and Olsen, 1998; Mattson and Riley, 1998; Smeriglio and Wilcox, 1999). In contrast to these results, there are few controlled studies of the effects of paternal drug exposure on reproduction and the development of viable offspring. In the clinical literature, behavioral and physiological deficits, including increased risk for drug abuse, have been documented in the children of fathers with a history of substance abuse, particularly alcoholism (Begleiter and Porjesz, 1988; Tarter et al., 1974, 1989). However, it is unclear whether these effects are the result of specific genetic differences, the direct biological effects of drug exposure prior to conception, socioeconomic and other environmental factors, or a complex interaction between all of the aforementioned variables. An animal model eliminates most of these confounding factors and allows for a more focused assessment of the direct, biological effects of drug exposure on conception and the generation of viable offspring.

Utilizing animal models, several investigators have shown that exposure of adult or adolescent male rodents to chronic opiate administration leads to adverse effects on the development of the offspring resulting from mating with drug-naive females (Cicero et al., 1991, 1995; Friedler and Cicero, 1987; Joffe and Soyka, 1982; Smith and Joffe, 1974). These effects include cognitive, physiological, and endocrine deficits and an altered pharmacological response to opiates. However, these deficits are generally not robust, vary markedly from one study to another, and independent replication has proven to be difficult. Thus, while an extensive amount of data suggests that paternal opiate or other drug exposure may have adverse effects on the normal development of offspring, it has been unusually difficult to characterize these effects and, most importantly, to elucidate

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the mechanisms that might be involved. What is particularly striking in this literature is the absence of a careful and systematic analysis of whether chronic paternal opiate administration influences the most important aspect of reproductive and fetal development: the conception of viable offspring.

It is well known that opiates influence sexual drive in male humans and rodents (Agmo and Paredes, 1988; Mumford and Kumar, 1979; Tokunaga et al., 1977), but what is not clear is whether chronic opiate administration influences species-specific sexual behavior that leads to successful fertilization and the subsequent development of hearty offspring. In the present studies, we have examined the influence of chronic paternal morphine administration on mating, fertilization, and the development of viable fetuses in male Sprague–Dawley rats. In the rat, the conception process is highly complex (Adler and Toner, 1986; Eddy, 1988; Lamb, 1988) and drug-induced insults can markedly disrupt one or more crucial steps in the sequence of events leading to pregnancy and the generation of viable offspring. Briefly, copulation must occur, but the entire mating sequence is crucial for successful fertilization, including ejaculation and the formation of a vaginal plug. Once these events occur, fertilization can take place and the embryo undergoes cell division for 4–5 days prior to implantation into the uterine wall. After implantation, resorption of the fetus is possible up to approximately the 14th day of pregnancy; thereafter, any abnormal fetuses are spontaneously aborted. There is ample evidence in the literature that drugs and environmental toxins can disrupt several points in the process leading to healthy offspring, including mating behavior and significant preimplantation or postimplantation loss of offspring (Anderson et al., 1983; Cohen, 1986; Eddy, 1988; Lamb, 1988; Matthews and Adler, 1978; Narod et al., 1988; Niswender and Nett, 1988). We are unaware of any studies in which the influence of chronic paternal morphine administration on the complex processes leading to favorable pregnancy outcome has been examined.

In the present studies, we have examined whether morphine administration to adult male rats adversely affects pregnancy outcome and at what point in the complex series of steps leading to viable offspring it exerts its effects.

2. Materials and methods

2.1. Animals and materials

Male and female Sprague–Dawley rats, 60–80 days of age, were obtained from Harlan Sprague–Dawley (Indianapolis, IN). The rats were housed singly in a room with a 12:12 light/dark cycle. Morphine sulfate was generously provided by the National Institute on Drug Abuse. All animal-related protocols were approved by the Institutional Animal Care and Use Committee.

2.2. Chronic opiate exposure and breeding protocol

Male rats were administered morphine ($n=99$) or saline ($n=51$) for 14 days in several replications. On Day 1, 10 mg/kg morphine or saline was injected subcutaneously twice daily with the injections 9 h apart. The morphine dose was increased daily by 5-mg/kg increments per injection until a maximum of 30 mg/kg twice daily was achieved. The rats were continued at 30 mg/kg twice daily until Day 14. On the afternoon of Day 14, the dosage was decreased to 20 mg/kg twice daily for the breeding period, which occurred on Days 15 and 16. This reduction in dose was done, on the basis of pilot studies, to minimize morphine's direct influence on sexual drive without inducing the development of withdrawal syndrome and the associated stress during breeding, which could be a confounding factor in these studies. Males were bred on the evening of Day 15.

Vaginal cytology was performed on 176 virgin, drug-naive female rats for at least two consecutive estrous cycles before inclusion in the study. If a female entered pseudopregnancy during vaginal cytology in the first estrous cycle, but then had a complete estrous in the second cycle, they were included in the study. Those females (26 of 176) who did not resume normal cyclicity (i.e., pseudopregnancy) were excluded from the study. Vaginal smears were collected each day between 09:00 and 10:00 h using 6-in. cotton-tipped applicators slightly moistened with sterile saline. The swab was then rolled onto a microscope slide. After drying, the slide was fixed in 90% methanol and stained with a modified Wright's stain to assess the stages of the estrous cycle.

Males were placed in the female's cage at late proestrus (12 h after the designation of proestrus by vaginal cytology) and were left with the female for a maximum of 48 h. Vaginal lavage was performed on the mornings of the first and second day after mating by well-established procedures (Lamb, 1988). A vaginal plug or a positive vaginal lavage was designated as Day 0 of pregnancy, and the male was removed. If mating was not evident (two negative vaginal lavages), the male was removed after 48 h, and this was designated as Day 0 of pregnancy for purposes of assessing male and female fertility parameters. Vaginal lavage was performed by inserting a tuberculin syringe filled with 0.1–0.2 ml of sterile saline and repeatedly (two or three times) filling the vagina and aspirating the fluid into the syringe. The lavage fluid was placed onto a microscope slide and examined under a microscope for the presence of sperm (Lamb, 1988).

2.3. Assessment of male fertility parameters

In a number of morphine-exposed and control males ($n=19$ in each group), fertility parameters were assessed. Males were euthanized by exposure to CO_2 at the termination of the breeding period. The left testis and epididymis,

Table 1
Mean (±S.E.M.) sperm counts in rats treated with morphine compared to saline-treated control rats

Sperm count		
Organ	Morphine	Saline
Testes	2.10×10^8 ($\pm 4.43 \times 10^7$)	1.55×10^8 ($\pm 9.43 \times 10^6$)
Cranial epididymus	8.60×10^7 ($\pm 4.45 \times 10^6$)	9.66×10^7 ($\pm 8.88 \times 10^6$)
Caudal epididymus	5.80×10^7 ($\pm 4.14 \times 10^6$)	5.30×10^7 ($\pm 5.02 \times 10^6$)

seminal vesicles, and prostate were removed, weighed, and immersion-fixed in Bouin's solution for 24 h. The tissues were then repeatedly rinsed in alcohol, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Sections were examined qualitatively under light microscopy.

The right cranial and caudal epididymis and right testis were also removed. The testes, a 1-cm section of the caudal epididymis and a 1.5-cm section of the cranial epididymis, were weighed. Sperm were extruded from the epididymis, placed on a microscopic slide, and sperm motility was assessed. Each tissue was then homogenized using a Polytron homogenizer. Sperm heads were counted in each sample using a hemocytometer. Sperm counts were compared as counts per organ and as counts per milligram of organ weight. Smears of caudal epididymal fluid were fixed in methanol, stained using Giemsa stain, and sperm morphology was assessed for evidence of sperm abnormalities. The number of abnormal sperm per 100 was recorded.

2.4. Assessment of female fertility and fetal outcome

All of the females mated with morphine-exposed or control (i.e., saline-injected) males were sacrificed 14 days after sperm were detected by vaginal lavage or after the 48-h breeding period without a positive lavage. The purpose for killing the females at Day 14, rather than waiting until delivery, was to assess pseudopregnancy and fetal resorptions, which can be reliably detected only at Days 12–15 of pregnancy in the rat. At the time of sacrifice, the females were anesthetized with methohexital (40 mg/kg ip). After anesthesia was achieved, the heart was exposed via a lateral thoractomy approach; 0.3–0.5 cc of 1% Evans blue solution was injected into the left ventricle to help visualize implantation sites and corpora lutea. After 15 min, the uterus, with the ovaries still attached, was harvested. A silk ligature was placed around the right horn of the uterus, excess fat was trimmed away, the uterus and ovaries were placed in 10% formalin and fixed for a minimum of 7 days.

With the aid of a dissecting microscope, extraneous fat was dissected from the ovaries and the uterus. Right and left ovaries were kept separate to correlate the number of primary corpora lutea to the number of implantation sites. The ovaries and uterus were blotted dry and weighed to the nearest 0.1 mg. After weighing, the character of the uterus was noted (i.e., pregnant; fluid filled, indicating pseudopregnancy; or normal) and the number of implantation sites, resorptions, and fetuses were counted in each horn. The fecundity index was defined as the percentage of pregnancies resulting from confirmed matings while the fertility index was the percentage of pregnancies resulting from total cohabitations regardless of confirmation of mating (Wise et al., 1994).

Fetuses from the pregnant rats were removed, blotted dry, weighed to the nearest 0.1 mg, and examined for any signs of abnormalities. All corpora lutea were dissected out of the ovaries under a dissecting microscope and were then weighed to the nearest 0.1 mg.

2.5. Statistical analysis

Statistically significant differences in fecundity and fertility indices and pseudopregnancy rates in the females were determined using simple and complex chi-squares (Bruning and Kintz, 1977). The remaining data assessing male and female fertility and fetal outcome was analyzed using a standardized Student's *t* test.

3. Results

3.1. Effects of chronic morphine exposure on male fertility

There were no significant differences in sperm motility detected in the male rats exposed chronically to morphine when compared to saline-injected controls (data not shown). There were also no significant differences in the sperm counts from the cranial and caudal epididymis or testis (Table 1). Assessment of sperm morphology yielded no significant differences in abnormalities between groups (data not shown). As shown in Table 2, prostate and seminal vesicle weights were significantly lower in morphine-treated rats than in controls. There was no significant difference in testis weight. Histological evaluation of testes, prostate, seminal vesicles, and epididymis showed no significant abnormalities. Mature sperm were found in numerous tubules in both groups. Qualitatively, there was no difference in primary or secondary spermatocytes or spermatogonia between groups. Leydig cells were normal in size and

Table 2
Effects of morphine on wet weights of testes, prostate, and seminal vesicles

Organ	Morphine	Saline
Testes (mg)	1789 ± 30	1793 ± 30
Prostate (mg)	581 ± 29*	877 ± 44
Seminal vesicles (mg)	793 ± 56**	1005 ± 70

* Significantly different ($P < .001$) than in saline-exposed males.
** Significantly different ($P < .05$) than in saline-exposed males.

Table 3
Reproductive and fetal parameters in response to chronic paternal opiate exposure

Parameter	Morphine	Saline
Cohabitations	99	51
Pregnancies	33	38
Positive vaginal lavages	38	36
Pregnancies with positive lavages	31	35
Fecundity Index	81.60%*	97.20%
Pseudopregnancies	40*	3
Number primary CL[a]/rat	18.84 ± 0.52	20.50 ± 0.55
Number implantations/rat	14.16 ± 0.64*	16.06 ± 0.44
Litter size/rat	13.13 ± 0.66	14.22 ± 0.59
Number resorptions/rat	1.10 ± 0.18	2.38 ± 0.50
Ratio of primary CL[a]: implantations	1.53 ± 0.17	1.31 ± 0.06
Ratio of implantations: fetuses	1.12 ± 0.03	1.24 ± 0.10
Mean fetal weight/litter (mg)	166.19 ± 4.50 mg	158.70 ± 1.90 mg

[a] CL denotes corpora lutea.
* Significantly ($P < .05$) different than in females mated with saline-exposed males.

distribution. There was no evidence of epithelial atrophy or edema in any of the accessory sex glands. The lumens of the epididymis of all animals contained morphologically normal, mature sperm.

3.2. Effects of chronic paternal opiate exposure on female fertility and fetal outcome

The data in this section represents the pooled results of several studies of chronic paternal opiate exposure: A total of 99 males were treated with morphine and 51 males with saline as described in Section 2. Vaginal cytology performed prior to mating revealed a mean estrous cycle length of 4.81 (± 0.04) days in the total of 150 females used in these studies (i.e., those mated with morphine- or saline-exposed males).

The outcome of the matings of male rats, chronically exposed to opiates or saline, with drug-naive females is shown in Table 3 and Fig. 1. As can be seen in this table and figure, 33 pregnancies resulted from 99 matings of morphine-treated males to drug-naive female rats, as compared to 38 pregnancies out of 51 matings in controls. The fecundity index, or pregnancies resulting from confirmed matings, of morphine-treated males (81.6%) was significantly lower than in controls (97.2%). Additionally, 40.4% of the females mated with morphine-treated males were found to be pseudopregnant, which was nearly 10 times higher than that found in controls (5.6%).

As shown in Table 3, the only significant difference between females mated with morphine-treated males when compared to controls in a number of reproductive param-

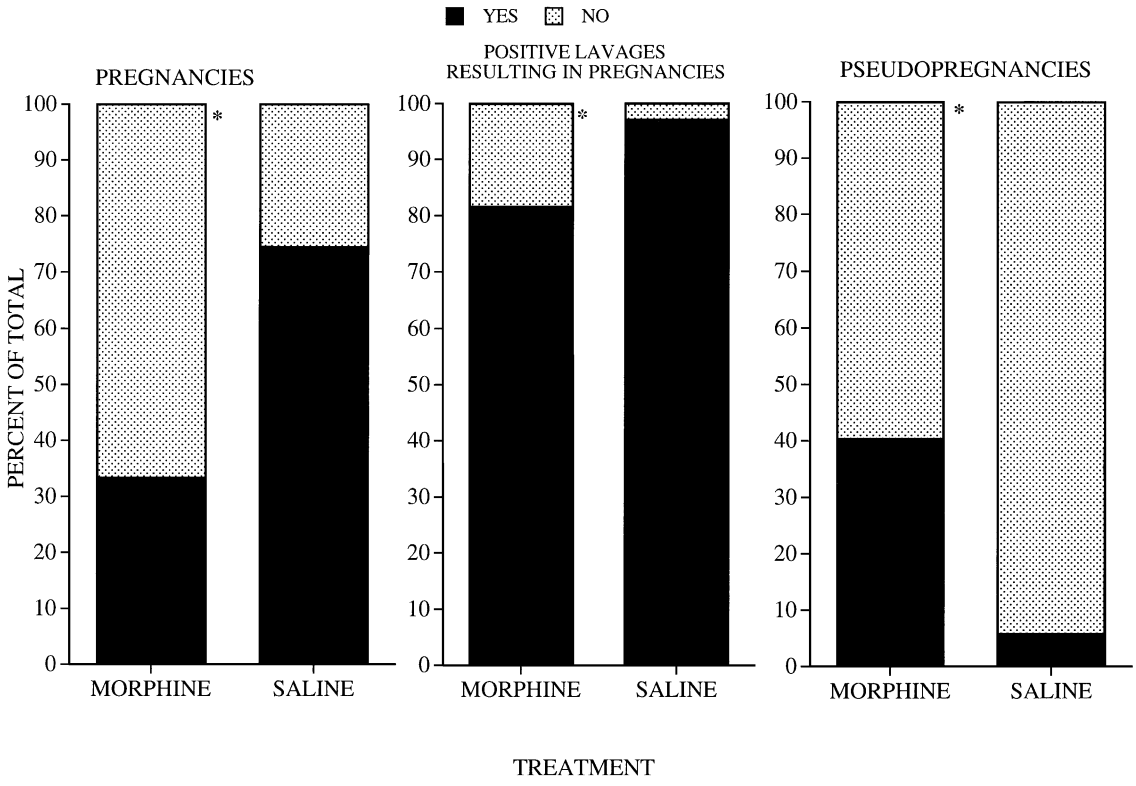

Fig. 1. Percent of females, mated with morphine- or saline-exposed males, in which pregnancies occurred (left panel), with pregnancies in the face of positive vaginal lavage (center panel), and with pseudopregnancies (right panel). See Table 3 for raw data.

eters was in the total number of implantation sites, which was significantly lower in females mated with morphine-treated males. Although the number of primary corpora lutea per rat in females and the total number of morphine derived fetuses were lower than that observed in controls these differences were not statistically significant. Similarly, there were no other statistically significant differences found in any other aspect of reproductive and fetal parameters (Table 3).

Physical examination of the surviving offspring of males chronically exposed to morphine resulted in no significant differences in fetal weight (Table 3) and no observable signs of deformations.

4. Discussion

The results of these studies indicate that chronic morphine exposure to male rats markedly influences fertility measures in matings with drug-naive females. The principal observations were: First, there was a pronounced increase in pseudopregnancies in females mated with males treated chronically with morphine when compared to controls; second, the number of pregnancies resulting from mating drug-naive females with morphine-treated males was significantly less than in controls; and, finally, there were also fewer implantation sites in females mated with morphine-treated males than in controls. Taken together, these observations suggest that the chronic morphine regimen used in these studies did not influence vaginal penetration by male rats, but markedly reduced successful conception. Specifically, the very large numbers of pseudopregnancies in female rats mated with males treated chronically with morphine indicate that vaginal penetration occurred since pseudopregnancies occur only as a result of vaginal stimulation. Thus, the males were apparently able to copulate, but there was a failure to successfully impregnate the females as indicated by the very low incidence of pregnancies in females mated with morphine-exposed males (33%) when compared to controls (74.5%). Moreover, the lower number of implantation sites in females mated with morphine-treated male rates suggests a primary defect in the ability of sperm to fertilize eggs in females or that there was significant preimplantation fetal loss in females mated with morphine-treated males.

Interestingly, there were no apparent differences in postimplantation loss since the number of resorption sites in females mated with morphine-exposed males did not differ from that observed in controls. The latter observations suggest that if copulation, fertilization, and implantation occurred, there was little subsequent effect of paternal opiate administration on fetal outcome. Collectively, these data suggest that the principal effect of paternal opiate administration is on fertility and/or preimplantation processes that lead to poor pregnancy outcome in drug-naive females.

Since motility and sperm counts from the testes and caudal and cranial epididymis were not affected by morphine treatment, and neither was sperm morphology, it appears that gross aberrations in sperm function cannot explain the low pregnancy rate in females mated with morphine-treated males. One potential explanation of these findings is that morphine influenced the secretions of the secondary sex organs, which play a pivotal role in fertilization. Seminal vesicle secretions provide a transport medium for sperm, which facilitate cervical passage of spermatozoa into the uterus (Blandau, 1945; Carballada and Esponda, 1993; Matthews and Adler, 1978) and, in combination with secretions from the prostate and coagulating glands, leads to the formation of the copulatory plug in the vagina of the female to prevent outflow of sperm. The possibility that morphine influenced the formation of a functional copulatory plug needs to be considered in view of recent observations (Carballada and Esponda, 1993) in which it was found that the formation of copulatory plugs by male rats, with partial surgical resection of the seminal vesicles, was cupped at the cervical end of the vagina. This ''cup'' was filled with spermatozoa and sperm, which could not be isolated from vaginal lavage after removal of the plug, indicating that ejaculation occurred but that the plugs prevented sperm from entering the vagina. The fact that we did not find positive lavages in rats in pseudopregnancy correlates well with findings of negative lavages with malformed plugs. As such, our results could indicate that a reduction in seminal vesicle secretions induced by chronic morphine administration may have led to a nonfunctional copulatory plug, which thereby prevented sperm from entering the vaginal tract, resulting in high pseudopregnancies and a lack of successful fertilization. While we know of no direct evidence that morphine adversely effects the formation of a vaginal plug, the findings that chronic morphine administration reduces the weights and secretions of the secondary sex organs in rats (Cicero et al., 1975a) and human males exposed chronically to methadone (Cicero et al., 1975b) is consistent with this interpretation.

In addition to an increased rate of pseudopregnancies, fewer total pregnancies resulted from morphine-treated males mated to drug-naive females compared to controls. Seven of the 38 morphine matings confirmed by the detection of sperm in the vagina did not result in pregnancy compared to only one of the 36 control matings that did not yield a pregnancy, resulting in a substantial difference in fecundity indices (81.6% vs. 97.2% in morphine-treated and control males, respectively). These findings indicate a complete conception failure in these females. There are three possible explanations for these findings. First, although sperm motility and counts in the primary and secondary sex organs were normal, we were unable to measure the actual volume of the ejaculate and the number or motility of sperm contained therein. It is possible, therefore, that there were insufficient viable and motile sperm to successfully impregnate females mated with mor-

phine-exposed males. Second, while we found evidence of vaginal penetration, as indicated by markedly enhanced pseudopregnancies in females mated with morphine-exposed males, it is possible that the frequency and quality of mating behavior — which are critical in the conception process — were reduced in male rats chronically treated with morphine. Fourth, it is also possible that female rats reacted differently to morphine-treated rats than controls in terms of successful copulatory behavior leading to a conception failure. Finally, it is possible that fetal loss occurred at the conception phase or was the outcome of preimplantation loss of a nonviable fetus. Further studies will be needed to explore which of these interpretations are correct.

No matter which of the foregoing explanations are correct, our data strongly suggest that if conception and fetal implantation in the uterine wall occurred, there was little effect on fetal outcome, since we observed no differences in resorption sites or a corresponding decrease in the number of fetuses in females mated with morphine-exposed males. These data suggest that the primary adverse effect of morphine on successful pregnancy outcome is at the preimplantation level (i.e., either a complete conception failure or loss of a nonviable fetus prior to implantation into the uterine wall).

An additional possibility to explain the much higher incidence rate of pseudopregnancies, in the absence of spermatozoa in the vagina, is that morphine induced subtle alterations in the male's sexual behavior. Specifically, it is clear that a female rat's cervix must be stimulated to induce pseudopregnancy (Niswender and Nett, 1988); this process requires intromission, but not necessarily ejaculation. Thus, our results could suggest that males copulated, but that for reasons that are not clear, they failed to ejaculate. If this occurred, this could account for the high number of pseudopregnancies and the absence of sperm in the vaginal tract. Although we and others have previously shown that large doses of morphine do not affect mating and ejaculation (Agmo and Paredes, 1988; Cicero et al., 1995), we know of no studies that have systematically examined the effects of chronic morphine administration on copulation and ejaculatory behavior in male rats.

Other studies that have examined the influence of morphine on pregnancy outcome have had variable results. Friedler (1985) reported no effect of paternal opioid exposure on reproductive indices such as fertility, gestation period, sex ratio, litter size, and stillbirths. Additionally, in previous studies (Cicero et al., 1991), we found no effects of chronic paternal exposure on reproductive and fetal outcome parameters such as the fertility index, fetal sex ratio, stillbirths, and fetal and neonatal growth rates. However, it should be noted that in all previous studies, the males were exposed to morphine for a prolonged period, but then drug administration was terminated for 3–7 days prior to the actual mating period. Thus, only the residual effects of chronic morphine exposure were examined in contrast to the present studies in which morphine was given both prior to and during mating. The contrasting age of drug exposure and time between exposure and mating difference between these studies and the present results could explain the differences in recorded effects.

On the basis of the results in this paper, it is clear that extremely important preimplantation effects of paternal morphine administration occur in rats treated chronically with morphine, which have not previously been noted. These effects need to be examined more systematically to elucidate mechanisms involved in these significant reproductive deficits. Moreover, our results also suggest that the implications of morphine- or heroin-induced defects in reproductive function (i.e., failure to impregnate and/or conceive) need to be examined in human drug abusers.

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