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Prenatal Stress Alters Morphine- and Stress-Induced Analgesia in Male and Female Rats

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KINSLEY, C. H., P. E. MANN AND R. S. BRIDGES. *Prenatal stress alters morphine- and stress-induced analgesia in male and female rats.* PHARMACOL BIOCHEM BEHAV **30**(1) 123–128, 1988.—Prenatal stress affects the expression of many opioid-regulated behaviors in adulthood, e.g., aggressive, maternal, regulatory, and sexual. In the present report we examined two forms of analgesia, morphine-induced (opioid receptor-mediated), and stress-induced [cold-water swim (CWS), nonopioid] analgesia in adult prenatally-stressed (P-S) male and female rats to determine whether and to what extent these analgesic responses might be altered. Timed-mated Sprague-Dawley females were exposed to heat and restraint stress (three daily 1/2 hour sessions, 0830, 1230, and 1630 hr) from days 15–22 of gestation. Control animals remained undisturbed throughout pregnancy. Between 120–150 days of age, baseline pain sensitivities were determined using a tail-flick monitor. P-S and Control animals were then exposed to 3.5 min cold-water swims (2°C) and pain thresholds were again determined at 30 min intervals for 120 min. P-S females exhibited significantly lower pain thresholds than Control females at the 30 and 60 min marks, whereas P-S and Control males did not differ. Six to eight days later, analgesia was measured for 180 min following morphine (5.0 mg/kg) administration. P-S females exhibited significantly greater analgesia at each time-point after morphine treatment than Controls. Conversely, P-S males were significantly less analgesic than Control males from 60 to 180 min. These data suggest that prenatal stress alters the status of endogenous opiate systems. Such prenatal stress-induced alterations in opiate function may help account for some of the behavioral effects reported in P-S animals.

Prenatal stress Opiates Sexual differentiation

PRENATAL stress disrupts sexual differentiation in rodents. Of the behaviors reported to be affected by prenatal stress, sexually dimorphic behaviors appear particularly vulnerable. For instance, when exposed to heat and restraint stress in utero, male rat offspring are demasculinized and feminized with respect to copulatory behavior [43], and, in mice, show a number of similar effects on the male-typical expression of behaviors like intermale aggression [28]. Recently, we demonstrated that intact prenatally-stressed (P-S) male rats exhibit enhanced maternal behavior toward neonates (i.e., shorter latencies to respond to young), relative to nonstressed Control males [27].

In females, the behavioral effects of prenatal stress are less apparent. Generally, however, the effects are associated with reductions in nestbuilding and pregnancy-induced aggression, and elevations in postpartum aggression and male sex ratio in mice and, in rats, reductions in fertility and fecundity and postpartum maternal behavior [12, 19, 29–31]. Prenatal stress also affects the maternal behavior of intact nulliparous female rats, with P-S females exhibiting longer latencies than Control females to respond to young [27]. Many of the effects reported in P-S females are similar to those reported for perinatally-androgenized females [17,18] and Herrenkohl and Scott [20] provided data to support that hypothesis, demonstrating a number of parallels between the two types of females.

The effects of prenatal stress have many features in common with the behavioral and physiological actions of opiates and endogenous opioids. This system modulates a wide variety of physiological and behavioral variables in both the male and the female, in particular, sexually dimorphic behaviors such as sexual, maternal, and aggressive [1, 6, 9, 14, 22]. It has recently been shown that the ontogeny of the mu (μ) type opioid receptor system follows a pattern of organization similar to that for the development of other sexually dimorphic systems [16], since testosterone (T) administration suppresses, and neonatal castration promotes, the development of the female pattern of opioid receptor formation. Because

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prenatal stress is believed to disrupt sexual differentiation through alterations of prenatal hormone activity [44,45], is associated with effects in the female which resemble perinatal androgenization [20], and alters other neurochemical activity [11,34], the present work was performed to investigate if and to what extent prenatal stress may disrupt some aspect of the development of opioid and nonopioid systems involved with analgesia in the adult animal.

METHOD

Animals

Female nulliparous Sprague-Dawley rats (CRL:CD(SD) BR) (Charles River Laboratories, Inc.) were timed-mated by placing them with stud males. The day that sperm was observed in the vaginal lavage was designated day 1 of pregnancy, and the females were isolated in 20×45×25 cm polypropylene cages, the floors of which were covered with wooden shavings. Food (Purina rat chow) and water were available ad lib and all animals were kept in light- (on from 0500–1900 hr) and temperature-(21–24°C) controlled testing rooms. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council [DHHS publication No. (NIH) 85-23, Revised, 1985].

Prenatal Stress Procedure

On the morning of gestation day 15, females were randomly assigned to one of two groups. One group [designated the prenatally-stressed (P-S) group] of pregnant animals (N=15) was exposed to a regimen of heat and restraint stress comprised of placing the female into a 6¾ in. Plexiglas restraint tube over which were poised two 100-watt flood lights. The stress procedure, which produces 350 foot candles of illumination and an ambient temperature within the restraint tube of approximately 38°C, began on day 15 of gestation and continued through day 22. There were three 30-minute stress sessions conducted each day at 0830, and 1230 and 1630 hr. The second group of females (designated the nonstressed Control group; N=15) was left undisturbed for the duration of their pregnancies. Following parturition the offspring from both Stressed and nonstressed Control mothers were cross-fostered to recently parturient dams. Litters were adjusted to 8–10 pups of both sexes and were weaned at 25 days of age. The animals were maintained in groups of 3–5 from the time of weaning to the time of isolation and testing. No more than two animals of either sex from any individual litter were used in the present experiments.

Tail-Flick Apparatus

The tail-flick monitor used in the present experiments consisted of a wooden platform under which was mounted a 150-watt projector light bulb which, when activated, shone through an aperture in the base of the platform producing both heat and light. The behavioral measure was the rapidity (latency in seconds) with which the tail was moved away from the noxious stimulus (intense heat). A minimum of three days of stable baseline tail-flick latencies were recorded for each animal prior to testing. Preventative measures were taken to preclude any tissue damage to the tail by stipulating an upper limit of six (6) seconds contact on the apparatus for cold-water swim, and eight (8) seconds for morphine-induced analgesia.

Cold-Water Swim (CWS) Procedure

Between 100–120 days of age, all animals were isolated. Females had vaginal smears taken daily. P-S and Control males, and females in diestrus, were placed in a plastic tank filled with ice water (2°C) for 3.5 min. Immediately following the CWS the animals were partially toweled-off and placed in holding cages until their respective tests for analgesia on the tail-flick monitor at 30, 60, 90, and 120 min postswim.

Morphine-Induced Analgesia

Six to seven days following CWS, all animals were assessed for morphine-induced analgesia. (Vaginal smears were taken daily after CWS to determine whether this acute stressor affected subsequent estrous cycles.) Prenatally-stressed and Control males and females (in diestrus) were weighed and treated with 5.0 mg/kg morphine sulfate SC. The dose was used because previous work from our laboratory has shown it to be effective in disrupting various maternal behaviors [9,14] and postpartum aggression [24]. Determinations of analgesia were taken at 30, 60, 90, 120, 150, and 180 min postinjection.

Statistical Analyses

For the purposes of the overall analysis, a two-way repeated measures analysis of variance (ANOVA) was employed, with Sex (male, female) by Treatment (prenatal stress, control) nested within the repeated measure, Time [30 min–120 min (Cold-Water Swim) and 30 min–180 min (Morphine Analgesia)]. In those cases where there was a significant interaction, Fisher's Least Significant Differences (LSD) test ($\alpha = p < 0.05$) was used to determine significance. A two-way ANOVA (Sex by Treatment) was used to analyze body weight, with post hoc comparisons performed with the LSD.

RESULTS

CWS Analgesia

Figure 1 portrays the mean change in tail-flick latencies for males and females following exposure to CWS. That significant differences in baseline pain thresholds were found between the males and females [males longer than females, $F(3,37)=12.01$, $p<0.001$] prior to CWS necessitated the use of difference scores in all analyses (Table 1). Difference scores were derived by subtracting each animal's baseline score from subsequent test scores. The repeated-measures ANOVA revealed a significant main effect of Time, $F(3,111)=49.65$, $p<0.001$. There was no main effect of Treatment or Sex, nor was any of the two-way interactions significant. The three-way interaction among Sex, Treatment and Time was significant, $F(3,111)=4.48$, $p<0.01$. Post hoc analyses with the LSD ($p<0.05$) indicated that at no time-point did P-S and Control males differ from each other. Both Control and P-S males showed significantly longer tail-flick latencies 30 min postswim, whereas only Control males displayed CWS analgesia 60 min postswim. Unlike males, Control and P-S females differed from each other at the 30 and 60 min time-points with the Control females displaying significantly longer tail-flick latencies. Both Control and P-S females displayed significantly longer tail-flick latencies relative to their baseline values at 30 min postswim, with Control females also displaying longer tail-flick latencies at 60 min.

Comparing across Sex, Control males had significantly

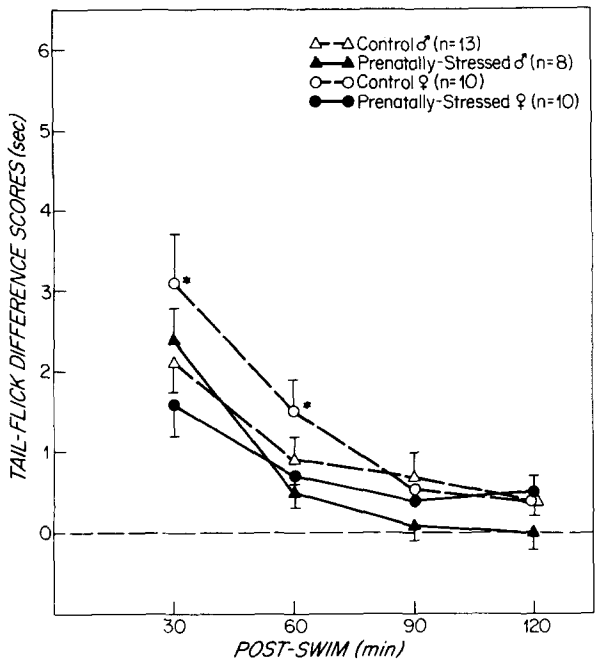

FIG. 1. Mean (±SEM) tail-flick latency difference scores for male and female prenatally-stressed (P-S) and Control rats following a 3.5 min immersion in a cold-water swim. *Significantly different ($p<0.05$), P-S females vs. Control females.

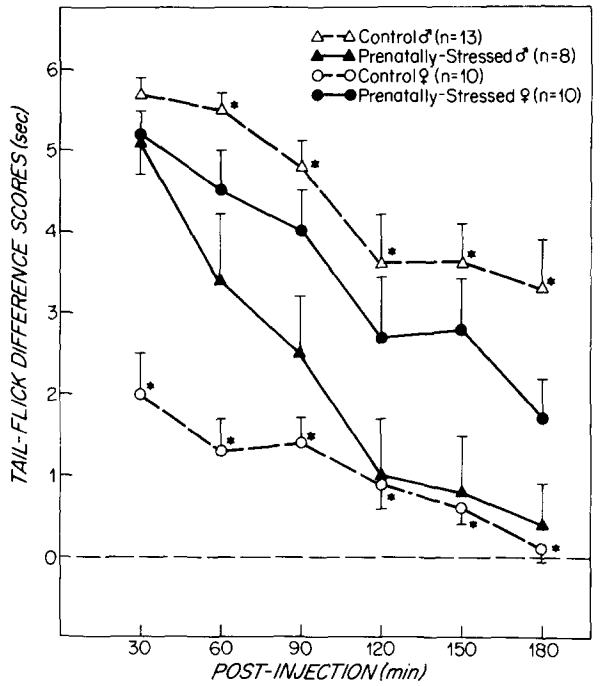

FIG. 2. Mean (±SEM) tail-flick latency difference scores for male and female prenatally-stressed and Control rats following an injection of 5.0 mg/kg morphine sulfate. *Significantly different ($p<0.01$), P-S vs. Control (within sex). See text for other significant comparisons.

shorter tail-flick latencies than Control females at 30 min. Prenatal stress reversed this effect as P-S males had significantly longer tail-flick latencies than P-S females at 30 min. Comparing across Sex and Treatment, Control females had significantly longer latencies than P-S males at 60 min only, whereas there was no difference between Control males and P-S females at any time-point postswim.

Morphine Analgesia

In the morphine-induced analgesia study, difference scores calculated from baseline values were again used for statistical analysis. Figure 2 portrays the tail-flick latency difference scores for P-S and Control males and females following treatment with 5.0 mg/kg morphine. Significant main effects were observed for Sex, $F(1,37)=11.95$, $p<0.01$, and Time, $F(6,222)=66.16$, $p<0.01$. The interaction between Sex and Treatment was significant, $F(1,37)=37.57$, $p<0.01$, as was Time by Treatment, $F(5,185)=3.01$, $p<0.05$. There was no main effect of Treatment, nor was the two-way interaction of Sex by Time and the three-way interaction of Treatment by Sex by Time significant. The post hoc analyses ($p<0.05$) revealed that there was no difference between P-S and Control males at 30 min. Beginning with the 60 min time-point, however, and continuing throughout the time course, P-S males showed significantly less analgesia than Control males, and returned to baseline values by 120 min, whereas Control males' latencies remained elevated beyond the 180 min mark. On the other hand, prenatal stress exerted the reverse effect in females. P-S females exhibited significantly longer tail-flick latencies than Controls beginning 30 min following the injection and at every time-point thereafter, and were elevated relative to their baseline values beyond 180 min. Control females returned to baseline levels within 120 min following the injection.

Comparing across Sex, Control males displayed markedly greater morphine analgesia than Control females for the duration of testing, with the males exhibiting latencies 3–4 seconds longer than Control females. Conversely, P-S females appeared more sensitive to morphine than P-S males, as they displayed a higher degree of analgesia beginning 90 min postinjection and continuing through 180 min. Comparing across Sex and Treatment, Control males had similar postinjection tail-flick latencies to P-S females differing only at the 180 min time-point. P-S males displayed significantly greater morphine analgesia than Control females only at 30 and 60 min postinjection.

Body Weights

Table 1 contains the body weight data for P-S and Control males and females. A two-way ANOVA indicated a significant main effect of Sex, $F(1,37)=218.31$, $p<0.0001$, as males were heavier than females. No main effect of Treatment was found. A significant two-way interaction of Sex by Treatment was found, $F(1,37)=7.81$, $p<0.01$. Follow-up analysis with the LSD revealed that P-S males weighed significantly more than Control males. There was no effect of Treatment on female body weights.

DISCUSSION

The present results demonstrate that prenatally-stressed (P-S) males and females exhibit differential analgesic responses to morphine and, for females alone, cold-water swim (CWS). There was no difference between P-S and Control males in CWS analgesia at any time postswim, whereas P-S females showed significantly less analgesia than Control females between 30–60 minutes. In the case of morphine analgesia, P-S males were significantly less analgesic than

TABLE 1

MEAN (±S.E.M.) BODY WEIGHTS AND BASELINE TAIL-FLICK LATENCIES FOR PRENATALLY-STRESSED AND CONTROL MALE AND FEMALE RATS

	Body Weight			Baseline Tail-Flick		
	Male		Female	Male		Female
Prenatally Stressed	522.5† (±8.03)	*	290.0 (±5.23)	2.35 (±0.17)	*	1.54 (±0.09)
Control	452.3 (±10.2)	*	277.0 (±5.24)	2.04 (±0.12)	*	1.47 (±0.07)

*$p<0.05$, males vs. females.

†$p<0.01$ P-S males vs. Control males.

Control males from 60–180 minutes; P-S females, however, exhibited a marked sensitivity to morphine relative to Control females, displaying significantly greater analgesia beginning at 30 minutes and extending to 180 minutes. Together, these data indicate that the regulation of stress- (in females alone), and opiate-induced analgesia (in males and females) is affected by exposure to prenatal stress. Furthermore, given the magnitude of the effects of morphine sulfate, these results suggest that the normal behavioral and physiological consequences of prenatal stress in males and females may be due to differences in opiate sensitivity, i.e., by the manner in which the animal responds to its endogenous opioids.

In the present study we found that prenatal stress did not alter CWS analgesia in males. Other work has shown that CWS is an environmental stressor which produces analgesia that is nonopioid-mediated since it is not cross-tolerant with morphine and is not blocked by the opioid receptor antagonists naloxone or naltrexone [7,8]. That prenatal stress did not affect responses to this acute stressor in the male suggests that the development of physiological systems mediating CWS analgesia may not be susceptible to the disruptions that accompany prenatal stress or, additionally, may have a different threshold than the female for activating changes.

Some aspect of opioid function is, however, altered by exposure to CWS. Seeger, Sforzo, Pert and Pert [40] report that opiate receptor binding in certain areas of the male rat brain involved in pain responsiveness is decreased following a 3.5 minute immersion in 4°C water bath, indicating endogenous opioid release and hence, opioid mediation of this stress-induced response. It is unclear, though, to what extent alterations in these opioid systems reflect a relation between opioid and nonopioid analgesia in the P-S male since no effect was observed in our behavioral measures.

Females, in contrast to males, did exhibit a moderate effect of prenatal stress on CWS analgesia. Specifically, P-S females were less analgesic after CWS relative to Controls at the two initial time-points postswim. These findings suggest that this (generally) nonopioid-mediated, acute stress-induced physiological response in the P-S female is dampened. Pollard [36] reported that P-S male and female rats exhibited a less acute increase in plasma corticosterone following rapid, intense stress in adulthood compared to nonstressed controls. These physiological responses are in contrast to behavioral responses which seem to indicate that prenatal stress exacerbates aspects of "emotionality" such as defecation and reactions to the open field [23, 32, 42], in addition to reducing the display of postpartum maternal behavior following exposure to a moderate stressor [12]. The observation of effects on both stress- and morphine-induced analgesia in the P-S female, but only morphine-induced analgesia in the P-S male, suggests that prenatal stress may have differential disruptive actions on the development of these two analgesia systems, one opioid, the other, nonopioid. To our knowledge, no organizational effects of gonadal steroid exposure have been reported for either morphine-induced analgesia or stress-induced analgesia, though sex differences for the latter in adulthood suggest some possibility of early effects [4, 10, 37, 38]. Until such parameters are examined it will remain unclear in what manner prenatal stress is affecting stress-induced analgesia in the female, and why it is not effective in the male.

The findings of differential analgesic responses to morphine in P-S animals are particularly striking. That P-S males were significantly less sensitive, and P-S females more sensitive to this potent opiate has a number of ramifications for the manner in which prenatal stress may exert its effects. Other data suggest that the events surrounding or associated with stress during the onset and elaboration of prenatal sexual differentiation may concurrently disrupt the development of the opioid system(s) which regulate the physiology and behavior of the adult animal. Specifically, Hijazi and Hammer found that the development of the mu (μ) opioid receptor system is sexually dimorphic, and that these effects are fairly permanent as they extend into adulthood [16,21]. Likewise, Watson, Weigand and Hoffman [44] reported that certain components of the endogenous opioid system (viz., enkephalinergic) may develop prenatally. Finally, Forman and Estilow [11] reported that perinatal treatment of male and female rats with steroids alters adult levels of beta-endorphin in pituitary and hypothalamus. Thus, by virtue of the disruptions of sexual differentiation which accompany prenatal stress, the normal development of opioid ligand or receptor systems may be altered. Owing to the perturbations in the development of this regulatory system, the normal modulatory role of opioids in behavioral and physiological systems may be less effective. Ward, Monaghan and Ward [46] reported that treatment with naltrexone during the prenatal stress regimen significantly reduced the disruptive effects of prenatal stress on male sexual behavior. The present data strongly implicate some effect on the development of opioid systems as well. Furthermore, we have preliminary data examining neuroendocrinological sensitivity to opiates which show that prolactin (Prl) release following an injection of morphine is greater in P-S females relative to Control females (Kinsley, Mann and Bridges, in preparation). These data suggest, and are in line with the present demonstration of, increased sensitivity to opiates in the P-S female rat. Therefore, alterations within the endogenous opioid system should be considered as contributory to the actions of prenatal stress on the physiology and behavior of adult rodents.

The differences that occurred in baseline pain thresholds between males and females confirm previous reports [35, 37, 38]. In general, male rats are less sensitive than females to nociceptive stimuli, an effect that can be eliminated by castration [4,33]. Gender differences in morphine analgesia have also been reported [3,10] and we have found similar differences. Control females were much less sensitive to the analgesic action of morphine than control males. When comparisons are made between P-S males and Control females, and between P-S females and Control males, some interesting relationships arise. Specifically, prenatal stress appeared to

make males more "female-like" with regard to analgesia in response to morphine. P-S males did not differ from Control females from 90–180 min. At 30–60 min, P-S males exhibited greater analgesia than Control females (which is in the direction of the normal sex difference), so the "female-like" influences are not complete. Likewise, P-S females were nearly identical to Control males, excepting the 180 min time-point at which Control males showed greater analgesia. If organizational effects of steroids influence morphine analgesia, then neonatal treatment of female rats with androgens might be expected to elevate morphine analgesia. Conversely, neonatal castration, which completely eliminates gonadal steroid contribution to sexual differentiation, would render male rats significantly less sensitive to morphine analgesia in adulthood. Prenatal stress behaviorally masculinizes the female (see Introduction), and also affects androgen stimulation in male rats during the so-called prenatal organizational period [44,45]. The P-S females in our study exhibited increased, and the P-S male reduced, sensitivity to morphine. Therefore, prenatal stress apparently affects morphine analgesia in the female by means of virilization of the substrates underlying analgesia, and reduces the response in the male by means of reduced prenatal steroid exposure.

If prenatal stress is acting to influence the development of endogenous opioid systems, what effect(s) would such changes have on certain behaviors? One response that is affected both by prenatal stress and morphine in adulthood is maternal behavior. In female rats the onset and maintenance of maternal behavior at various reproductive stages (ranging from virgin to primparous) is under an inhibitory influence of opiates and is naloxone-reversible ([9, 14, 24, 25, 39], Mann, Kinsley and Bridges, unpublished observations). Prenatal stress also affects the onset of maternal behavior in intact, virgin rats [27]. Relative to controls, P-S females exhibited a protracted latency to respond maternally when exposed to foster young, whereas P-S males showed a more rapid onset of the behavior. Sex differences were similarly reversed by prenatal stress, generally rendering males more "female-like" and females more "male-like." The present work demonstrates that P-S males are significantly less sensitive to morphine when assessed for analgesia; P-S females, on the other hand, were significantly more sensitive to morphine analgesia. If differences in the exhibition of certain behaviors, in particular, sensitization latencies to respond to young, are based on differences in opioid regulatory systems, prenatal stress might bring about such differences through alterations of those systems.

With regard to possible mechanisms underlying the effects of prenatal stress on opiate sensitivity, insensitivity to gonadal steroids may play an integral role. Chatterjee *et al.* [10] demonstrate that the presence of testosterone potentiates the effects of morphine on analgesia. Since males deprived of T due to castration show less responsiveness to T in adulthood [13], the alterations in T-exposure as a consequence of prenatal stress [44,45], and/or reductions in the levels of circulating T in adult P-S males [2], may combine to affect morphine sensitivity in P-S males in the direction we observed.

In the case of P-S females, equally interesting effects on steroid sensitivity may determine their reactions to opiates. In an earlier study, Selye [41] reported that estradiol (E2) pretreatment to female rats could "protect" the animal from lethal doses of the opiate ethylmorphine. We have previously shown that the P-S female is less sensitive to E2 in terms of prolactin release [26]. If this reduced sensitivity to exogenous E2 extends to physiological responses of exposure to endogenous E2, P-S females might lack the "protection" of this steroid hormone and be more susceptible to the analgesic properties of morphine.

The body weight data in Table 1 show that, as expected, males—regardless of treatment—are significantly heavier than females. What is surprising is the observation that P-S males are heavier than Control males. Given the facilitatory role of organizational androgenic effects on body weight and food intake in rats [5], the present finding is counterintuitive in that P-S males are exposed to lower levels of androgens [44,45], and therefore would be expected to weigh less than their Control counterparts. Other work in the mouse has reported that P-S males are heavier than Controls, though in this case, the animals had been approximately 120–130 days of age at the time of body weight determinations, slightly older than the average age for behavioral testing in mice [28]. The animals in the present experiment were similarly somewhat older and at present it is unclear to what extent age may interact with prenatal stress to bring about such differences.

The present results, in summary, demonstrate that opioid function and, to a lesser degree, stress-induced responses, are altered as a consequence of prenatal stress in male and female rats. The exact manner in which prenatal stress brings about such effects will help to shed additional light on the process of sexual differentiation of neurochemical regulatory systems and their importance for behavior.

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