

Sex Chromosome Complement Affects Nociception and Analgesia in Newborn Mice

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Abstract: In animal studies of nociception, females are often more sensitive to painful stimuli, whereas males are often more sensitive to analgesia induced by μ -agonists. Sex differences are found even at birth, and in adulthood are likely caused, at least in part, by differences in levels of gonadal hormones. In this report, we investigate nociception and analgesia in neonatal mice and assess the contribution of the direct action of sex chromosome genes in hotplate and tail withdrawal tests. We used the 4 core genotypes mouse model, in which gonadal sex is independent of the complement of sex chromosomes (XX vs XY). Mice were tested at baseline and then injected with μ -opioid agonist morphine (10 mg/kg) or with the κ -opioid agonist U50,488H (U50, 12.5 mg/kg) with or without the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (0.1 mg/kg). On the day of birth, XX mice showed faster baseline latencies than XY in tail withdrawal, irrespective of their gonadal type. Gonadal males showed greater effects of morphine than gonadal females in the hotplate test, irrespective of their sex chromosome complement. U50 and morphine were effective analgesics in both tests, but MK-801 did not block the U50 effect. The results suggest that sex chromosome complement and gonadal secretions both contribute to sex differences in nociception and analgesia by the day of birth.

Perspective: Sex differences in pain may stem not only from the action of gonadal hormones on pain circuits but from the sex-specific action of X and Y genes. Identification of sex chromosome genes causing sex differences could contribute to better pain therapy in females and males.

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Key words: Pain, sex difference, hotplate, tail withdrawal, sex chromosomes, neonate.

In various species including humans, pain perception and sensitivity to analgesic drugs can differ in either sex. Females are generally more sensitive to pain and/or differ from males in neural circuits mediating nociception.^{5,18,30} In mice, a variety of *N*-methyl-D-aspartate (NMDA) antagonists block the acute analgesic effects of μ - or κ -agonists or development of tolerance to morphine.^{6,12,33,34,37,41} However, specific NMDA antagonists block the morphine analgesia only in females, whereas other NMDA antagonists block analgesia in either sex.³⁷ Estradiol activates a female-specific

mechanism that is absent in males, is insensitive to NMDA antagonists, and is mediated by the melanocortin-1 receptor.^{33,34}

In animals including mice, μ -agonists such as morphine are more effective in males than females,^{11,12,27,32,35} although the literature is complex and contradictory.¹² Activational and organizational effects of steroids contribute to sex differences in opioid analgesia.^{9,10,12,25,32} For example, estradiol can reduce morphine analgesia¹² and testosterone can increase opioid analgesia because of activational effects.^{33,42} In addition, treating neonatal female mice with testosterone permanently masculinizes their response to morphine, and castrating males at birth demasculinizes them (organizational effects).^{9,25} Analgesia produced by the selective κ -opioid receptor agonist U50,488H (U50) shows sex differences in the same direction as the μ -opioid receptor agonist morphine.^{34,40} U50 can be more potent in males than in females, and the U50 effects are blocked specifically in males by MK-801.^{34,40} However, organizational and activational effects of gonadal ste-

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roids do not account for all of the sex differences in nociception and drug-induced analgesia.

Sex differences are also caused by "sex chromosome effects," which are direct actions of X and Y genes on nongonadal cells.¹⁻³ In this study, we used the "4 core genotypes" (FCG) model to study the biological origins of sex differences in nociception and analgesia in neonatal mice. In this mouse model, the complement of sex chromosomes (XX vs XY) is independent of the gonadal sex (testes vs ovaries) of the mouse.^{3,7,8,16,20,21,38}

We recently observed that neonatal XX mice of either gonadal sex have higher expression in brain of prodynorphin mRNA than XY mice (unpublished data). That finding suggested that dynorphin levels might be higher in XX than XY mice, which might alter κ -opioid nociception. μ - and κ -receptors are found in neonatal rodent spinal cord and brain.³⁹ We therefore asked whether neonatal mice show hormonal and sex chromosome effects on nociception and μ - and κ -mediated analgesia and whether the NMDA antagonist MK801 could block any U50 effect. We found that morphine and U50 were both effective analgesics at this age but that the NMDA antagonist MK-801 did not block the effects of U50 as it does in adults.⁴⁰ Groups differing in sex chromosome complement and in gonadal sex showed differences in the 2 assays of nociception.

Methods

Mice

In FCG mice, the testis-determining *Sry* gene is deleted from the Y chromosome, producing the "Y minus" chromosome, Y^- , so that the Y^- chromosome no longer determines gonadal sex.^{3,26,28} An *Sry* transgene is inserted onto an autosome, producing $XY^- Sry$ males. Breeding these males with XX females produces 4 types of progeny. XX females, XY^- females, $XXSry$ males, and $XY^- Sry$ males. We define "male" and "female" according to gonadal sex. Comparing the phenotype of males ($XXSry$ and $XY^- Sry$) and females (XX and XY^-) tests for the effects of presence or absence of *Sry*. The effects of *Sry* are thought to be mediated primarily by sex differences in gonadal secretions, although direct effects of *Sry* on the brain also occur.¹⁷ On the other hand, comparing XX mice (XX females and $XXSry$ males) with XY^- mice (XY^- females and $XY^- Sry$ males) tests for the differential effects of sex chromosome complement (XX vs XY). The FCG model tests simultaneously for the effects of gonadal steroids, for sex chromosome effects, and their interaction (eg, hormonal effects that occur only in XY but not XX mice). In the present experiment, we used randomly bred MF1 mice that were kindly provided to us by Paul Burgoyne (National Institute for Medical Research, London, UK), in which the MF1 X chromosome of all mice was invariant (ie, had no allelic variation across animals, a substrain produced by breeding male mice to their XO mothers). The Y^- chromosome derives from strain 129.²⁶

Mice were quasi-randomly assigned to groups, attempting to maximize the number of groups repre-

sented within each litter. The sex and sex chromosome complement of each mouse was determined by PCR amplifying from genomic DNA a Y chromosome sequence (to detect the presence/absence of the Y^- chromosome), *Sry* (to detect the *Sry* transgene), and a control gene.

Experimental Subjects and Procedures

Experimental subjects were male ($n = 175$, 91 $XXSry$ and 84 $XY^- Sry$) and female ($n = 146$, 67 XX and 79 XY^-) FCG MF1 mice, bred in our colony. Animals were tested on the day of birth (P1) between 8 AM and noon. Animals were housed in a light-controlled (L:D 12:12, lights on at 7 AM) and temperature-controlled ($21 \pm 1^\circ\text{C}$) environment. $XY^- Sry$ males were each housed with pairs of XX females for 2 weeks. Dams were then separated from males and singly housed for the duration of the pregnancy, with no disruption except for the routine cage maintenance. Dams were given free access to standard laboratory food and tap water. As parturition approached, the cages were observed daily for the presence of pups. On the day of birth, pups were removed from the dam, weighed, and placed in individual cardboard containers. The containers were kept on a warming pad for the duration of the testing session. Two tests of nociception were performed as described below. Pups within each litter were randomly assigned to one of the drug conditions. Immediately after hot plate and tail withdrawal baseline assessment, the pup was injected with a drug (see below) and returned to its container. Pups were retested on both pain assays 15, 45, and 90 minutes after injection by an experimenter who was blinded to the drug condition and sex/sex chromosome complement. All procedures were approved by the UCLA Chancellor's Animal Research Committee and conformed to applicable national and international guidelines.

Tests of Nociception

Mice were tested on 2 different behavioral tests of thermal nociception as described by Sternberg et al:⁴¹ the hotplate and tail withdrawal assays, which show diverse sensitivity to drugs and sex. Both the hotplate and tail withdrawal assays have been used extensively in adult rodents and have also been adapted for use in neonates previously.⁴¹

In the hotplate test, the experimenter held the pup between the thumb and forefinger in an upright position and gently placed one hind foot of the mouse on the surface of the hot plate (52.5°C ; AccuScan Instruments, Columbus, OH). Latency to remove the foot from the surface was recorded, with a 15-second cutoff time for nonresponsive animals. The procedure was repeated on the opposite foot after a 10-second interval, and an average of the latencies for both feet was used in the analysis. Foot withdrawal was measured at time $t = 0$ (before injection) and 15, 45, and 90 minutes later.

In the tail withdrawal test, the pup was held in the same manner, and the distal tip of the tail was lowered into a water bath maintained at 50°C . The latency to

vigorous tail withdrawal was recorded, with a 15-second cutoff time. The test was repeated after a 10-second interval, and the latencies were averaged. The tail withdrawal test was performed 1 minute after the hotplate test, at each of the time points (0, 15, 45, and 90 minutes).

Drugs

To study the acute effects of morphine, pups were injected subcutaneously (s.c.) with morphine at 1 of 3 doses, 0, 1, or 10 mg/kg, immediately after the baseline tests of hotplate and tail withdrawal at time $t = 0$. Pups were tested again on both assays at 15 and 45 minutes after injection. Group sizes were 10 to 15 for each group (ie, 1 sex chromosome complement in 1 sex at 1 drug treatment dose).

To study the effect of a κ -opiate agonist and interactions with the NMDA system, pups were injected subcutaneously with the NMDA antagonist MK-801 (MK; 0.1 mg/kg; Sigma, Indianapolis, IN) or saline vehicle (SAL) immediately before injection of the κ -agonist U50,488H (12.5 mg/kg; Sigma) or saline vehicle (SAL). Pups were tested again on both assays at 15, 45, and 90 minutes after injection. Although NMDA systems that mediate opioid analgesia are reported not to have matured at birth in rats,^{23,45,46} we rationalized that testing for NMDA system involvement in U50 analgesia in neonatal mice was warranted because of the robust sex differences reported in the effects of NMDA antagonists on U50 analgesia in adult mice.⁴⁰

Morphine, MK, and U50 were dissolved in saline in a volume of 10 mL/kg. Group sizes were 7 to 20 (usually 10–15) for each combination of sex, sex chromosome complement, and drug regime.

Statistical Analysis of Data

The experimental design included 3 between factors (sex chromosome, gonadal sex, and drug) and 1 within factor (time). Baseline hot plate and tail withdrawal latencies were analyzed with a 2-way ANOVA with factors of sex (male vs female) and sex chromosome (XX vs XY). The response to analgesic drugs was analyzed by using a mixed-factorial, 4-way ANOVA on nociceptive latencies with 1 within factor (time) and 3 between factors of sex chromosome, sex, and drug. The initial analysis of data showed that the effect of 1 mg/kg morphine did not differ from the effect of saline. Therefore, for all subsequent statistical tests, we combined the data for the 0 and 1 mg/kg morphine doses to increase statistical power. Thus, the drug groups in the morphine study were morphine 0 and 1 vs morphine 10. In the U50 study, the 4 drug groups were saline-saline (sal), saline-U50 (U50), MK-saline (MK-sal), and MK-U50. In the morphine study and in the study of U50 effects on tail withdrawal test, because some groups differed in their baseline latencies, we used the dependent variable of the percentage of maximum possible effect of morphine (%MPE = $100 \times (\text{latency} - \text{baseline}) / (15 - \text{baseline})$). In the U50 study, however, there were no group differences in hot-

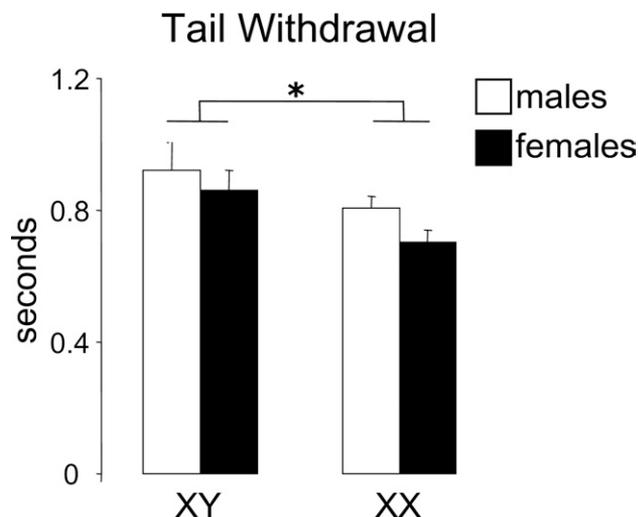


Figure 1. In the baseline tail withdrawal tests for all experiments, XX mice showed faster latencies than XY mice irrespective of their gonadal sex ($*P = .02$). Mean latencies were XY male, 0.92; XY female, 0.86; XX male, 0.80; XX female, 0.70 seconds.

plate baseline latencies, so we used response latency in seconds as the dependent variable. The significance level was set at $\alpha = 0.05$. Post hoc tests were performed by Tukey test.

Results

Hotplate and Tail Withdrawal Baselines

In the baseline measurements of the hotplate test, there were no group differences of experimental subjects due to gonadal sex or sex chromosome complement in the morphine or the U50 experiments ($P > .05$). In the tail withdrawal assay, however, there was an overall main effect of sex chromosomes on tail withdrawal baseline latencies (XY > XX, $F_{(1,317)} = 5.14$, $P = .02$, Fig 1, XY $n = 163$ and XX $n = 158$).

Morphine Study: Analgesia in Hotplate and Tail Withdrawal Assays

Hotplate

Hotplate tests showed effects of sex but not sex chromosome. As expected, morphine significantly increased %MPE for foot withdrawal on the hotplate (main effect of drug: ($F_{(1,131)} = 130$; $P = .000001$). In the 4-way ANOVA, there was a significant main effect of sex ($F_{(1,131)} = 7.06$; $P = .009$): Males had higher %MPE than did females (Fig 2A). Interestingly, there was a trend toward significance in the sex by drug interaction ($F_{(1,131)} = 3.55$; $P = .06$) because males had greater analgesia (higher %MPE) than did females in response to morphine ($P = .02$, Tukey test comparing 10 mg/kg males and females) (Fig 2B).

Tail Withdrawal

Tail withdrawal showed effects of sex and sex chromosome complement. Morphine significantly increased

Morphine Study

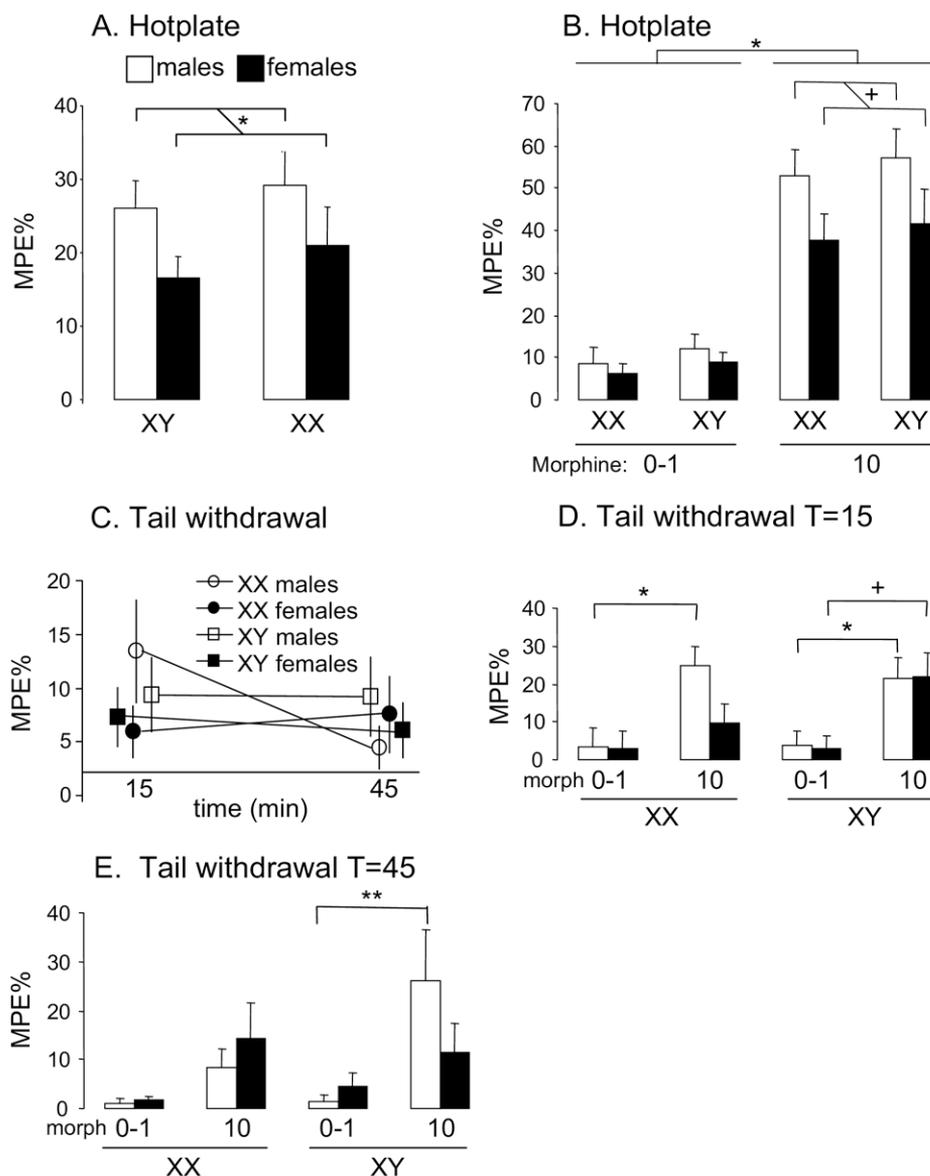


Figure 2. Results of the hotplate and tail withdrawal assays in the morphine study. **A**, Averaging over all drug (including saline) and time conditions in the hotplate test, males had a higher %maximum possible effect (%MPE) than females ($*P = .009$). **B**, Graphs show %MPE averaged across time. In the hotplate test, morphine at 10 mg/kg had significant analgesic effect ($*P = .000001$) relative to the 0 to 1 mg/kg morphine group. The response to morphine tended to be higher in males than females irrespective of their sex chromosome complement ($P = .06$ sex by drug interaction; +, $P = .02$ comparison of males vs females at 10 mg/kg). **C**, Graphs show results averaged across all drug doses, including saline, so that the main effects of sex and sex chromosome complement can be seen. In the tail-withdrawal test, the %MPE over time differed as a function of both sex and sex chromosome complement because XX males had a more variable response than the other 3 groups (sex by sex chromosome by time interaction, $P = .035$). **D** and **E**, In the tail withdrawal test, there was a statistically significant interaction of time, sex chromosome, sex, and drug ($P = .015$), meaning that the effects of sex chromosome, sex, and drug were different at different times. To illustrate those differences, the %MPE are graphed separately for $t = 15$ and $t = 45$ minutes. At $t = 15$ minutes after morphine injection, there was a differential effect of morphine on XX vs XY females (+, $P = .07$ in XY females and not significant for XX females) but not between XX and XY males ($*$ both $P < .05$). At 45 minutes after injection ($t = 45$), the effect of morphine was significant only in XY males ($**P = .0002$).

%MPE in the tail withdrawal test (main effect of drug: $F_{(1,131)} = 30.06$; $P < .000001$) (Fig 2C). In the 4-way ANOVA, XX and XY mice and male and female mice responded differently over time (significant interaction between time, sex chromosome, and sex, $F_{(1,131)} = 4.54$; $P = .035$), which can be seen in Fig 2C as a lower %MPE for XX

males at $t = 45$ than at $t15$. There was also a significant interaction of time by sex chromosome by sex by drug ($F_{(1,131)} = 6.03$; $P = .015$). That significant effect is illustrated by group differences in Figs 2D and 2E. Several factors appear to explain the statistically significant interaction. At $t = 15$, morphine nearly increased %MPE in

XY females but not XX females (XY females treated with morphine vs controls, Tukey test, $P = .07$). In males, at $t = 15$ (Fig 2D) and $T = 45$ (Fig 2E), morphine increased %MPE in XY males (vs controls: $P = .045$ at $t = 15$ and $P = .0002$ at $t = 45$), whereas morphine increased latency in XX males only at $t = 15$ (vs control animals: $P = .014$).

U50 Study: Effects of Acute U50 and MK on Hotplate and Tail Withdrawal Assays

Hotplate

Hotplate tests showed effects of sex chromosome complement. As expected, the drugs significantly influenced latencies for foot withdrawal from the hotplate (main effect of drug: ($F_{(3,166)} = 31.3$; $P = .000001$): U50 significantly increased hotplate latencies, but MK did not block U50 analgesia (U50 vs sal, $P < .0001$; U50-MK vs sal, $P < .0001$) (Fig 3A). In the overall 4-way ANOVA, there was a trend in the main effect of sex ($F_{(1,166)} = 3.49$; $P = .06$): Males tended to have higher hot plate latencies than females (males, mean \pm SEM = 4.52 ± 0.207 ; females, 3.60 ± 0.215). Interestingly, the time by sex chromosome interaction was significant ($F_{(3,496)} = 3.42$; $P = .02$) because XY mice differed from XX only at $t = 90$, when XX mice had longer latencies than XY regardless of their treatment ($P < .05$, Tukey) (Fig 3B). (In other words, at $t = 90$, XX mice of all treatment groups considered together had longer latencies than XY mice of all treatment groups considered together.)

Tail Withdrawal

There were no significant main effects or interactions involving sex or sex chromosome complement. Drugs significantly affected %MPE in the tail withdrawal test (main effect of drug: $F_{(3,166)} = 16.4$; $P < .000001$) (Fig 3C). U50 with or without MK was effective in increasing tail withdrawal %MPE when compared with saline animals (U50 vs sal and U50-MK vs sal: $P < .00001$, Tukey test); and MK alone was no different than saline.

Discussion

We report that sex chromosome complement (XX vs XY) and gonadal sex (male vs female) both influence the development of nociception and/or response to analgesic drugs by the day of birth in mice. In the FCG model using MF1 mice, neonatal XX mice showed greater basal nociception than XY mice in a test-dependent fashion (faster tail withdrawal but no difference in hotplate latencies), irrespective of their gonadal sex. Moreover, morphine was an effective analgesic at this age but had a greater effect on gonadal males than females in the hotplate test, suggesting that differences in gonadal secretions before birth or on P1 caused these sex differences. The tail withdrawal test in the morphine study showed complex interactions of sex, sex chromosome complement, and time. We found that U50 had analgesic effects at this age on hotplate and tail withdrawal, which were not blocked by MK-801 and mostly did not differ according to gonadal sex or sex chromosome comple-

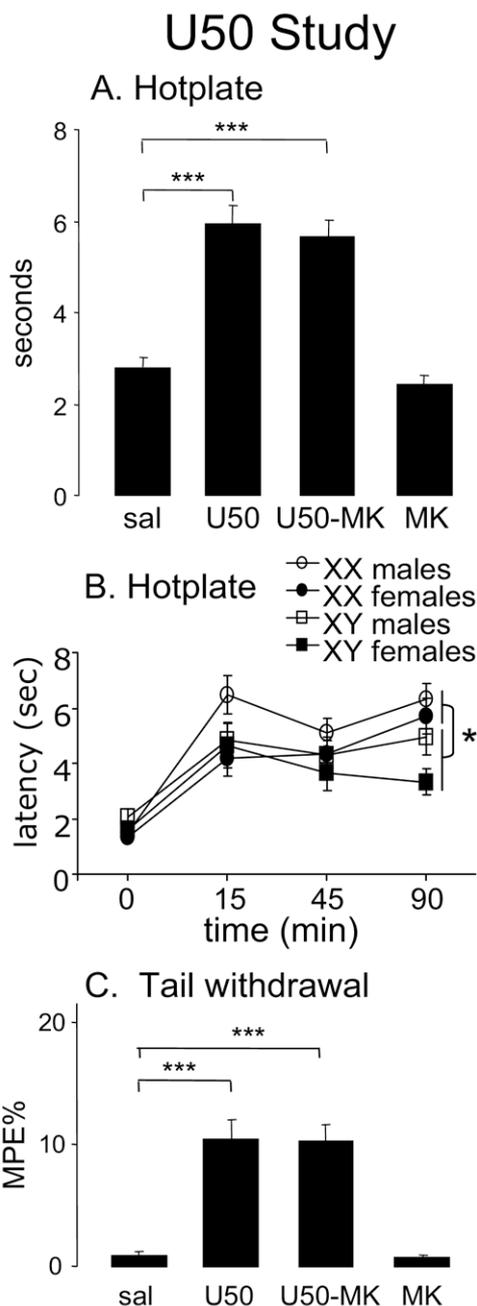


Figure 3. Hotplate and tail withdrawal assays in the U50 study. **A**, Averaging across all groups before and after injection in the hotplate test, the U50 group had longer latencies than control animals (U50 vs sal, $***P < .0001$), but MK did not block the effect of U50 (U50-MK vs sal, $***P < .0001$). **B**, Hotplate latencies as a function of time and sex and sex chromosome complement. There was a significant interaction of time and sex chromosome complement—XX and XY mice had longer latencies than XX mice only at $t = 90$ ($*P < .05$). To show this interaction, which did not depend on drug treatment, data from all drug treatment groups, including saline, are combined for each of the FCG groups. **C**, Averaging across time and sex and sex chromosome complement, U50 with or without MK significantly increased latencies in the tail withdrawal test (U50 vs sal, and U50-MK vs sal, both $***P < .00001$).

ment. In the study on the effects of U50 on the hotplate test, XX and XY mice differed only at 1 time point. In general, the results suggest that both gonadal hormones and sex chromosome complement contribute to sex dif-

ferences in nociception and analgesia in newborn mice, although the effects of sex chromosome complement are modest and often limited to specific subsets of conditions tested. In some cases, the 2 effects interact, suggesting that gonadal hormones have different effects in XX and XY mice. This is the first demonstration of sex chromosome effects on nociception and morphine analgesia in neonatal mice.

The differences between XX and XY groups are attributable to differences in the action of X or Y genes. Such differences include the effects of Y genes and differences in the expression of X genes resulting from XX-XY differences in gene dose⁴⁴ or genomic imprinting of the X chromosome.^{2,13} The sex chromosome effects could be exerted directly on the brain to induce changes in nociception, or elsewhere in the body. It is possible, for example, that XX and XY males, or XX and XY females, differ from each other in the level of prenatal sex steroid secretions coming from the gonads or from other organs such as the adrenals or the brain itself. However, the pattern of the present and previous results argues against the idea that the sex chromosome effect on baseline tail withdrawal latencies are mediated by differences in gonadal hormones. XX and XY mice differed in their baseline response in the tail withdrawal test irrespective of gonadal sex. Because FCG gonadal males are similarly masculinized in numerous brain and behavioral traits,^{16,20,29,43} including the response to morphine in hotplate tests of the present study, it is likely that both types of males experience similar male-typical levels of testicular hormones and that both female groups do not. In the tail withdrawal tests reported here, gonadal males and gonadal females did not differ in baseline latencies, suggesting that their large differences in gonadal secretions did not cause sexual differences of that response by P1 under the present conditions. In contrast, sex chromosome complement did cause a significant difference, irrespective of gonadal sex, which is therefore not likely a result of XX vs XY differences in gonadal secretions in mice with either type of gonad. It is more likely that the effects of X or Y genes are exerted on nongonadal tissues such as the brain or other components of the nociception pathways.

Sternberg et al⁴¹ found sex differences in basal nociception and analgesic effects of morphine in neonatal CD-1 mice. Female neonates had longer latencies in a tail withdrawal test using parameters similar to those used here. In the same study, females tended to have shorter latencies than males in the hotplate test. These results contrast with the present results in which no effect of gonadal sex was found in either test. Sternberg et al⁴¹ also found that analgesic effects of morphine lasted longer in males than in females when tested in the tail withdrawal paradigm. In the present study, males were more sensitive than females to the analgesic effects of morphine in the hotplate test. These 2 findings, although not equivalent, agree with the general finding that the analgesic effects of morphine are greater and last longer in males rather than in females.^{32,41} When we tested the FCG neonates in the tail withdrawal test, mor-

phine lasted longer in XY males than in XX males (Fig 2E), whereas it did not have a significant analgesic effect in females (only a trend in XY females, Fig 2D). The differences between the results of Sternberg et al⁴¹ and ours could be attributed to several factors. The 2 studies used different strains (CD-1 vs MF1). Moreover, many of the statistical tests and conclusions of Sternberg et al⁴¹ are based on combined analysis of P1 and P8 pups, in contrast to our focus only on P1. Moreover, we cannot exclude the idea that there are differences between normal XY males with an endogenous *Sry* gene encoded on the Y chromosome and FCG mice in which gonadal males have an *Sry* transgene encoded on an autosome.¹⁶

At birth, the neural circuits underlying nociception are incompletely developed. Studies in rats show that by the time of birth, nociceptive afferent C fibers express TRPV1 receptors mediating thermal pain, project into the dorsal horn, and show responses to nociceptive stimuli similar to adult C fibers.¹⁹ Central connections of polymodal pain receptors are established but immature on the day of birth. Neonatal rats have thresholds for withdrawal from heat that are lower than adults, and the dorsal horn neurons are more excitable. Especially immature at the time of birth are the descending inputs to the dorsal horn from the brainstem. For example, stimulation of periaqueductal gray (PAG), which induces analgesia in adults, does not have this effect until 3 weeks after birth.¹⁹ Similarly, the biphasic response to formalin injection into the paw, which is thought to reflect the function of the descending pathways, does not mature until P15.¹⁹ Thus, the nociceptive responses and sex or sex chromosome effects that we have observed here probably are mediated predominantly spinally or peripherally, assuming that the maturation of circuits is similar in rats and mice. μ -receptors are expressed in the rat dorsal horn and dorsal root ganglia by P1 and thus represent likely sites of action of morphine, which has an analgesic effect in this and previous studies.³⁶ Moreover, κ -receptors are expressed in the rat spinal cord by the time of birth³⁹; thus, the analgesic actions of U50 found here may be spinal, although peripheral analgesic effects of U50 have been reported and are also a possibility.⁴ Extensive studies in rats also demonstrate sensitivity of neonatal rodents to κ - and μ -agonists.^{22,24,31} The lack of effect of MK801 found here is probably explained by the lack of maturity of descending NMDA circuits in the brain (eg, PAG) or at their termination onto spinal nociception circuits. The absence of an MK801 effect found here in mice is compatible with evidence in rats that NMDA receptor systems, and their involvement in nociception, mature in the first few weeks after birth.^{23,45,46}

Sex differences have been reported in the analgesic effects of U50 and MK blockade. Intact adult male mice have been found to be more sensitive than females to analgesia produced by U50.⁴⁰ Moreover, aged females had a male-like response to U50. We did not observe a differential effect of U50 or MK on males and females (no significant effect of gonadal sex or interaction of gonadal sex with other variables). Age and strain of animals might have contributed to the difference in results.

Sternberg et al⁴⁰ used as experimental subjects male and female young adult mice and old female mice from the C57BL/6 strain, in contrast to our use of neonatal MF-1 FCG mice.

The biological origins of sex differences in pain and analgesia are likely to be complex. On the one hand, it is abundantly clear from previous studies that sex differences are widespread, that specific gonadal steroids alter the response to pain and analgesics in adulthood (activational effects), and that perinatal sex differences in levels of testosterone produce long-lasting organizational differences.^{12,32} We have recently also found that gonadectomized adult XX mice respond faster than XY mice at baseline in hotplate tests on C57BL/6 FCG mice.²¹ That finding, together with the present evidence, suggests that the complement of sex chromosomes is also a factor contributing to sex differences in pain and analgesia. It is likely that the gonadal steroid effects interact in complex ways with the sex chromosome effects. The current results offer evidence for such complex interactions, for example, because the sex chromosome effect in the tail withdrawal paradigm depended on gonadal sex. In XY males, the effects of morphine were evident at

both time points, whereas morphine decreased tail withdrawal latency in XX males only at $t = 15$ (Figs 2D and 2E). This result suggests that testosterone, secreted in males before or after birth, might have a different effect in XX than in XY mice. Alternatively, the levels of testosterone present in XX and XY males might differ. Among the females, the effects of morphine were not significant at both time points in this test. Such interaction between sex chromosome and gonadal sex is consistent with previous literature showing that males are usually more sensitive than females to morphine.^{32,41} The effects of sex chromosome complement could be to enhance or counteract the effects of gonadal steroids.^{14,15,38} It will be important in future studies to manipulate the level of gonadal hormones in FCG mice directly, at different times of life, to determine how hormonal and sex chromosomal influences interact and the role they play in the maturation of pain circuits.

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