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Genetic variation in morphine analgesic tolerance: A survey of 11 inbred mouse strains

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Abstract

The present study assessed the analgesic potency of morphine in 11 inbred mouse strains before and after chronic morphine treatment. Using the 49 °C tail-withdrawal test, significant strain differences in morphine AD_{50} estimates derived from cumulative dose–response curves were noted prior to tolerance induction on Day 1. AD_{50} estimates were reassessed on Day 4, after three daily systemic morphine injections for 3 days using an escalating dose schedule (10, 20, and 40 mg/kg sc). In 9 of 11 strains, morphine potency was significantly reduced from 2-fold to as much as 11-fold. Two strains (129P3 and LP) displayed no evidence whatsoever of tolerance development. Neither initial baseline withdrawal latency nor morphine analgesic sensitivity was significantly correlated with tolerance magnitude. Also observed were strain-dependent alterations (mostly hyperalgesia) in baseline tail-withdrawal latencies as a result of chronic morphine treatment. The magnitude of hyperalgesia and analgesic tolerance was significantly correlated among strains, implicating common genetic substrates and supporting their proposed association. The present work demonstrates that the presence and magnitude of morphine analgesic tolerance is genotype-dependent and identifies strains with widely divergent liabilities that should facilitate identification of trait-relevant genes.

Keywords: Morphine; Tolerance; Inbred strains; Analgesia; Genotype; Hyperalgesia

1. Introduction

Prolonged or repeated morphine administration can result in analgesic tolerance, a diminution of morphine analgesic potency, magnitude, and/or duration. The development of tolerance to morphine, and concomitantly cross-tolerance to other clinically useful opioids, renders subsequent effective pain control difficult and unpredictable. Similar to morphine analgesia, there exists inter-individual variability among humans in response to chronic opioid treatment. Whereas some patients require rapidly escalating opioid doses in conjunction with escalating pain, others find adequate analgesia from stable opioid doses for periods of up to several months (Foley, 1993).

Studies comparing rodent strains have demonstrated the important contribution of genetic background to individual differences in the development and magnitude of tolerance (Oliverio and Castellano, 1974; Ho et al., 1977; Gwynn and Domino, 1984; Mas et al., 2000). These studies however were based on data obtained exclusively from the same three inbred strains, typically compared two at a time, thereby limiting the analysis of variability in tolerance liability but also precluding the ability to perform a correlation analysis with other pain-related phenotypes that may be predictive of tolerance itself. Furthermore, there was substantial variability in methodology, including the duration of morphine treatment, the nociceptive assay utilized and/or parameters within even a given nociceptive assay. In almost all studies, only single morphine doses were tested after tolerance induction, precluding the generation of dose–response curves and the subsequent calculation of potency estimates upon which accurate comparisons can be made. The present study attempts a more compre-

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hensive and systematic analysis of the genetic contribution to inter-individual differences in morphine analgesic tolerance. The morphine tolerance liability of 11 inbred mouse strains were determined by assessing the loss of relative analgesic potency through comparisons of half-maximal morphine analgesic dose (AD_{50}) estimates obtained before and after chronic morphine treatment. By comparing many strains in a single study under identical experimental protocols, the present strain survey facilitates comparison between strains previously studied as well as those previously untested, and allows for the correlation of tolerance with other phenotypes such as baseline nociceptive sensitivity and morphine analgesia both before and after chronic morphine treatment.

2. Methods

2.1. Subjects and drugs

Male and female adult mice ($n = 18–20$ mice per sex per strain) of the following inbred strains were obtained from The Jackson Laboratory (Bar Harbor, ME): 129P3, A, AKR, BALB/c, C3H/He, C57BL/6, CBA, DBA/2, LP, SJL, and SWR (all "J" substrains). All mice were housed four to a cage with same sex/strain mates in the College of Staten Island Animal Facility. Mice were allowed free access to food (Purina chow) and water in a temperature-controlled (22 °C) environment maintained on a 12:12-h light/dark cycle (lights on at 07:00 h). All testing was performed following an acclimation period of at least one week after arrival and at 7–9 weeks of age. Morphine sulfate was generously supplied by the National Institute for Drug Abuse (Rockville, MD) and was dissolved in 0.9% physiological saline. All injections were delivered via the subcutaneous route in a volume of 10 ml kg^{-1}.

2.2. Nociceptive assay

All testing was conducted near mid-photophase (10:00–15:00 h) to reduce circadian effects on nociceptive and analgesic sensitivity (Kavaliers and Hirst, 1983). Although the large numbers of mice used required testing to continue over several weeks, both strain and sex were completely counterbalanced so that an equal number of mice per strain per sex were tested together. Following a 30-min habituation to the testing room, mice were assessed for baseline nociceptive sensitivity on the 49 °C tail-withdrawal test. In this assay of acute, thermal nociception, the mouse is gently restrained and the distal half of the tail is immersed in water maintained at 49.0 ± 0.2 °C by an immersion circulator pump (Fisher Isotemp Model 71). Latency to reflexive withdrawal of the tail was measured twice to the nearest 0.1 s, with each determination separated by a minimum of 20 s. The two determinations were later averaged. The tail-withdrawal test was chosen because of its stability even after repeated exposures to noxious water temperatures (D'amour and Smith, 1941). A cut-off latency of 15 s was employed to prevent the possibility of tissue damage and was used to calculate AD_{50} values.

2.3. Dose–response data and tolerance induction

To greatly reduce the number of mice required for these studies, morphine analgesic potency was determined using cumulative dose–response curves. Immediately following baseline latency assessment, subjects were injected with an initial 1.0 mg kg^{-1} dose of morphine, followed in succession with increasing ($\sim$0.25 log) doses (2.0, 3.6, 6.5, 11.7, and 21.0 mg). These doses have been demonstrated to provide reliable morphine potency estimates in a variety of mouse strains (Kest et al., 1995, 2000). To ensure that all mice received the same cumulative dose of drug on Day 1, all mice were injected with identical morphine doses. Those displaying cut-off latency values prior to the others differed only in that they were not subjected to further nociceptive testing. For some individual mice in tolerance sensitive strains, morphine injections at the highest dose (21.0 mg) were repeated until cut-off latency was achieved on Day 4. Tail-withdrawal latencies were retested 30 min after each dose, and the subsequent dose of morphine injected immediately thereafter. Baseline latencies and dose–response data were collected prior to (Day 1) and after (Day 4) tolerance induction.

Immediately following dose–response studies on Day 1, subjects were injected with morphine three times daily for 3 days according to an escalating daily dosage schedule (10, 20, and 40 mg kg^{-1} on Days 1, 2, and 3, respectively). To assess for possible repeated handling/injection effects, control mice of each strain received an equal number of saline injections throughout, except on Day 4, when morphine dose–response curves were collected in them similarly to mice undergoing tolerance induction.

Analgesia at each dose was expressed as a percentage of the maximum possible analgesic effect (% MPE) as calculated by the formula: % analgesia=[(postmorphine latency − baseline latency)/(cut-off latency − baseline latency)] × 100. The use of this transformed dependent measure takes into account the cut-off latency and the individual baseline latency for each subject, so that any variation in baseline values between test Days 1 and 4 will not bias the quantification of analgesia obtained each day.

2.4. Data analysis

Two-way ANOVA was used to examine the main effects of strain and sex, and their interaction, on tail-withdrawal baseline latencies. Subjecting Days 1 and 4 latencies to repeated measures ANOVA assessed changes in baseline latencies after chronic morphine treatment. Half-maximal analgesic doses (AD_{50}) and associated 95% confidence

intervals (CI) were calculated for dose–response data using the method of Tallarida and Murray (1981). AD_{50} values with nonoverlapping CIs were considered significantly different.

Estimates of narrow-sense trait heritability were calculated by comparing the ANOVA-derived between-strain variance to the total variance: $h^2 = V_A/(V_A + V_E)$; where V_A is the allelic (between-strain) variance and V_E is the error or environmental (within-strain) variance. For baseline nociception, raw Day 1 tail-withdrawal latencies for each mouse were used. The estimation of heritability of Days 1 and 4 analgesic sensitivity and tolerance was less straightforward, since these measures are normally calculated based on group data, and individual data is required for heritability estimation. We therefore estimated the Days 1 and 4 AD_{50} in each mouse as the lowest cumulative morphine dose producing $\geq 50\%$ analgesia, and estimated tolerance in each mouse as the ratio Day 4 AD_{50}:Day 1 AD_{50}. These estimates featured higher within-strain variance than the group-based estimates, but were highly accurate, correlating with the group-based estimates at r=.88–.97. The increased within-strain variance will, however, tend to underestimate the true heritability of the analgesia and tolerance phenotypes.

To assess genetic codetermination between phenotypes, baseline withdrawal latencies and morphine AD_{50} strain means obtained prior to and following chronic morphine treatment, and their subsequent differences, were ranked from smallest to highest according to effect size. Correlation coefficients were then obtained using Spearman's r followed by Bonferroni correction for multiple comparisons. In all statistical tests, a threshold α criterion level of .05 was employed.

3. Results

3.1. Baseline withdrawal latencies

There was a significant main effect of strain and sex (both $P<.001$) on baseline tail-withdrawal latencies, but no significant interaction (P=.40). Therefore, baseline tail-withdrawal latencies obtained before (Day 1) and after (Day 4) chronic morphine treatment are presented for all strains (collapsed across sex) and by sex (collapsed across strains) in Table 1. Withdrawal latencies varied significantly between strains on both Days 1 and 4 (both $P<.001$). Heritabilities of Days 1 and 4 baseline latencies were estimated as $h^2 = 0.36$ and 0.35, respectively, which are very similar to those obtained previously for this trait (Mogil et al., 1999a).

In addition, there was a significant main effect of the difference in latencies between Days 1 and 4 ($P<.001$) and a significant interaction of this repeated measure with strain ($P<.001$). Pairwise comparisons using Student's t-test revealed significant decreases in baseline latencies on Day 4 relative to Day 1 in an overwhelming majority of strains (Table 1). Exceptions were noted for mice of the 129P3 and A strains, for whom no changes in Day 4 withdrawal latencies were observed, and for SWR mice, in which Day 4 latencies were significantly increased. Considering strain means, the correlation between baseline nociceptive sensitivity on Days 1 and 4 approached significance (r=.69), but there was no correlation between Day 1 latency and latency alterations from Days 1 to 4 ($r = -.35$) (see Table 3). There was no significant alteration in baseline withdrawal latencies obtained on Day 4 relative to Day 1 in saline-treated control mice of any strain, suggesting that such alterations in morphine-treated mice were likely due to chronic drug administration and not possible confounding factors such as repeated handling, injection, or testing.

Table 1
Nociceptive sensitivity before and after chronic morphine treatment in 11 inbred mouse strains

Strain	Baseline latency[a]		Latency change[b]
	Day 1	Day 4	
129P3	2.4±0.1	2.1±0.1	−13%
A	2.4±0.1	2.4±0.2	0%
AKR	2.9±0.2	2.2±0.1	−24%*
BALB/c	3.1±0.2	2.5±0.2	−32%*
C3H/He	1.8±0.1	1.5±0.1	−19%*
C57BL/6	2.2±0.1	1.5±0.1	−17%*
CBA	1.9±0.1	1.3±0.1	−32%*
DBA/2	2.5±0.1	2.1±0.1	−16%*
LP	2.8±0.1	2.5±0.1	−11%*
SJL	2.2±0.1	1.8±0.1	−18%*
SWR	1.8±0.1	2.2±0.1	+22%[†]
Males	2.5±0.1	2.1±0.1	−16%[#]
Females	2.2±0.1	2.0±0.1	−9%

[a] Mean latency (s) ± S.E.M. to withdraw distal half of tail from 49 °C water.
[b] Percent reductions on Day 4 relative to Day 1 values.
* Significant decrease ($P<.05$) in baseline withdrawal latencies.
[†] Significant increase ($P<.05$) in baseline withdrawal latencies.
[#] Significantly different in latency change relative to females.

3.2. Morphine analgesia and tolerance

Morphine analgesic potency determined using AD_{50} estimates derived from cumulative dose–response curves did not differ between sex either before or after chronic morphine treatment in any strain tested. Thus, male and female cumulative dose–response data from Days 1 and 4 were pooled for each strain. As illustrated in Fig. 1, all strains displayed an increase in analgesia with increasing doses of morphine. However, the dose–response function can be seen to differ between strains. Indeed, derived AD_{50} values presented in Table 2 reveal significant potency differences between strains on Day 1, with a 6.7-fold difference between BALB/c (AD_{50}: 2.8, 95% CI: 2.3–3.5) and SWR (AD_{50}: 18.7, 95% CI: 15.6–22.0) mice, the most and least sensitive strains, respectively.

Heritability of Day 1 AD_{50} values was estimated at $h^2 = 0.31$, which is very similar to that obtained previously for this trait (Bergeson et al., 2001). Morphine analgesic dose–response curves obtained on following chronic morphine treatment, on Day 4 ($h^2 = 0.44$), are also illustrated in Fig. 1 alongside those obtained on Day 1. Significant rightward shifts were observed for 9 of the 11 strains tested on Day 4 relative to Day 1, demonstrating a loss of morphine analgesic potency. For two strains (129P3 and LP), the dose–response functions for Days 1 and 4 were practically indistinguishable. The heritability of tolerance in these strains was estimated at $h^2 = 0.28$, indicating that more than one-quarter of the observed variability among strains can be attributed to the additive effect of inherited genetic alleles. For reasons described above, this is likely an underestimate.

AD_{50} and relative potency estimates derived from strain morphine dose–response curves are presented in

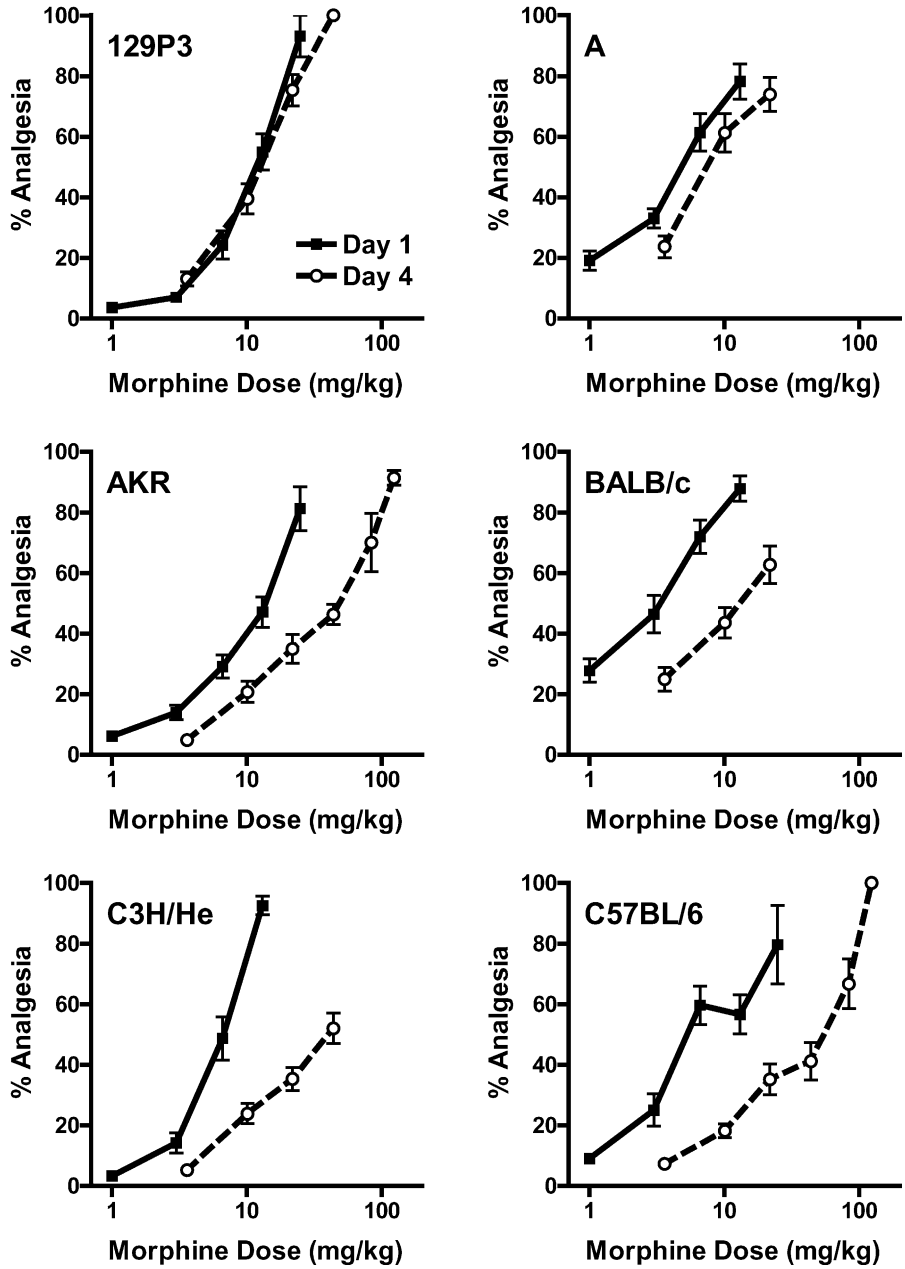

Fig. 1. Morphine dose–response relationships on the 49 °C tail-withdrawal assay for 11 inbred mouse strains before and after chronic morphine administration. Key to figures is contained in the first graph (top left). Morphine was injected three times daily for 3 days according to an escalating dose schedule (10, 20, and 40 mg kg^{-1} on Days 1, 2, and 3, respectively). Symbols represent strain mean percentages (±S.E.M.) of the maximum possible analgesia (% analgesia) at each cumulative dose on Days 1 and 4. Significant rightward curve shifts, indicative of analgesic tolerance, were observed for all strains except 129P3 and LP.

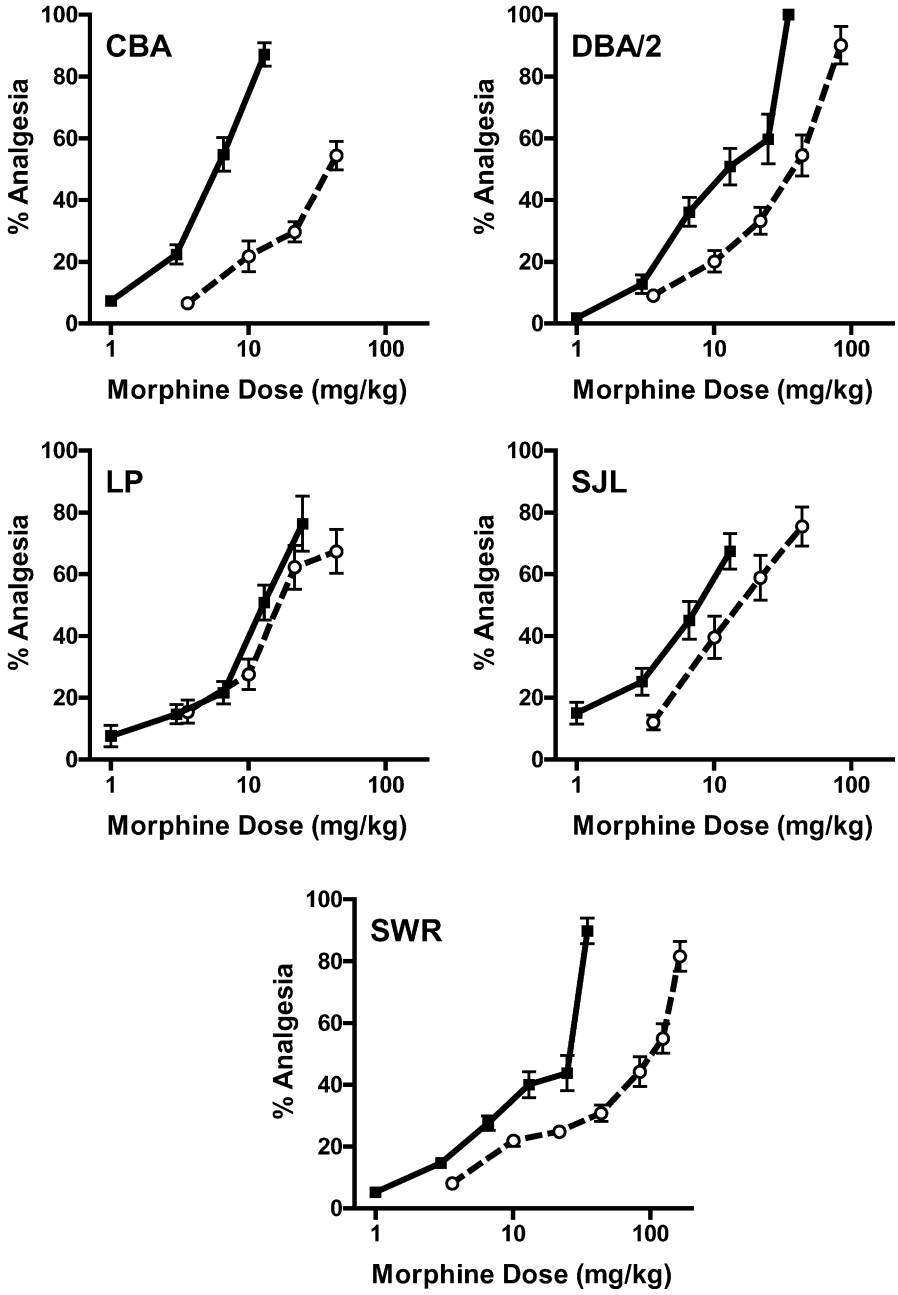

Fig. 1 (*continued*).

Table 2 and confirm these visual impressions. Day 4 AD_{50} values for all strains, except 129P3 and LP, were significantly increased relative to AD_{50} values obtained prior to chronic morphine injections on Day 1, indicative of tolerance. Even in strains where significant tolerance was observed, there was considerable interstrain variation in magnitude. Relative potency estimates in Table 2 show that potency reductions ranged from as low as approximately 2-fold in A and SJL mice, respectively, to as great as approximately 7-, 8-, and 11-fold losses in C57BL/6, C3H/He, and CBA mice, respectively. Day 4 morphine AD_{50} estimates obtained from saline-injected control mice did not differ from the Day 1 morphine AD_{50} estimates in experimental mice of any strain, nor did we observe any overt behavioral differences at any time (data not shown). Thus, strain differences in tolerance can not be attributed to response variability to repeated handling and/or injection.

There was no significant correlation between tolerance and baseline withdrawal latencies on Day 1 or 4, or between tolerance and initial morphine analgesic potency on Day 1. Tolerance was, however, significantly corre-

Table 2
Morphine analgesic potency before and after chronic morphine treatment in 11 inbred mouse strains

Strain	AD_{50} estimate[a]		Potency shift[b]
	Day 1	Day 4	
129P3	13.7 (11.3–16.5)	11.4 (9.9–13.2)	0.8
A	4.4 (3.6–5.4)	8.4 (6.7–65.4)	1.9 *
AKR	13.0 (10.9–15.4)	42.3 (35.7–50.1)	3.3 *
BALB/c	2.8 (2.3–3.5)	12.5 (9.5–16.5)	4.5 *
C3H/He	5.4 (4.7–6.3)	42.8 (35.3–51.8)	7.9 *
C57BL/6	6.8 (5.5–8.6)	48.7 (39.3–60.4)	7.2 *
CBA	5.0 (4.4–5.8)	53.9 (43.1–67.4)	10.8 *
DBA/2	13.8 (11.1–16.7)	39.6 (32.3–43.7)	2.9 *
LP	15.0 (11.9–18.8)	18.4 (14.4–23.5)	1.2
SJL	7.2 (5.6–9.3)	15.7 (12.5–19.7)	2.2 *
SWR	18.7 (15.6–22.0)	93.8 (80.2–109.7)	5.0 *

[a] Values are morphine dose (mg kg^{-1}) and 95% confidence interval endpoints (parentheses).
[b] Calculated as AD_{50} Day 4/AD_{50} Day 1 for that strain.
* Significant reduction in analgesic potency ($P < .05$).

lated with baseline latency changes resulting from chronic morphine treatment and with Day 4 AD_{50} values (see Table 3).

4. Discussion

Studies of morphine tolerance utilizing inbred strains are relatively few, making comparisons and confirmations between studies difficult. Compounding the problem is the use of the same few strains, but with comparisons typically limited to two strains in each study. Among those for whom data are available are C57BL/6 and BALB/c mice, for which we observed approximately equal magnitudes of tolerance. Similar findings were obtained by treating subjects with both higher (Eidelberg et al., 1975) and lower (Oliverio et al., 1975) morphine doses for, respectively, longer and shorter periods of time than those presently employed. Our observation of greater morphine tolerance in C57BL/6 relative to DBA/2 mice is also complimentary with the more rapid onset of tolerance observed in the C57BL/6 strain when an intracerebroventricular route was used to inject morphine (Frigeni et al., 1981). Although C57BL/6 mice have been differentiated from DBA/2 mice by their greater brain, spinal cord, and plasma morphine concentrations after chronic morphine treatment (Gwynn and Domino, 1984), we are of the opinion that a plausible explanation for greater morphine concentration can be advanced for either the more or less tolerant strain. Indeed, morphine tolerance for both strains in that study was of equal magnitude. In general, attempts to ascribe magnitude of tolerance to strain differences in morphine pharmacokinetic parameters have been unsuccessful (Gwynn and Domino, 1984; Mas et al., 2000), implying the existence of differential alleles at genes playing a pharmacodynamic role in tolerance liability.

Although the studies cited above were all performed using the hot-plate test of nociception, we are still unable to suggest that strain differences in sensitivity to tolerance, as revealed by our hot water tail-withdrawal test, may be generalized to other nociceptive assays since discrepancies exist. Using the hot-plate test to measure analgesia, DBA/2 mice were found to be 14% more tolerant than BALB/c mice (Oliverio and Castellano, 1974), whereas we found BALB/c mice to be approximately 50% more tolerant than DBA/2 mice on the tail-withdrawal test. Also, in contrast to our observation that there is an approximately 2.5-fold greater reduction in morphine potency in C57BL/6 mice relative to DBA/2 mice, these strains displayed equal levels of tolerance on the hot-plate test (Gwynn and Domino, 1984). Note, however, that in another study, C57BL/6 and DBA/2 differences consistent with our findings were observed using the hot-plate test (Frigeni et al., 1981). Certainly, differences in methodology such as morphine dose, injection route and schedule, and quantification of analgesia (i.e., area-under-the-curve vs. % analgesia vs. quantally defined responders), can contribute to variable findings between studies. Therefore, by utilizing many strains in a single study under identical experimental protocols, the present survey advances our understanding of variability associated with genotype in morphine tolerance.

Similar considerations may also underlie discrepant findings in our studies comparing responses of male and female mice. For example, significant sex differences in Day 1 morphine AD_{50} estimates were previously observed in 4 of the presently tested 11 strains, including C57BL/6 mice (Kest et al., 1999). Although there was no overall Strain × Sex interaction in the present study, planned contrasts did reveal a sex difference in analgesia for this particular strain. However, important differences between these studies remain, including the systemic vs. central routes of drug delivery. In contrast to the present subcutaneous delivery of morphine, intracerebroventricular microinjections of drug will not distribute along the entire neuraxis and recruit spinal and supraspinal analgesic mech-

Table 3
Spearman's rank (r_s) correlation coefficients between inbred mouse strains for nociceptive sensitivity and morphine analgesia before and after chronic morphine administration

	Baseline latencies[a]		Latency change[a]	AD_{50} estimates[b]		Potency shift[b]
	Day 1	Day 4		Day 1	Day 4	
Baseline latencies—Day 1	–					
Baseline latencies—Day 4	.69	–				
Latency change	−.35	−.38	–			
AD_{50} estimates—Day 1	−.05	.14	.02	–		
AD_{50} estimates—Day 4	−.54	−.51	.80 *	.27	–	
Potency shift	.48	.57	−.76 *	.35	−.79 *	–

[a] See Table 1 for definition of parameters.
[b] See Table 2 for definition of parameters.
* $P < .05$ after Bonferroni correction for multiple comparisons.

anisms shown to act in synergy (Roerig and Fujimoto, 1988). There may consequently be differences in analgesic responses elicited by the two different injection routes, disallowing simple comparisons between studies, including those on sex differences. Our present data also contrast with sex differences in morphine analgesic tolerance reported for CD-1 mice (Kest et al., 2000). This discrepancy may be simply related to differences in the strains used, indicating that sex differences may be more salient in CD-1 mice. Furthermore, as noted above, quantification of analgesia can contribute to variable findings between studies, and this is particularly true with regards to sex differences in morphine tolerance. Sex differences in tolerance in CD-1 mice were observed when analgesia is compared using ED_{50} estimates based on quantal data (% responders) but not % MPE (Kest et al., 2000). In contrast, analgesia in the present study quantified analgesia using % MPE values rendered into AD_{50} estimates to facilitate comparison with our previous (Kest et al., 1999) and ongoing studies of inbred mice.

The present data identify two strains, 129P3 and LP, that appear refractory to morphine tolerance. Previous studies have demonstrated that 129S6 mice (previously referred to as 129/SvEv), another 129 substrain, develop little or no morphine tolerance (Kolesnikov et al., 1998; Crain and Shen, 2000). Thus, although our description of 129P3 mouse insensitivity can be considered a partial replication of previous studies in the highly related 129S6 mouse, it is also a partial extension of these previous findings since 129 substrains display allelic variation at any number of genetic loci (Simpson et al., 1997). An important finding of the present study is that 129 mice are not unique in their resistance to tolerance development, since LP mice also displayed no tolerance, and other strains (e.g., A and SJL) exhibited the phenomenon only weakly. With regard to the absence of tolerance liability in 129S6 mice, previous research has yielded the following possible mechanisms. Crain and Shen (2000) have provided evidence that deficiencies in GM1 ganglioside-regulated excitatory opioid function mediate both the enhanced analgesic sensitivity and reduced tolerance to morphine. In the present study, however, Day 1 AD_{50} estimates for 129P3 mice were among the highest of all strains tested, indicating low morphine analgesic sensitivity. It should be admitted that this finding is in contrast to other data collected in our lab with these mice, showing relatively high sensitivity to systemic (unpublished data) and intracerebroventricular (Kest et al., 1999) morphine. We have at present no good explanation for the discrepancy, but note that the findings were obtained in different laboratories (see Crabbe et al., 1999). Kolesnikov et al. (1998) found functional deficiencies in the *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptor system of the 129S6 strain, and/or the biochemical cascade activating nitric oxide synthase consequent to its activation. The importance of this signaling system in morphine tolerance has been previously delineated (Elliott et al., 1995). Whether the lack of morphine tolerance development in 129P3 mice is due to similar deficiencies in NMDA/nitric oxide activation remains to be addressed. With regard to understudied LP mice, they were similar to 129P3 mice in displaying low initial morphine analgesic sensitivity. However, the overall strain correlation between the initial analgesic potency of morphine (Day 1) with the loss of potency after tolerance induction was not significant, consistent with the dissociation of analgesia and tolerance in many previous reports (Eidelberg et al., 1975; Gwynn and Domino, 1984; Kolesnikov et al., 1998).

An additional finding of the present study was that 3 days of repeated morphine injection strain-dependently altered baseline nociceptive thresholds. Tail-withdrawal latencies obtained prior to dose–response studies on Day 4 for 7 of 11 strains tested were significantly decreased by 11% or more relative to predrug Day 1 values, and by as much as 32% in C57BL/6 and CBA mice. These increases in nociceptive sensitivity stand in contrast to the significant decrease in nociceptive sensitivity of SWR mice, whose withdrawal latencies were increased 22%, and 129P3 and A mice, who were altogether refractory to change. Thus, although previous studies have reported increased nociceptive sensitivity on various assays in response to chronic morphine treatment (Mao et al., 1994; Hoffmann et al., 1998; Celerier et al., 1999; Vanderah et al., 2001; Li et al., 2001), to our knowledge the present data are the first to demonstrate that the presence, magnitude and even direction of these alterations in mice are strain-dependent. Furthermore, the lack of a significant correlation between Day 1 ($r = -.35$) baseline withdrawal latencies and their subsequent alteration by chronic morphine injection on Day 4 suggests their independent genetic mediation, and argues against the possibility that the phenomenon is confounded by Lord's (1967) paradox (i.e., the "law of initial values"). Germane to the present report, hyperalgesia following chronic opioid administration has been advanced as contributing to opioid analgesic tolerance (Mayer et al., 1999; Vanderah et al., 2001). Specifically, opioids may maintain their analgesic efficacy after repeated administration, but this analgesia is offset by a concomitant increase in pain sensitivity (Celerier et al., 1999). All strains considered, we found a significant genetic correlation between strain rankings for changes in baseline latencies (i.e., hyperalgesia) and analgesic tolerance. Genetic correlation of these or any two traits directly implies, although does not prove, similarity of their physiological substrates (see Mogil et al., 1999b). However, further studies are needed to confirm our results for two reasons. First, hyperalgesia can be observed 1–3 days after even a single 30 mg kg^{-1} morphine injection, even in the absence of a withdrawal-precipitating antagonist (Grilly and Gowans, 1986). Thus, hyperalgesia in the present study may have resulted from one or more spontaneous "mini-withdrawal" episodes elicited and interspersed among the lengthy inter-injection intervals during tolerance induction. Second, our 49 °C water bath for the tail-withdrawal test elicited mean withdrawal latencies of between 1.8 and 3.1 s, towards the lower limits of physiologically allowable

responses. Thus, our ability to detect greater reductions within strains, and differences between strains, may have been obfuscated by a floor effect. We are therefore currently comparing these same 11 strains for changes in baseline nociception using mice receiving continuous subcutaneous morphine infusion from implanted osmotic minipumps, thereby circumventing possible spontaneous withdrawal between injections, and milder water bath temperatures that substantially lengthen initial response latencies.

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