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Heritability of nociception I: Responses of 11 inbred mouse strains on 12 measures of nociception

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Abstract

It is generally acknowledged that humans display highly variable sensitivity to pain, including variable responses to identical injuries or pathologies. The possible contribution of genetic factors has, however, been largely overlooked. An emerging rodent literature documents the importance of genotype in mediating basal nociceptive sensitivity, in establishing a predisposition to neuropathic pain following neural injury, and in determining sensitivity to pharmacological agents and endogenous antinociception. One clear finding from these studies is that the effect of genotype is at least partially specific to the nociceptive assay being considered. In this report we begin to systematically describe and characterize genetic variability of nociception in a mammalian species, *Mus musculus*. We tested 11 readily-available inbred mouse strains (129/J, A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C58/J, CBA/J, DBA/2J, RIIIS/J and SM/J) using 12 common measures of nociception. These included assays for thermal nociception (hot plate, Hargreaves' test, tail withdrawal), mechanical nociception (von Frey filaments), chemical nociception (abdominal constriction, carrageenan, formalin), and neuropathic pain (autotomy, Chung model peripheral nerve injury). We demonstrate the existence of clear strain differences in each assay, with 1.2 to 54-fold ranges of sensitivity. All nociceptive assays display moderate-to-high heritability ($h^2 = 0.30–0.76$) and mediation by a limited number of apparent genetic loci. Data comparing inbred strains have considerable utility as a tool for understanding the genetics of nociception, and a particular relevance to transgenic studies. © 1999 International Association for the Study of Pain. Published by Elsevier Science B.V.

Keywords: Algesiometry; Inbred strains; Mouse genetics; Pain models; Strain differences

1. Introduction

A great deal of variability has been noted in pain responses among individual humans (e.g. Libman, 1934; Sherman, 1943; Beecher, 1946; Wolff et al., 1965; Chen et al., 1989), and across family and ethnic groups (e.g. Woodrow et al., 1972; Violon and Giurgea, 1984; Edwards et al., 1985; Bachiocco et al., 1993). Although this observation is generally interpreted as reflecting shared socialization rather than shared genes, it is also consistent with the possibility of genetic predisposition. It is particularly hazardous to ignore the possibility of constitutional effects on pain sensation in man in light of the emerging literature on the genetics of other complex psychosocial phenomena in humans, and of the growing body of information regarding pain genetics in animals.

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Increasing attention is especially being paid to behavior genetics of the mouse (*Mus musculus*). Mice are advantageous for such investigations because: (1) their small size renders them amenable to large genetic experiments (2) there exist a large number of inbred mouse strains with carefully catalogued pedigrees (see Festing, 1996) (3) the genetic linkage map for this species is more dense than for any other non-human mammal (Dietrich et al., 1996) and (4) transgenic knock-out technology is practical in this species.

With respect to studies of pain, we stress that a mouse is not a small rat; a number of relevant species differences have been noted (e.g. Taber, 1974; Yoburn et al., 1991; Rupniak et al., 1993). Moreover, a mouse is not a mouse. Early genetic studies revealed a surprising degree of genetic variation among common mouse stocks (see Klein, 1975). This variation is the basis of genetic studies, and a concern for the generalization of the findings obtained from experiments using a single mouse strain. Given this fact, knowledge of the basal nociceptive sensitivity of commonly used inbred mouse strains is decidedly lacking (see Mogil et al., 1996b for a review). To our knowledge, only a handful of studies have investigated nociceptive sensitivity across a large panel of mouse strains (Brown and Hughes, 1962; Ramabadran et al., 1982; Mogil et al., 1996a; Elmer et al., 1997), and even these have used only a limited range of nociceptive assays. Since there is an increasing interest in more clinically-relevant assays of pain in animals, and since genetic sensitivity to nociception appears to depend on the assay (Mogil et al., 1996a; Elmer et al., 1997) we undertook to test a large number of inbred mouse strains for responses in a broad range of murine assays of nociception.

The aims of this effort are three-fold. First, we wished to provide a comprehensive data set of inbred mouse strain nociceptive sensitivity for use by the pain research community in designing future experiments. Second, we wished to examine the degree of heritability, and the genetic complexity, of various types of nociception. Finally, we wished to examine cross-genotype correlations among nociceptive measures, a parameter that may indicate which types of pain share common underlying mechanisms. These issues are addressed in this and in our companion paper (Mogil et al., 1999).

2. Methods

2.1. Subjects

In all experiments we used male mice of the following strains, obtained from The Jackson Laboratory (Bar Harbor, ME): 129/J (129; Stock #690), A/J (A; #646), AKR/J (AKR; #648), BALB/cJ (BALB/c; #651), C3H/HeJ (C3H/He; #659), C57BL/6J (C57BL/6; #664), C58/J (C58; #669), CBA/J (CBA; #656), DBA/2J (DBA/2; #671), RIIIS/J (RIIIS; #683) and SM/J (SM; #687). All mice were shipped by air at 4–7 weeks of age and were acclimated to the vivarium for at least 1 week prior to testing or surgery at 7–10 weeks of age. Mice were housed in grouped cages in temperature-controlled environments, maintained on a 12:12 h light–dark cycle, and fed and watered ad libitum. Cage position on the rack shelves was randomized. Each mouse was only used in a single assay. It was a practical impossibility to test all strains simultaneously to eliminate testing day effects as an environmental confound, but steps were taken, insofar as possible, to standardize testing conditions. All experiments were performed near mid-photophase to minimize circadian variability (Kavaliers and Hirst, 1983).

2.2. Nociceptive assays (listed in alphabetical order by abbreviation)

2.2.1. Abdominal constriction (writhing) tests (acetic acid, AC_{AA}; magnesium sulfate, AC_{MS})

In these assays of chemical nociception (e.g. Siegmund et al., 1957; Hendershot and Forsaith, 1959; Koster et al., 1959), a noxious substance is injected into the peritoneal cavity, wherein it activates nociceptors directly and/or produces inflammation of visceral (subdiaphragmatic organs) and subcutaneous (muscle wall) tissues. Although many substances (e.g. acetic acid, acetylcholine, adenosine triphosphate, bradykinin, hypertonic saline, magnesium sulfate, phenylquinone) will produce the characteristic 'writhing' response that is scored in this assay, we chose to use two contrasting compounds: acetic acid (AC_{AA}) and magnesium sulfate (AC_{MS}).

For AC_{AA}, the most commonly used of these assays, 0.6% glacial acetic acid was injected intraperitoneally (i.p.) in a volume of 10 ml/kg. For AC_{MS}, 120 mg/kg magnesium sulfate was injected using the same route and volume. Acetic acid produces inflammation and inflammatory nociception with delayed onset (≈5 min) and long duration (≈30 min). In contrast, magnesium sulfate injection yields writhing almost immediately, with a duration rarely exceeding 5 min. Due to the lack of effect of anti-inflammatory analgesics in mice injected with magnesium sulfate, and the absence of prostaglandin metabolites in the peritoneal fluid, Gyires and Torma (1984) proposed that AC_{MS} assays for acute, non-inflammatory, prostaglandin-independent nociception.

We confirmed the presence inflammation in AC_{AA} in our mice, and its apparent absence in AC_{MS}, in consultation with a veterinary pathologist (C.A. Lichtensteiger, pers. commun.)

Prior to injection, mice were habituated for 1 h to the testing room, and for 10 min to individual Plexiglas observation chambers ($27 \times 17 \times 12$ mm) placed on corn-cob bedding. For the first 20 min following acetic acid injection and first 5 min following magnesium sulfate injection, the number of abdominal constrictions or writhes – lengthwise stretches of the torso with a concomitant concave arching of

the back – were counted and totaled. Some of these data were reported previously (Elmer et al., 1997).

2.2.2. Autotomy following hindlimb denervation (AUT)

Autotomy is an animal model of denervation dysaesthesias and pain of the sort present in anesthesia dolorosa and phantom limb (Wall et al., 1979). Under pentobarbital anesthesia (50 mg/kg, i.p.), the sciatic nerve was exposed unilaterally at mid-thigh, with an equal number of mice operated on the right and left sides. The nerve was tightly ligated with 5–0 silk, and cut across, 0.5–1.0 mm distal to the ligature, using a sharp blade. About 5 mm of the distal stump was excised to further suppress regeneration. In the same leg, the saphenous nerve was exposed near the knee, and likewise ligated and cut. Following surgery, wounds were closed in layers using silk sutures and Michel clips, and the mice were treated with topical and systemic antibiotics. Following recovery from anesthesia, animals were returned to the vivarium.

Complete denervation of the hindlimb was verified 1–7 days postoperatively (d.p.o.) by the absence of a flexion response to pinching the paw, including the medial and lateral toes. The extent of autotomy was rated weekly using the scale of Wall et al. (1979). Briefly, one point was given for the removal of one or more toe nails, and an additional point given for injury to each half phalange for a total maximum possible score of 11. Autotomy scores at the 35th d.p.o. were used for analyses.

2.2.3. Carrageenan hypersensitivity (CAR_{HT})

Injection of lambda carrageenan – a mucopolysaccharide derived from the Irish sea moss, *Chondrus* – produces inflammation, hypersensitivity, and some apparent spontaneous pain with a peak effect at 3–5 h post-injection (see Winter et al., 1962; Tonussi and Ferreira, 1992). These effects are sensitive to reversal by corticosteroids, non-steroidal anti-inflammatory drugs and opiates (Winter et al., 1962; Otterness and Bliven, 1985; Tonussi and Ferreira, 1992).

Carrageenan (2%; 20 mg/ml; Sigma) was suspended by sonication in saline, and injected subcutaneously in a volume of 20 μl into the right plantar hindpaw using a 50 μl microsyringe with a 27-gauge needle. Mice were tested for thermal sensitivity of both hindpaws on the test of Hargreaves et al. (1988) as described below (see Section 2.2.6) at 4.0 ± 0.5 h post-injection. There was little evidence of spontaneous pain (e.g. no licking/biting, scratching) in any strain. Hypersensitivity was quantified as a percentage using the formula: [(uninjected paw latency – injected paw latency)/uninjected paw latency] × 100. Although unilateral injuries have been reported to alter sensitivity in remote locations including contralateral sites (Levine et al., 1985), carrageenan does not appear to produce changes in nociceptive threshold in the contralateral hindpaw of the rat (Kayser and Guilbaud, 1987). In the present study we also demonstrate a very high correlation ($r = 0.92$) between strain means on Hargreaves' test for contralateral (i.e. uninjected) hindpaw values and basal (i.e. no injection) values.

2.2.4. Formalin test (F_{early}/F_{late})

Originally described by Dubuisson and Dennis (1977), and since modified for use in mice (Takahashi et al., 1984; Hunskaar et al., 1985; Murray et al., 1988), this assay measures behavioral responses to the subcutaneous injection of a dilute solution of formalin (37% w/w formaldehyde) into the hindpaw. Formalin injection produces a biphasic response: an acute, nociceptive 'early' phase (F_{early}) and a tonic, inflammatory 'late' phase (F_{late}), separated by a quiescent period in which there is no apparent pain behavior (see Tjolsen et al., 1992; Porro and Cavazzuti, 1993 for reviews). Unlike with carrageenan, the behavioral responses in this assay are spontaneously emitted and not a measure of sensitivity to an applied thermal or mechanical stimulus. One alleged advantage of the formalin test is that acute and sub-acute/tonic pain can be studied in the same subject after a single noxious event. Correspondingly, we have treated F_{early} and F_{late} as two separate measures of nociception, although we realize that they are not completely independent measures.

Mice were placed on a table top within Plexiglas cylinders (30 cm high; 30 cm diameter), and allowed to habituate for 30 min. Then, 25 μl of 5% formalin was injected subcutaneously into the plantar surface of the right hindpaw using a 50 μl microsyringe with a 30-gauge needle. Mice were then returned to the cylinders, and behavioral observations were begun immediately. The total time spent licking/biting the right hindpaw over the next 60 min was measured with a stopwatch and recorded to the nearest second in 5-min blocks; we have determined that in this species licking/biting is the only appropriate nociception-related dependent measure (Sufka et al., 1998). F_{early} was defined as the number of seconds spent licking/biting the affected hindpaw during the first 0–10 min post-injection of formalin; F_{late} as time spent licking/biting the hindpaw during the period 10–60 min post-injection. These data have been reported previously (Mogil et al., 1998).

2.2.5. Hot-plate test (HP)

A slightly modified version of the technique of Eddy and Leimbach (1953) (but originally described by Woolfe and MacDonald, 1944) was used. Mice were placed on a metal surface maintained at 53.0 ± 0.2°C (IITC Model PE34M-HC); a temperature in the middle of the range commonly reported in the literature (50–56°C). Locomotion of the mouse on the plate was constrained by 20-cm high Plexiglas walls to an area 14 × 14 cm. Latency to respond to the heat stimulus was measured to the nearest 0.1 s. Mice remained on the plate until they performed either of two behaviors regarded as indicative of nociception: hindpaw lick or hindpaw shake/flutter (Hammond, 1989; Espejo and Mir, 1993). No escape attempts (jumping) were observed in the present

study; we rarely observe this behavior in mice except after multiple hot-plate exposures and/or higher hot-plate temperatures. We used as criteria only hindpaw responses since forepaw licking and lifting are components of normal grooming behavior. Each mouse was tested only once since repeated testing in this assay leads to profound latency changes (e.g. Collins and Whitney, 1978; Gamble and Milne, 1989; Plone et al., 1996).

2.2.6. Hargreaves' test of thermal nociception (HT)

This assay (Hargreaves et al., 1988) is used to measure thermal hypersensitivity in the presence of neuropathy or inflammation, and also to measure basal sensitivity to noxious thermal stimulation.

Each mouse was placed in an individual chamber ($12.5 \times 12.5 \times 12.5$ cm) of a 12-chamber device (IITC Model 336) having transparent-Plexiglas outer walls to allow experimental observation, opaque-plastic inner walls that visually isolated each mouse from all others, and a 3/16th-inch-thick glass floor. Mice were acclimated for at least 2 h before testing. The stimulus was a high-intensity beam (setting = 3, $\approx$45 W) from a projector lamp bulb located 6 cm below the glass floor, and was aimed at the plantar surface of the mid-hindpaw of an inactive mouse. Paw-withdrawal latency was measured to the nearest 0.1 s. Response latencies in this test are robust against changes associated with repeated testing (Hargreaves et al., 1988), and thus we tested each mouse six times on each hindpaw, over a 4-h testing period. No significant effects of side were noted in any strain, so all 12 determinations were averaged.

2.2.7. Chung peripheral nerve injury model (PNI_{HT}, PNI_{VF})

In this neuropathic pain model (Kim and Chung, 1992), one of the three spinal nerves serving the hindlimb is tightly ligated. This procedure leaves residual, partial innervation of the hindlimb, avoiding total anesthesia and consequent autotomy. Surgery was carried under gaseous anesthesia with halothane (3.5% for induction and 1.7% for maintenance) in O_2. Following midline skin incision at L4-S2, paraspinal muscles were separated from the spinous processes and the transverse process of L6 was exposed on the left side and carefully removed with a small Rongeur. The L4-L6 segmental spinal nerves were exposed distal to the dorsal root ganglion (DRG) and the L5 spinal nerve was tightly ligated with 6–0 silk thread about 5 mm distal to the DRG, taking special care to avoid possible damage to the L4 spinal nerve. Following surgery, wounds were closed in layers using silk sutures and Michel clips. Following recovery from anesthesia (within 10–15 min), animals were returned to the vivarium.

Both hindpaws of each mouse were tested for thermal sensitivity 7 d.p.o., using Hargreaves' test (PNI_{HT}) with procedures identical to those described in Section 2.2.6. Hypersensitivity was quantified as a percentage using the formula: [(unoperated side paw withdrawal latency – operated side paw withdrawal latency)/unoperated side paw withdrawal latency] $\times$ 100.

A separate group of nerve injured mice were tested for mechanical sensitivity on the operated side, using calibrated von Frey filaments (PNI_{VF}; see Section 2.2.9). The preoperative threshold was determined by averaging the values of the last 3 days of 7 consecutive days of testing. The postoperative threshold was determined by averaging the values of the 4th and 7th d.p.o. Hypersensitivity was quantified as a percentage using the formula: [(average preoperative paw withdrawal threshold – average postoperative paw withdrawal threshold)/average preoperative paw withdrawal threshold] $\times$ 100. We determined that sham surgery did not alter mechanical thresholds in any strain (data not shown).

2.2.8. Tail-withdrawal test (TW)

This assay (Janssen et al., 1963) is a modified version of the classic tail-flick test developed by D'Amour and Smith (1941). Mice were lightly restrained in a cloth/cardboard 'pocket' into which the mice voluntarily entered. The protruding distal half of the tail was then dipped into a bath of circulating water thermostatically controlled at 49.0 ± 0.2°C (VWR Model 1110). Latency to respond to the heat stimulus with vigorous flexion of the tail was measured to the nearest 0.1 s. Three separate withdrawal latency determinations (separated by $\geq$20 s) were averaged. Since latencies in this test are known to be affected by tail skin temperature (Tjolsen and Hole, 1993), careful attention was paid to ensure that the ambient temperature was maintained at 22–23°C.

2.2.9. von Frey filament test of mechanical sensitivity (VF)

Mice were placed under a transparent plastic box ($4 \times 4 \times 7$ cm) on a metal mesh floor. Mechanical sensitivity was measured by determining the median 50% foot withdrawal threshold with von Frey monofilaments (bending forces: 0.3, 0.7, 1.6, 4.0, 9.8, 22.0 and 53.9 mN; applied using a single, steady >1-s application) using the up-down method (Chaplan et al., 1994). The threshold was determined on 7 consecutive days and the average value of the last 3 days was used for analyses.

2.3. Statistical analyses and calculation of genetic parameters

Data from the 12 dependent measures of nociception ('traits': AC_{AA}, AC_{MS}, AUT, CAR_{HT}, F_{early}, F_{late}, HP, HT, PNI_{HT}, PNI_{VF}, TW, VF) in each mouse strain were analyzed separately, to determine trait heritabilities, number of effective factors and strain differences (see Table 1). Raw data sets were subjected to one-way analyses of variance (ANOVAs).

Narrow-sense trait heritability was determined by comparing the between-strain variance to the total variance. Since animals are isogenic (i.e. genetically identical) within individual inbred strains, between-strain variance provides a measure of additive genetic ('allelic') variation (V_A),

Table 1

Sample sizes (n), standardized range of strain means, ANOVA results, estimates of heritability (h^2) and number of effective factors (#EF) mediating each nociceptive measure

Assay	n	Range[a]	F-value [b]	h^{2}[c]	#EF[d]
AC_{AA}	6	3.9	$F_{10,55} = 16.95$	0.76	2.8
AC_{MS}	6–12	17.2	$F_{10,70} = 9.97$	0.59	2.3
AUT	9–23	54.0	$F_{10,153} = 26.03$	0.63	2.2
CAR_{HT}	6–8	10.1	$F_{10,71} = 3.02$	0.30	3.4
F_{early}	7–13	2.5	$F_{10,94} = 5.89$	0.39	3.4
F_{late}	7–13	13.9	$F_{10,94} = 8.02$	0.46	2.8
HP	8–13	1.6	$F_{10,108} = 15.30$	0.59	2.5
HT	6–7	1.8	$F_{10,56} = 14.20$	0.72	2.9
PNI_{HT}	6	7.1	$F_{10,55} = 4.35$	0.45	2.3
PNI_{VF}	7–16	4.5	$F_{10,91} = 7.55$	0.45	3.6
TW	10–20	1.2	$F_{10,166} = 11.46$	0.41	2.9
VF	7–16	2.6	$F_{10,91} = 20.67$	0.69	1.9
Median		4.2		0.52	2.8

[a](largest mean − smallest mean)/smallest mean.
[b]All corresponding P-values are < 0.003.
[c]Calculated as $[V_A/(V_A + V_E)]$ (see text).
[d]Calculated as $[(\text{largest mean} - \text{smallest mean})^2/(4 \times V_A)]$ (see text).

whereas within-strain variance ('error variance') represents environmental variability (V_E). An estimate of narrow-sense heritability (h^2) for each trait was obtained using the formula: $h^2 = V_A/(V_A + V_E)$ (Falconer and Mackay, 1996). Since strains were chosen randomly, these values are likely accurate estimates of the true trait heritabilities (Hegmann and Possidente, 1981).

To estimate the number of apparent effective factors (EF; i.e. genetic loci) mediating each trait, we used the following formula adapted for use in inbred strains (see Falconer and Mackay, 1996): EF = [(highest strain mean − lowest strain mean)^2/(4 × V_A)]. Note that this calculation assumes that each locus is unlinked, and exerts equal and additive effects, with no epistatic interactions. Since these assumptions are unlikely, the calculation almost certainly underestimates the actual number of loci involved.

Strain differences were analyzed in several ways. First, using ANOVA, we demonstrated the significant main effects of genotype in all traits. Then, we partitioned the mouse strains into three classes, using an algorithm that maximizes the within-groups sum of squared deviations from the group means for a specified number of classes (Fisher, 1958). For each nociceptive assay we partitioned strains into 'sensitive', 'average' and 'resistant' classes. No restrictions were placed on the number of strains in each class. Standardized strain mean data for the 12 nociceptive measures were also used to define the 'distinctiveness' of each strain; to determine to what extent each strain differs from the average of all strains examined.

Finally, strain means were subjected to correlation and multivariate analyses to identify genetic relationships between the nociceptive assays suggestive of common physiological mediation. Results of this effort are presented in our companion manuscript (Mogil et al., 1999).

3. Results

Strain means in each nociceptive measure are shown in Fig. 1. There was a great deal of response variability across the inbred mouse strains examined. Moreover, the relative responsiveness of the strains differed depending on the nociceptive assay in question; no strain was consistently highest or lowest in all or even most of the assays. ANOVA revealed a significant main effect of genotype for each of the 12 measures (see Table 1), with P-values ranging from 0.003 to 9.7×10^{-12} (not shown). Corresponding values for heritability (h^2) ranged from 0.30 to 0.76 (Table 1).

The between-strains variability can be accounted for on the basis of allelic variation at a reasonably small number of genetic loci (i.e. oligogenic transmission). The minimum EF for each measure ranged from 1.9 to 3.6 (Table 1).

Table 2 shows the breakdown of the 11 strains as 'sensitive', 'average' or 'resistant' responders in each nociceptive measure. Each strain displayed a unique profile of responsiveness.

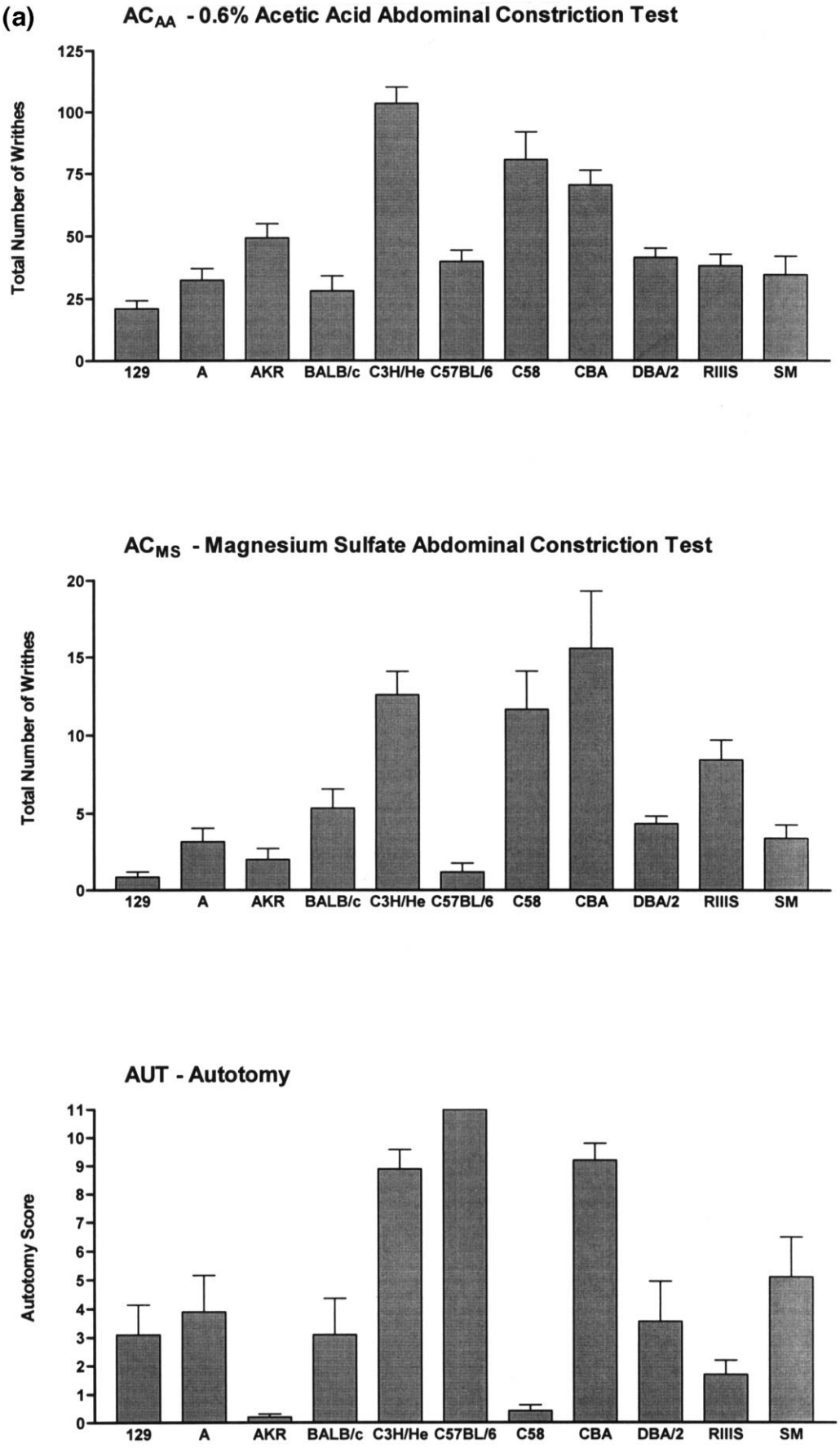
Mean absolute values of standardized strain means (i.e. mean $|z\text{-scores}|$) on all nociceptive measures are given in Table 3. Large numbers indicate that a particular strain is highly distinctive phenotypically relative to other strains.

Several of the nociceptive assays employed involved repeated measures or time-course data (AC_{AA}, F_{late}, HT, PNI_{VF}, TW, VF). No significant strain x repeated measure interactions were noted in any case (except for F_{late}; see below). In the AC_{AA} test, peak writhing occurred between 5 and 10 min post-injection; no obvious temporal differences were noted between strains. In F_{late}, strain-dependent temporal effects were observed: less sensitive strains exhibited delayed peak responding. This phenomenon has been discussed in a previous manuscript (Mogil et al., 1998).

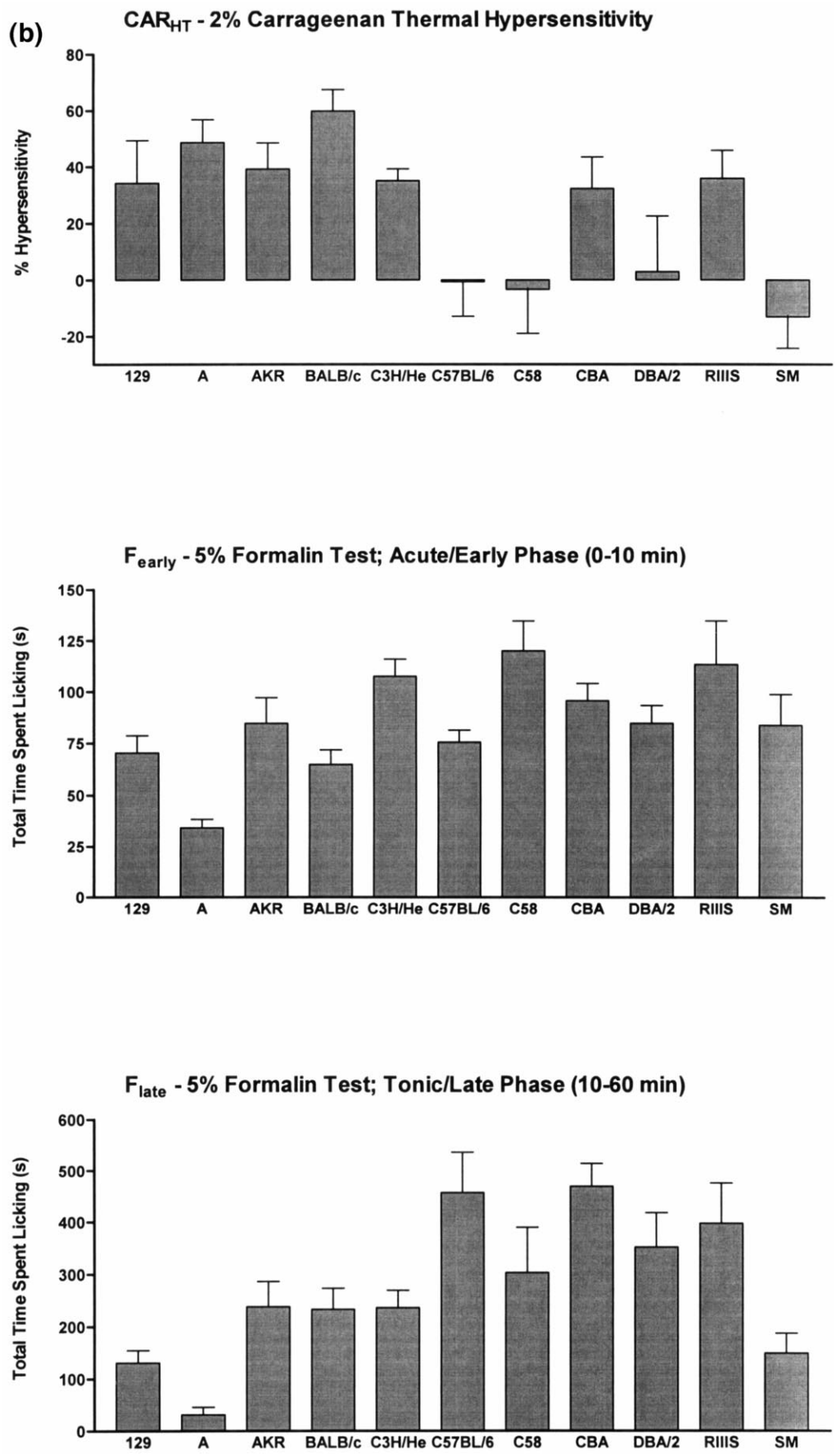
The usefulness of these data depends on the accuracy and reliability of the strain means obtained. Certain trade-offs possibly affecting accuracy were made for pragmatic reasons. For instance, in some assays relatively small within-strain sample sizes were used so that a larger number of nociceptive assays could be investigated. Also, four of the assays (AC_{AA}, AUT, PNI_{VF} and VF) were conducted in laboratories other than that of the first author. This necessarily introduced additional environmental variance which could, in principle, interact with genotype to produce phenotypic differences among strains. This disadvantage was balanced against the advantage of having more experienced investigators perform the assays.

To estimate the reliability of the strain means obtained, several of the assays were repeated on different groups of particular mouse strains at an interval of weeks or months. Three such replications were carried out, in one case in different laboratories, and in each a high degree of repeatability was obtained: (1) In the AUT assay, three independent groups of C3H/He mice (n = 10, 11, 17) and three independent groups of C58 mice (n = 6, 10, 17) were tested at an interval of months. Mean AUT scores obtained were

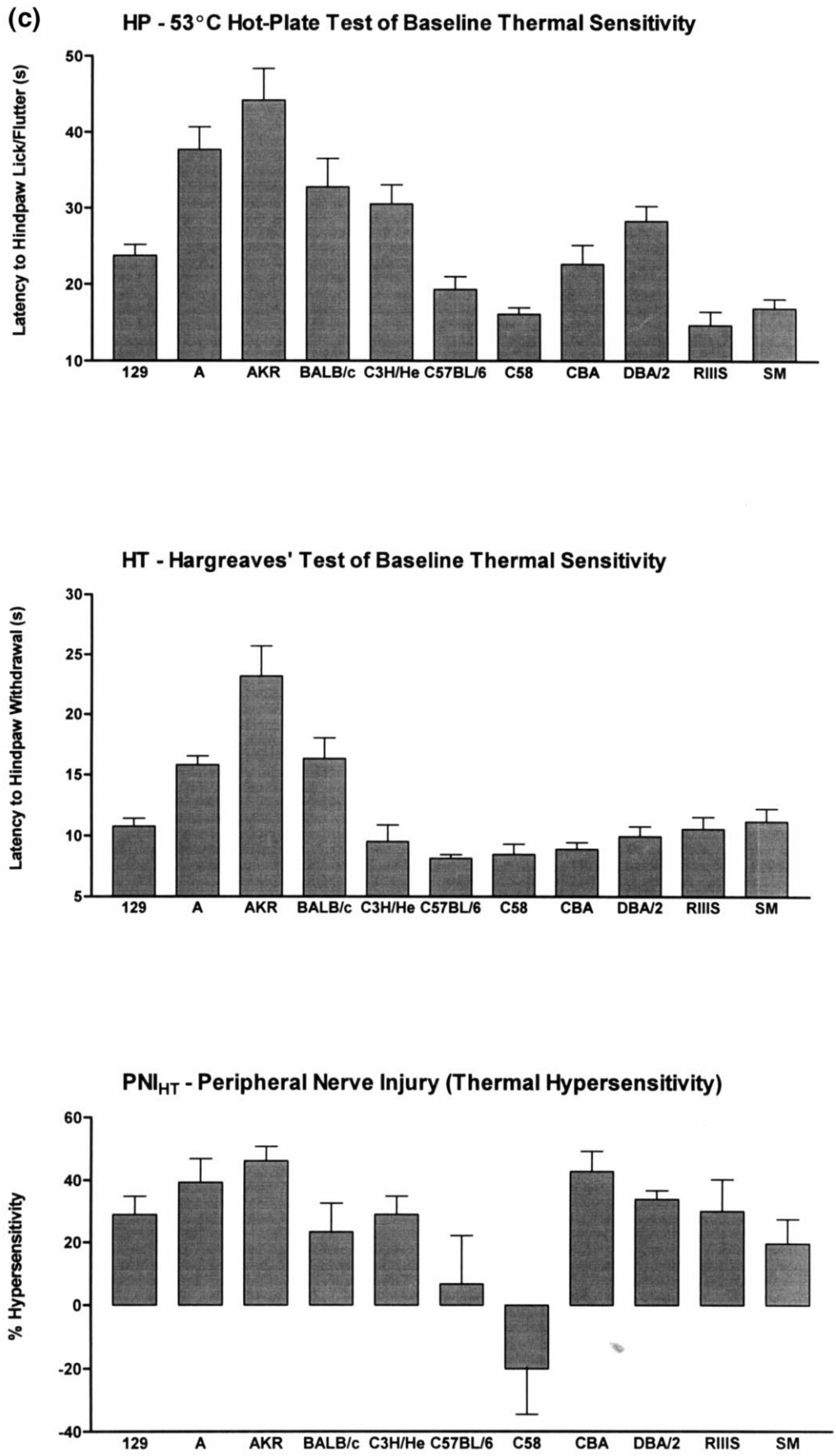
1a.


(a)
AC$_{AA}$ - 0.6% Acetic Acid Abdominal Constriction Test
Total Number of Writhes
0
25
50
75
100
125
129
A
AKR
BALB/c
C3H/He
C57BL/6
C58
CBA
DBA/2
RIIIS
SM
AC$_{MS}$ - Magnesium Sulfate Abdominal Constriction Test
Total Number of Writhes
0
5
10
15
20
129
A
AKR
BALB/c
C3H/He
C57BL/6
C58
CBA
DBA/2
RIIIS
SM
AUT - Autotomy
Autotomy Score
0
1
2
3
4
5
6
7
8
9
10
11
129
A
AKR
BALB/c
C3H/He
C57BL/6
C58
CBA
DBA/2
RIIIS
SM

1b.


(b)
CAR$_{HT}$ - 2% Carrageenan Thermal Hypersensitivity
% Hypersensitivity
80
60
40
20
0
-20
129 A AKR BALB/c C3H/He C57BL/6 C58 CBA DBA/2 RIIIS SM
F$_{early}$ - 5% Formalin Test; Acute/Early Phase (0-10 min)
Total Time Spent Licking (s)
150
125
100
75
50
25
0
129 A AKR BALB/c C3H/He C57BL/6 C58 CBA DBA/2 RIIIS SM
F$_{late}$ - 5% Formalin Test; Tonic/Late Phase (10-60 min)
Total Time Spent Licking (s)
600
500
400
300
200
100
0
129 A AKR BALB/c C3H/He C57BL/6 C58 CBA DBA/2 RIIIS SM

1c.


(c)
HP - 53°C Hot-Plate Test of Baseline Thermal Sensitivity
Latency to Hindpaw Lick/Flutter (s)
50
40
30
20
10
129 A AKR BALB/c C3H/He C57BL/6 C58 CBA DBA/2 RIIIS SM
HT - Hargreaves' Test of Baseline Thermal Sensitivity
Latency to Hindpaw Withdrawal (s)
30
25
20
15
10
5
129 A AKR BALB/c C3H/He C57BL/6 C58 CBA DBA/2 RIIIS SM
PNI$_{HT}$ - Peripheral Nerve Injury (Thermal Hypersensitivity)
% Hypersensitivity
60
40
20
0
-20
-40
129 A AKR BALB/c C3H/He C57BL/6 C58 CBA DBA/2 RIIIS SM

11, 11 and 8.2 for the C3H/He strain, and 0.3, 0.4 and 0.9 for the C58 strain. Data from the three replications in these strains were pooled for Tables 1–3 and Fig. 1. (2) HT data were collected in untreated mice of all 11 strains, and again, independently, as comparison data for the injected hindpaw in the CAR_{HT} and PNI_{HT} experiments. Across-strain correlations in these replicate measurements were very high ($r = 0.72–0.92$). (3) Basal TW data were collected in eight of these strains (all but C58, RIIIS, and SM), in an unrelated study conducted in a different laboratory (data not shown). Strain means were highly correlated with those obtained here ($r = 0.87$).

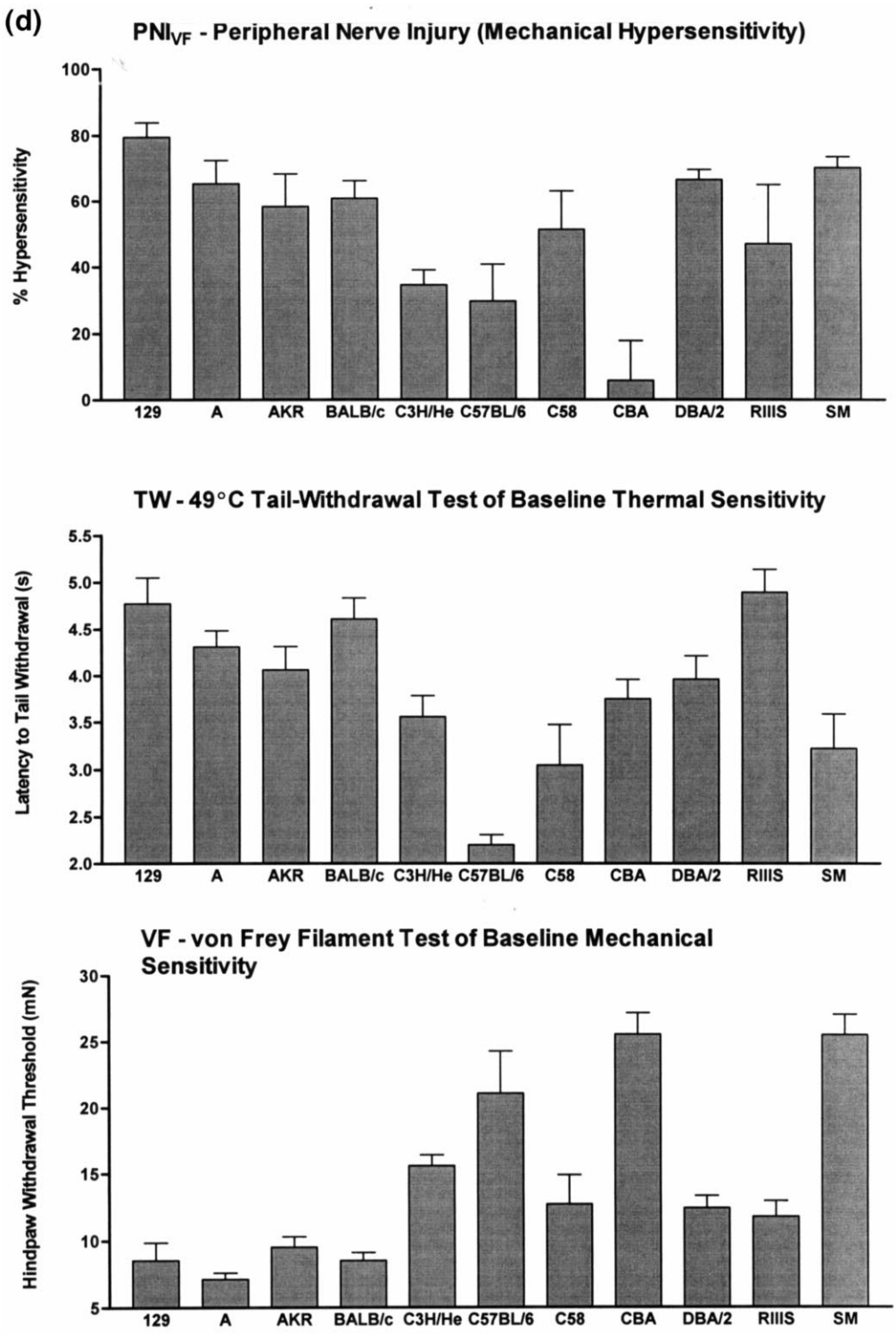


Fig. 1. Responses of 11 inbred mouse strains (see abscissae) on 12 behavioral measures of nociception (AC_{AA}, AC_{MS}, AUT, CAR_{HT}, F_{early}, F_{late}, HP, HT, PNI_{HT}, PNI_{VF}, TW, and VF). In each, bars represent the mean ± SEM of 6–23 mice (see Table 1).

4. Discussion

We have presented a systematic survey of the relative sensitivity of 11 inbred mouse strains using 12 different behavioral measures of nociception. Five (AC_{MS}, HP, HT, TW, VF) measure responsiveness of the intact somatosensory system to acute chemical, thermal, or mechanical stimuli ('nociceptive pain'). Four of the measures (AC_{AA}, CAR_{HT}, F_{early}/F_{late}) reflect responses to noxious chemicals known to provoke tissue inflammation. In three of these, behavioral responses are emitted spontaneously by the animal, and in one (CAR_{HT}) we measured changes in sensitivity to a thermal stimulus applied to the inflamed skin. The remaining three assays (AUT, PNI_{HT}, PNI_{VF}) involve neuropathic pain, chronic changes in sensory processing consequent to nerve injury. Both spontaneously-emitted behavior (autotomy) and sensitivity to applied thermal and mechanical stimuli were examined.

The main result was that genotype significantly affected performance in all of the nociceptive measures. That is, all of these behavioral traits have a significant heritable component in mice. Comparing across traits, each mouse strain had its own characteristic pattern of responsiveness. Here we discuss these basic indications of heritability, and consider the utility of inbred mouse strains in pain research.

In a companion paper (Mogil et al., 1999), we use more advanced statistical tools to compare the behavioral traits with one another. Together, the outcomes have implications for discovering underlying commonalties of mechanism, and for identifying the controlling genes.

4.1. Genetic versus environmental influences on nociceptive sensitivity

All of the inbred strains were derived by repeated brother–sister matings for >20 generations. As a result, genetic heterozygosity among members of the same strain is virtually zero. Except for rare de novo mutations, each individual mouse is genetically identical to (i.e. a clone of) every other member of its strain (see Silver, 1995). Variability among members of an inbred strain, therefore, must by definition arise from environmental sources. A corollary is that divergent inbred strain means represent predominantly genetic variability.

A potential caveat to this assertion is that interstrain variation may derive, in part, from interactions of genotype and environment. In general, the mechanism by which the protein product of a gene affects behavior is not simple. On the one hand, genetic variation could be related to proteins with direct and obvious connections to pain sensitivity, such as membrane channels responsible for afferent excitability, opioid receptors, or even factors determining the thickness of the cornified layer of the skin. On the other hand, such a gene could act through a highly indirect route with considerable potential for interaction with environmental variables. Examples are genes having to do with aspects of the animal's development or learning.

4.2. Influence of stress-induced antinociception on basal nociceptive sensitivity

A genetic-environmental interaction quite possibly relevant to the present study is stress-induced antinociception (SIA). It has been noted that habituation to the nociceptive testing apparatus or observation chamber can increase subsequent sensitivity on that test (Vidal et al., 1984; Abbott et al., 1986; Milne and Gamble, 1989; Aloisi et al., 1995; Plone et al., 1996). This phenomenon has been attributed to gradual habituation of SIA produced by the initially novel testing paradigm. Indeed, exposure to a novel environment has been directly shown to produce SIA (Siegfried et al., 1987; Kavaliers and Innes, 1988; Foo and Westbrook, 1991). Several of the nociceptive assays used here (AC_{AA}, AC_{MS}, CAR_{HT}, F_{early}/F_{late}, TW) also involve brief restraint and/or injection, which can also produce SIA (see Porro and Carli, 1988). It is not known whether SIA induced by novelty, restraint, or injection interacts with genotype, although this is a real possibility. It is known that 'emo-

Table 2
Qualitative rankings of all 11 inbred strains on each nociceptive measure

Strain	AC_{AA}	AC_{MS}	AUT	CAR_{HT}	F_{early}	F_{late}	HP	HT	PNI_{HT}	PNI_{VF}	TW	VF
129	–	–	0	+	0	–	0	+	0	+	–	0
A	–	–	0	+	–	–	–	0	+	+	0	+
AKR	–	–	–	0	0	0	–	–	+	+	0	+
BALB/c	–	–	0	+	0	0	–	0	0	+	–	+
C3H/He	+	+	+	0	+	0	0	+	0	0	0	0
C57BL/6	–	–	+	–	0	+	+	+	–	0	+	–
C58	0	+	–	–	+	0	+	+	–	0	+	0
CBA	0	+	+	+	+	+	0	+	+	–	0	–
DBA/2	–	–	0	–	0	+	0	+	0	+	0	0
RIIIS	–	0	0	+	+	+	+	+	0	0	–	0
SM	–	–	0	–	0	–	+	+	0	+	+	–

+, Sensitive; 0, average; –, resistant. Assignment of strains to these classes was achieved using Fisher's (1958) method, which minimizes the within-groups sum of squared deviations from the group means to arrive at an optimal partitioning.

Table 3

Genetic distinctiveness of 11 inbred strains with respect to nociceptive sensitivity

Strain	Mean |z-score| (SEM)[a]
129	0.72 (0.12)
A	0.95 (0.15)
AKR	0.82 (0.22)
BALB/c	0.71 (0.11)
C3H/He	0.72 (0.17)
C57BL/6	1.10 (0.14)
C58	0.93 (0.15)
CBA	1.01 (0.19)
DBA/2	0.37 (0.08)
RIIIS	0.63 (0.11)
SM	0.74 (0.15)

[a]Mean absolute values of standardized strain means (i.e. |z-scores|) on all nociceptive measures. Large numbers indicate genetic distinctiveness relative to other strains. Thus, the DBA/2 strain is the least distinctive (i.e. the most representative), and the C57BL/6 strain the most distinctive (i.e. the least representative) of the 11 inbred strains considered. These data closely reflect the strains' genealogical origins (Atchley and Fitch, 1991).

tional' mouse strains react differently to novel environments than do 'non-emotional' strains. In light of this possible caveat we asked whether results in our nociceptive assays correlate with emotionality measures (e.g. open field locomotion, elevated plus maze open arm entries) obtained in some of the same inbred strains by Trullas and Skolnick (1993) ($n = 6$ and 7 common strains, respectively). Correlations ranged from $r = -0.31$ to $r = 0.47$; none were significant.

4.3. Heritability of nociceptive sensitivity and number of apparent effective factors

Heritability in the narrow sense refers to the extent to which traits are determined by the genes inherited from the parents. It can be measured as the proportion of total trait variance attributable to genotype disregarding dominance, which does not exist among inbred strains (Falconer and Mackay, 1996). Heritability values of the 12 traits examined ranged from 0.30 to 0.76, (median = 0.52; see Table 1), where heritability of 1.0 means full determination by genotype (e.g. eye color) and heritability of 0.0 means full determination by environmental factors (e.g. 'mother tongue'). These values, which should be considered rough estimates, are similar to those of many other complex traits presently studied by behavioral geneticists (see Plomin, 1990).

The number of genetic loci effectively mediating the traits considered here range from 1.9 to 3.6, with a median of 2.8. As noted above, these estimates are extremely rough, and almost certainly underestimate the true number of genes responsible for the across-strain variability of these traits. Nonetheless, oligogenic mediation (few relevant genes) rather than polygenic mediation (many relevant genes) is implied in most cases. This conclusion is in fact consistent with prior biometric studies of various pain-related traits including basal nociceptive sensitivity, morphine antinociception, swim SIA, and neuropathic pain sensitivity as measured by autotomy (Devor and Raber, 1990; Mogil et al., 1995a,b). The high degree of heritability and the small number of apparent effective genetic loci render these traits highly amenable to genetic dissection using quantitative trait locus (QTL) mapping techniques (Soller et al., 1976; Lander and Botstein, 1989). It should be noted that the 'number of genes' refers not to the number constructing the pain-relevant circuitry, but rather to the number contributing to variability in the system's functioning. Only those genes that exist in alternate allelic forms, and have been fixed in allelically different homozygous states by inbreeding, can contribute to inbred strain variation. Similarly, only this subset of genes can be identified using QTL mapping strategies. However, we submit that it is precisely these genes that represent the most attractive targets for scientific and clinical advancement.

4.4. Implications for pain genetics: QTL mapping

We envision three major uses of the present data. First, our inbred strain survey can aid in choosing appropriate progenitor populations for QTL mapping studies. Second, the nociceptive sensitivity of certain inbred strains has important implications for the interpretation of existing transgenic knock-out (KO) studies and for the design of future studies. Third, these data represent the initial entries in what we hope will become a database of pain-related inbred strain information.

QTL mapping is the application of genetic linkage analysis to complex, quantitative traits (Lander and Botstein, 1989; Gora-Maslak et al., 1991). In this recently developed technique, segregating genetic populations (e.g. F_2 hybrids, backcrosses, recombinant inbred strains) are produced, tested on the phenotype of interest, and then 'genotyped', that is, assessed for the presence of alternate, codominantly-inherited alleles at polymorphic DNA sequence markers spanning the entire genome. QTL mapping can identify approximate chromosomal locations of genes contributing to variability between the progenitor populations ancestral to the segregating population. Chances of success using this method are maximized when the number of genes involved is small, and when progenitor populations display markedly different phenotypes (Soller et al., 1976; Lander and Botstein, 1989). Our data can inform the choice of appropriate nociceptive assays and strains for use in these efforts.

We have already performed the first QTL mapping studies of direct relevance to pain, investigating morphine antinociception (Belknap and Crabbe, 1992; Belknap et al., 1995), basal HP test sensitivity (Mogil et al., 1997a), and swim SIA (Mogil et al., 1997b). All three efforts employed recombinant inbred strains and F_2 hybrids derived from C57BL/6 and DBA/2 strains. The present data suggest, for

instance, that a new QTL mapping study of HP test sensitivity using extreme responders AKR and RIIIS (see Fig. 1) would be worthwhile.

4.5. Implications for pain genetics: KO studies

One major reason for the current interest in mouse genetics is the proliferation of mouse transgenic KO models, including those of relevance to pain (see Mogil and Grisel, 1998 for review). Although this technique is a powerful way to produce disruptive mutations of cloned genes, it is presently subject to several limitations (e.g. Gerlai, 1996; Lathe, 1996; Routtenberg, 1996; Mogil and Wilson, 1997). One such limitation is imposed by the fact that the embryonic stem (ES) cell lines used to carry the targeted mutation are all derived from various substrains of the 129 strain (Simpson et al., 1997). Standard KO development protocols involve breeding transgenic chimeras with the more fecund C57BL/6 strain, resulting in the placement of the null mutation on a mixed 129 × C57BL/6 background.

Two potential confounds arise from this strategy (Gerlai, 1996; Mogil and Wilson, 1997). First, it is difficult to separate by homologous recombination the 129-derived transgene from tightly linked genes. Even after repeated backcrosses to C57BL/6, a step most often omitted in the competition to publish, the wild-type and KO populations will differ in their inheritance of so-called 'hitchhiking donor gene' alleles (Crusio, 1996). KO mice will inherit 129-derived alleles at these genetic loci whereas wild-type mice will inherit C57BL/6-derived alleles. Observed phenotypic differences between wild-type and KO mice could, therefore, be due to the targeted mutation, to allelic variation at one or more of the many unidentified hitchhiking genes, or to an interaction between them (e.g. Schauwecker and Steward, 1997; Kelly et al., 1998).

A second potential confound arises since the background genes from the parental strains can interact with the targeted mutation ('epistasis'), importantly affecting the observed phenotype (e.g. Threadgill et al., 1995). This concern – which is also relevant to forward genetic strategies such as mutagen screenings that historically use the C57BL/6 genetic background – recently prompted the convening of an NIH workshop with the goal of providing a database of the behavioral phenotypes of inbred mouse strains, so that rational decisions could be made regarding the choice of background genotype in such efforts (Crawley, 1996).

Our data can be regarded as particularly worrisome in this context. Among the 12 nociceptive dependent measures we investigated, 129 and C57BL/6 mice displayed differential phenotypes in 8 (see Table 2). The hitchhiking donor gene confound is most problematic when KO mice display '129-like' phenotypes, since these phenotypes can be misinterpreted as effects due to the null mutation. Some existing KO studies of pain (see Mogil and Grisel, 1998) are potentially subject to this concern based on the present data (Malmberg et al., 1997a,b; Murata et al., 1997; Robertson et al., 1997). The conclusions of other studies are reinforced by these data since the KO mice display 'C57BL/6-like' phenotypes less likely to be associated with hitchhiking 129-derived alleles (Matthes et al., 1996; Rupniak et al., 1997; Sora et al., 1997; Xu et al., 1997). It should be noted that the 129 substrain tested herein was 129/J, whereas ES cell lines are derived from many different 129 substrains (Simpson et al., 1997). It remains unknown to what extent nociceptive phenotypes differ among 129 substrains.

Of the strains we tested, the C57BL/6 strain is one of the most sensitive overall, and the most genetically distinct with respect to the 12 nociceptive phenotypes (Table 3). We have also shown that C57BL/6 is among the least sensitive strains with respect to opiate antinociception (Belknap et al., 1995; Mogil et al., 1996a; Elmer et al., 1997). We contend that the distinctive responses of C57BL/6 mice to nociception renders it unlikely that the phenotypic expression of targeted or mutagen-induced mutations in this strain may be adequately generalized to the species as a whole (Mogil and Wilson, 1997).

We recommend, therefore, that instead of C57BL/6, mutation studies of pain-related traits, including KO designs, be conducted using a moderate-scoring and genetically non-distinct strain as the genetic background. Consultation of Tables 2 and 3 reveals the DBA/2 mouse as the best single choice. This strain exhibits average responsivity on six of the nociceptive assays and by far the lowest degree of genetic distinctiveness. DBA/2 mice are also advantageous due to their ready availability, relatively low price and reasonable fecundity (see http://www.jax.org/resources/). If mutant mice will only be tested on a limited number of nociceptive assays, Table 2 could be used to identify average-responding strains.

4.6. Studies of pain using inbred strains

The comparison of inbred mouse strains presents many advantages for the study of the genetic basis of pain. Inbred mice are readily available to the entire research community through commercial breeders. Inbred mice possess naturally-occurring alleles at trait-relevant genetic loci, albeit fixed in a homozygous state. This contrasts to null mutant (i.e. KO) mice that may never have existed in nature or were quickly selected against. Moreover, focus on single gene mutations artificially isolates the effects of one gene from its modifiers (i.e. ignores epistasis), and risks overestimation of the actual role of the targeted gene (Frankel and Schork, 1996).

A dense characterization of inbred strain comparisons, followed by QTL mapping, congenic strain development and positional cloning (e.g. King et al., 1997) if necessary, is a powerful alternative research approach. With the rapidly increasing knowledge of the mouse (and the human) genome, these strategies can map traits with sufficient precision such that the target gene(s) can be intuited from among a modest number of candidate genes on the

basis of known pain physiology. One is not limited to studying already-cloned genes, however, as one of the major aims of QTL mapping and positional cloning is the identification of novel trait-relevant genes that are not necessarily predictable on the basis of our current knowledge.

In addition to gene mapping efforts, important advances can be made by searching for additional correlated traits. Such correlated traits could be other behavioral assays of nociception, or any number of anatomical, physiological or neurochemical measures.

For instance, do the levels of a peptide in the spinal cord of these strains correlate with sensitivity to one or another of the nociceptive assays? The isogenicity of all individuals of an inbred strain obviates the need to replicate all parameters within the framework of a single research project. One can simply apply the new assay to the 11 inbred mouse strains, and then compare the results obtained to those reported here. Although a strain survey of all 11 strains would yield the most statistical power to investigate correlations, a simpler, and still potentially effective approach would be to consult Table 2 and choose representative sensitive, average, and resistant strains.

A full understanding of the pleiotropic effects of genes affecting pain behavior requires a much fuller characterization of inbred mouse strains than we have carried out here. We encourage other investigators to apply their expertise to this effort by characterizing some or all of these 11 strains on accessible parameters of all sorts. Ultimately, we would like to collaborate on the creation of a rich and widely useful database of pain-related inbred strain information.

4.7. Human relevance

A great deal of clinical evidence and experience attests to the wide individual variability in sensitivity to pain, vulnerability to painful pathologies, and response to analgesics (see Section 1). There are some examples, particularly in the realm of painful pathologies, where a clear pattern of inheritance has been identified (e.g. Golik et al., 1988; Kirkpatrick, 1989; Dyck et al., 1993; Battie et al., 1995). In every case, however, the trait inherited has been assumed to be susceptibility to the precipitating disease (e.g. nerve entrapment, diabetes mellitus, intervertebral disc herniation), rather than pain susceptibility per se. The possibility of differential predisposition for the disease to be painful, once inherited, must also be considered. It should be noted that a number of inherited painful pathologies including hereditary sensory neuropathy type I (Nicholson et al., 1996), familial hemiplegic migraine (Ophoff et al., 1996), and painful congenital myotonia (Rosenfeld et al., 1997) have recently yielded to an explanation on the genetic level, as has congenital insensitivity to pain (Indo et al., 1996). In the latter three cases, sequence analysis revealed the syndromes to be due to single base pair transversion or deletion mutations.

As in other fields, the discovery of genes associated with painful pathology in humans could provide valuable tools for diagnosis, prognosis, counseling and treatment. Such information might also go a long way towards removing the stigma from individuals who suffer more than the average from particular painful conditions. Identification of relevant genes in mice is likely to greatly accelerate the discovery process given the high degree of homology (‘synteny’) between the mouse and the human genomes (Nadeau and Reiner, 1989).

We are aware of only one recent study that has investigated the determination of non-pathological, experimental pain in humans. MacGregor et al. (1997), testing monozygotic and dizygotic twins for pressure pain threshold, found only a slight excess in twin/co-twin correlation for monozygotic twins corresponding to a heritability of only 10%. This finding reinforces the important role of environmental influences including family dynamics and learning of pain behavior (Violon and Giurgea, 1984; Edwards et al., 1985; Bachiocco et al., 1993; Lester et al., 1994). It is unknown at present whether genetic or environmental influences predominate in other experimental pain modalities and pathological pain conditions. Indeed, it should be noted that mice are an uncommonly genetically-variable species, and the demonstration of high heritability of nociception in this mammalian species does not necessarily predict a similar situation in humans.

Even if genetic factors are ultimately demonstrated to play only a minor role in the determination of pain sensitivity in humans, genetic approaches to the study of pain may still prove highly valuable. Such studies, for example, may illuminate those components of pain processing circuitry in mice and humans that are especially amenable to alteration; knowledge likely to be useful for the development of novel analgesic strategies. The use of these data to identify pain-related genes, and their protein products, could open new directions for research on mechanisms underlying pain.

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