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The effect of genotype on sensitivity to electroacupuncture analgesia

You Wan[a,b], Sonya G. Wilson[b], Ji-Sheng Han[a], Jeffrey S. Mogil[b,*]

[a]*Neuroscience Research Institute, Peking University Health Science Center, Beijing 100083, People's Republic of China*
[b]*Department of Psychology and Neuroscience Program, University of Illinois at Urbana-Champaign, 603 E. Daniel Street, Champaign, IL 61820, USA*

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Abstract

Individual differences in sensitivity to pain and analgesia are well appreciated, and increasing evidence has pointed towards a role of inherited genetic factors in explaining some proportion of such variability. It has long been known by practitioners of acupuncture, an ancient modality of analgesia, that some patients are 'responders' and others 'non-responders.' The present research was aimed at defining the inherited genetic influence on acupuncture analgesia in the mouse, using 10 common inbred strains. Two pairs of metallic needles were inserted into acupoints ST 36 and SP 6, fixed in situ and then connected to the output channel of an electric pulse generator. Electroacupuncture (EA) parameters were set as constant current output (intensity: 1.0–1.5–2.0 mA, 10 min each; frequency: 2 or 100 Hz) with alteration of a positive and negative square wave, 0.3 ms in pulse width. Tail-flick latencies evoked by radiant heat were measured before, during and after EA stimulation. Narrow-sense heritability estimates of 2 and 100 Hz EA were 0.37 and 0.16, respectively. We found that the C57BL/10 strain was the most sensitive, and the SM strain was the least sensitive to both 2 and 100 Hz EA. However, the relative sensitivities of other strains to these two EA frequencies suggested some genetic dissociation between them as well. These results demonstrate a role of inherited genetic factors in EA sensitivity in the mouse, although the low-to-moderate heritability estimates suggest that environmental factors may be of greater importance in predicting who will benefit from this analgesic modality.

Keywords: Antinociception; Pain; Acupuncture; Mice; Inbred strains; Genetic

1. Introduction

Acupuncture is a 3000-year-old practice developed in China, whose efficacy for producing analgesia is now becoming more well-accepted in the West (e.g. National Institutes of Health, 1997). It is well known that the analgesic effect of acupuncture varies from patient to patient, such that some can be classified as responders and others as non-responders. It would obviously be tremendously useful to patients and clinicians alike to be able to predict who would benefit from this particular analgesic modality.

Although most clinical acupuncture involves the manual manipulation of needles, the passage of electrical current through needles inserted into 'acupoints' – up to 365 in humans, located on hypothetical channels called meridians – produces more clinically efficacious analgesia, and is also a more standardized stimulus for scientific study. Andersson and Holmgren (1975) noted that low-frequency (2 Hz) and high-frequency (100 Hz) electroacupuncture (EA) produced dissociable effects in humans. Similarly, the neurochemical mediation of EA analgesia (EAA) from low- and high-frequency stimuli has been dissociated. Although some debate exists regarding the opioid mediation of EAA in rats (Bossut et al., 1991; Chapman et al., 1983), one of our laboratories has frequently and consistently demonstrated the involvement of opioid peptides and receptors in EAA. EAA from 2 Hz stimulation is associated with the release of β-endorphin and met-enkephalin into the cerebrospinal fluid, and can be blocked by pretreatment with μ- and δ-opioid receptor antagonists (see Ulett et al., 1998). By contrast, EAA from 100 Hz stimulation is associated with the release of dynorphin, and blocked by κ-opioid receptor antagonists (see Ulett et al., 1998).

Individual differences in EAA in outbred rats, the most common subject for non-human acupuncture research, have been consistently noted. For example, in a study of 168 Wistar rats, Liu and colleagues (unpublished data) found 100 Hz EAA ranging from non-existent to maximal, and a cluster analysis readily identified two modes. Of interest is the fact that 'low-responders' to EAA were also found to be insensitive to analgesia resulting from low-dose (0.5–3 mg/kg, s.c.) morphine administration or electrical stimulation of the dorsal periaqueductal gray, and 'high-responders' to

* Corresponding author. Tel.: +1-217-333-6546; fax: +1-217-244-5876.
E-mail address: jmogil@s.psych.uiuc.edu (J.S. Mogil).

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EAA were found to respond readily to these other opioid-mediated analgesias (Takeshige et al., 1983; Tang et al., 1997). Some evidence has been collected suggesting that this variability may be related to the activity of anti-opioid peptides. Specifically, functional reductions of the anti-opioid peptides cholecystokinin-8 (CCK-8) (Faris et al., 1983) and orphanin FQ/nociceptin (Mogil et al., 1996a) can convert the EAA (and low-dose morphine analgesia) of low-responder rats to high-responder status (Tang et al., 1996; 1997; Tian et al., 1998). Supporting the notion that cholecystokinin may play a role in mediating individual differences in EAA in rats is the observation that the P77PMC rat strain, derived from Wistar rats and demonstrating congenital audiogenic seizures and reduced brain CCK-8 content, shows increased sensitivity to 100 Hz EAA (Zhang et al., 1997).

Interindividual variability in response to analgesic manipulations is by no means restricted to acupuncture. For example, Lasagna and Beecher (1954) documented the inability of 10 mg morphine to relieve pain in up to 33% of human patients. The existence of responders and non-responders to non-steroidal anti-inflammatory drugs (NSAIDs) is also very well recognized (e.g. Scott et al., 1982). Although environmental factors no doubt account for much (perhaps even the majority) of such variability, studies using inbred strains of rodents have consistently noted robust strain mean differences in analgesic sensitivity (see Mogil, 1999), implying a role of inherited genetic factors.

Therefore, using 10 inbred strains of mice, the present study aimed to investigate the relative importance of genetic factors in explaining observed variability in EAA. In addition, we hoped to identify contrasting extreme-responder strains that could be exploited for future gene mapping efforts.

2. Methods

2.1. Subjects

Breeding pairs of the following inbred mouse strains were purchased from The Jackson Laboratory (Bar Harbor, ME): 129P3 (129), A, AKR, BALB/c (B/c), C3H/He (C3H), C57BL/6 (B6), C57BL/10 (B10), C58, RIIIS (R3) and SM (all 'J' substrains). Offspring were weaned at 18–21 days of age, and subsequently housed with their same-sex littermates (up to four per cage; mice with no same-sex littermates were tested) in wire-topped polycarbonate shoebox cages, and maintained under a 12:12 h light/dark cycle (lights on at 07:00 h) and an ambient temperature of 22 ± 1°C. Mice were fed (Purina chow) and tap-watered ad lib. Care was taken to avoid any systematic environmental variability in the housing and testing of these strains; for example, cage locations were randomized. Experimentally naïve mice of both sexes, 6–10 weeks of age, were used in all experiments. At least two different litters of each strain were tested so as not to confound true genotype differences with litter effects (e.g. litter size, sex ratio, maternal influences) (Blizard, 1992).

Control studies were run using male and female outbred Swiss Webster (Hsd:ND4) or CD-1® (Hsd:ICR) mice bred and housed similarly from breeders obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN).

2.2. Animal fixation

Mice were restrained in 8-cm long, cylindrical Plexiglas restrainers, and partially immobilized therein by pressing cotton balls over the sacral region. Small holes in the anterior end of the restrainer provided ventilation. The hind legs of the mouse extended out through the two rear openings. After the mouse entered the restrainer, a round metal piece was used to cover the posterior entrance, except for a hole out through which the mouse's tail extended. This fixation allowed the insertion of acupuncture needles into the hind legs and freed the tail for nociceptive testing. Mice so restrained were attached to the top of a rack suspended 25 cm above a table top, allowing simultaneous testing of eight mice per session. As much as practically possible based on mouse availability, multiple strains and both sexes were represented in each testing session.

2.3. Insertion and connection of needles

Each stainless steel acupuncture needle (0.15 mm diameter) was bent into an 'L' shape. The proximal end was soldered to a wire that was connected to one of the output channels of an electric stimulator, HANS (Han's Acupoint Nerve Stimulator; Neuroscience Research Institute, Peking University Health Science Center, China). The bent distal ends of two needles per mouse were inserted into acupoints on the hind leg: (1) Zusanli (ST 36), near the knee joint, 2 mm lateral to the anterior tubercle of the tibia; and (2) Sanyinjiao (SP 6), near the ankle joint, at the level of the superior border of the medial malleolus between the posterior border of the tibia and the anterior border of the Achilles tendon. Needles were inserted 3 mm in depth in ST 36 and just penetrable in SP 6. After insertion, the needles were fixed in situ with adhesive tape.

2.4. Nociceptive testing

Nociceptive threshold to acute, thermal stimulation was measured using the tail-flick test (D'Amour and Smith, 1941). Focused light from 12.5 W projection bulb was applied directly to the middle of the tail (3 mm diameter). The projection bulb was turned off as soon as the mouse flicked its tail, and a digital timer connected in series measured the tail-flick latency (TFL) to an accuracy of 0.1 s. Before the experiment began, voltage was adjusted to produce 4–5 s basal TFLs in outbred mice, and then remained constant thereafter. During the experiment, room temperature was carefully monitored to be 22 ± 1°C, to

minimize the possible influence of ambient temperature on TFLs (Tjolsen and Hole, 1993). We used a cut-off latency of 10 s in order to avoid the possibility of tissue damage to the superficial tail.

In order to habituate mice to the restrainer, experiments began at least 30 min after insertion and fixation of needles. Then, TFL was assessed three times at 5-min intervals and the mean value of the three assessments was taken as the basal nociceptive threshold. This averaging was justified statistically by the lack of a significant repeated measures effect over the three basal TFLs, or a significant strain x repeated measures interaction.

2.5. EA parameters

EA was applied after the basal threshold determination. The EA parameters were set as follows: constant square wave current output (pulse width: 0.6 ms at 2 Hz, 0.2 ms at 100 Hz); intensities ranging from 1.0–1.5–2.0 mA; at a frequency of 2 or 100 Hz. Stimulation time was 10 min at each intensity, totaling 30 min of stimulation. TFLs were measured 10, 20, and 30 min after EA onset. When the TFL was being tested, the corresponding output channel to that mouse was temporarily turned off, and turned on again once the measurement was complete. Since EAA is known to outlast the stimulation itself, post-EA TFLs were measured at the 40, 50 and 60 min time points.

Mice of each strain and sex were divided randomly into 'EA' and 'Control' groups. After basal thresholds were determined, electrical stimulation was applied in EA group only. Half of the EA group received 2 Hz stimulation; the other half received 100 Hz stimulation. After testing, mice were returned to the vivarium. Ten days later, all EA group mice were tested again with the opposite frequency; Control group mice again received sham testing.

2.6. Statistical analyses

For purposes of analysis, EAA was expressed as percentage of the maximum possible effect (%MPE), as calculated by the following formula: %MPE = [(peak EA latency − baseline latency)/(cut-off limit − baseline latency)] × 100. The use of %MPEs takes into account the cutoff and individual baseline latencies, so that these will not bias the quantification of analgesia. We chose to use peak TFL value from the six EA and post-EA time points (10–60 min) for statistical analysis. We also tried other dependent measures, including area under the curve (AUC) of the raw TFL data (from 0 to 30 and 0 to 60 min) and percentage change from baseline. Correlation analysis indicated that all indices were highly correlated with each other except AUC, which may be due to the lower basal TFLs of B6 and SM strains. Peak %MPEs were thus calculated for the EA and Control groups. Control group mice from every strain tested exhibited virtually no analgesia, but in order to isolate the specific analgesic effect of EA, we calculated a 'differential peak %MPE' value for each EA group mouse, subtracting the mean peak %MPE of the Control mice of that strain from that of the individual EA group mouse.

Basal TFLs and EAA values were analyzed via two-way ANOVA (strain × sex). Raw TFLs over the time course of the data collection were analyzed by repeated measures ANOVA. Individual strain differences were assessed by Tukey's post-hoc test where appropriate, and sex differences by *t*-test. The criterion level of significance in all cases was $P < 0.05$.

Since individual members of inbred strains are isogenic, genetically identical at virtually all loci, between-strain variance provides an estimate of additive genetic variation (V_A), whereas within-strain variance represents environmental variability (V_E) (Falconer and Mackay, 1996). Thus, we estimated narrow-sense heritability (h^2) of 2 and 100 Hz EAA from a one-way ANOVA of EA group mice as $h^2 = V_A/(V_A + V_E)$. These estimates are likely to be fairly accurate since strains were chosen randomly (Hegmann and Possidente, 1981).

3. Results

3.1. Basal nociceptive thresholds: strain and sex differences

As expected, strain and sex significantly affected basal TFLs ($F_{9,596} = 32.64$, $P < 0.001$; $F_{1,596} = 50.57$, $P < 0.001$, respectively). Also not unexpectedly, we observed a significant strain × sex interaction ($F_{9,596} = 2.10$, $P < 0.05$), an interaction that approached significance in a previous strain survey using the related 49°C hot water tail-immersion/withdrawal assay (Kest et al., 1999). Table 1 shows basal TFLs in all mice (EA group and Control group data combined). B6 and SM mice were the most sensitive strains, exhibiting significantly shorter TFLs than all other strains. Although female mice were more sensitive to thermal nociception overall, the sex difference was only significant in the 129, AKR, B6, B/c, C3H, and C58 strains. These data are very similar to those obtained previously on the tail-withdrawal test, where we found significant sex differences in AKR, B6 and C3H mice, with 129 and B/c strains showing strong trends in the same direction (Kest et al., 1999). Narrow-sense heritability of basal thermal nociception on the tail-flick test (both sexes combined) was estimated at $h^2 = 0.32$, which is similar to that obtained for the 49°C tail-withdrawal assay ($h^2 = 0.41$) (Mogil et al., 1999b). The correlation between strain sensitivities on these two related assays was found to be $r = 0.81$, $P < 0.01$.

3.2. EAA strain survey

A pilot study (data not shown) revealed that order of testing (2 Hz → 100 Hz versus 100 Hz → 2 Hz) did not affect EAA magnitude. Also, comparison of data from strain survey subjects receiving either order of frequency presentation showed no order effect; thus, data from all subjects

Table 1
Basal nociceptive sensitivity of 10 inbred mouse strains

Strain	Sex	*n*	Basal TFL (s)[a]
129	M + F	51	4.59 (0.07)
	M	34	4.70 (0.10)
	F	17	4.39 (0.09)*
A	M + F	79	4.64 (0.08)
	M	51	4.71 (0.10)
	F	28	4.51 (0.14)
AKR	M + F	66	4.64 (0.09)
	M	42	4.98 (0.11)
	F	24	4.06 (0.10)*
B10	M + F	61	4.30 (0.11)
	M	26	4.37 (0.18)
	F	35	4.25 (0.13)
B6	M + F	72	3.46 (0.08)†
	M	37	3.66 (0.12)
	F	35	3.24 (0.08)*
B/c	M + F	80	4.60 (0.07)
	M	39	4.83 (0.11)
	F	41	4.39 (0.08)*
C3H	M + F	53	4.34 (0.09)
	M	21	4.67 (0.17)
	F	32	4.12 (0.08)*
C58	M + F	46	4.10 (0.10)
	M	32	4.24 (0.11)
	F	14	3.76 (0.14)*
R3	M + F	46	4.49 (0.10)
	M	29	4.57 (0.14)
	F	17	4.36 (0.10)
SM	M + F	60	3.44 (0.05)†
	M	28	3.54 (0.07)
	F	32	3.37 (0.07)

[a] Basal radiant heat tail-flick latency (TFL) means, averaged over three assessments, spaced at 5-min intervals. The first measurement occurred after a 30 min habituation period. Values in parentheses represent SEM. *Significantly more sensitive than male mice of that strain, $P < 0.05$. †Significantly more sensitive than all strains not so denoted, $P < 0.05$.

were analyzed and presented together. Unlike for basal TFLs, neither the main effect of sex nor the strain × sex interaction on EAA was found to be significant, so data from both sexes were combined for further analyses. Fig. 1 shows time-course data (raw TFLs) from all strains for both 2 and 100 Hz EAA. As can be seen, whereas TFLs of SM mice given EA are virtually identical to those of the Control group in this strain, TFLs of the EA group of many other strains are significantly elevated. Repeated measures ANOVAs on raw TFLs revealed only one instance of a significant effect of repeated measure in Control group mice: C3H at 2 Hz ($F_{8,104} = 2.93$, $P = 0.005$). Repeated measures ANOVAs on raw TFLs in EA group mice were significant in every strain and frequency condition except 129/100 Hz, AKR/100 Hz and SM/2 Hz. Considering these results together, one can conclude that significant EAA was seen in all but three conditions, and only one significant EAA condition (C3H/2 Hz) is potentially confounded by Control group increases.

EAA – expressed as differential peak %MPE – at both 2 and 100 Hz frequencies was found to be strain-dependent, although much more so at 2 Hz ($F_{9,127} = 7.25$, $P < 0.001$, $F_{9,127} = 1.96$, $P < 0.05$, respectively). Corresponding heritability estimates for 2 and 100 Hz EAA are $h^2 = 0.37$ and 0.16, respectively. As can be easily seen in Fig. 2, for both frequencies, B10 and SM were extreme sensitive and resistant strains, respectively. However, the strain rankings were not identical for the two frequencies.

4. Discussion

4.1. Genetic versus environmental influences on EAA

The major finding of the present study is that genotype significantly affects EAA in mice, indicating a heritable component to this phenomenon. All mice tested were inbred, isogenic and homozygous at all genetic loci (i.e. they are virtual clones of each other; see Silver, 1995), and they were born and raised in the same environment in as identical a fashion as was practically possible. Thus, the observed strain mean differences in EAA very likely derive from the divergent genetic background of each strain, and specifically due to allelic differences at as yet unknown genes.

However, it must be noted that the heritability estimate for 2 Hz EAA was moderate, and that for 100 Hz EAA very low, attesting to the primacy of environmental factors in mediating EAA variability, even in the mouse. Since the environment was held constant, in that mice were bred together in our vivarium and tested identically and concurrently, the possibility of gene × environment interactions playing a large role must be seriously considered. For example, Sudakov et al. (1996) reported that strain differences between Fischer-344 and Wistar Albino Glaxo/G rats in morphine analgesia disappeared after cross-fostering, suggesting that maternal behavior (a genetically-based factor, of course) was a more important influence than the genotype of the subjects actually tested. Maternal behavior has been recently shown to produce differences in behavioral and biochemical stress responses that can be transmitted (non-genomically) from one generation to the next (Francis et al., 1999). Postnatal social environment also has been shown to affect morphine analgesia in mice. In one study, two different kinds of outbred CD-1® mice were tested (Alleva et al., 1986). The mice were maintained from birth to weaning either in litters containing only male pups (MM), or in litters containing both male and female pups (MF). On postnatal day 40, morphine analgesia was assessed on the hot-plate test; MF males displayed significantly longer latencies than MM males.

Thus, although we attempted to control for many environmental factors, some, e.g. maternal behavior, litter size and composition – were beyond our practical control (also see Crabbe et al., 1999). It should be noted, however, that some of the within-strain variance in this study arose from day-to-day effects (e.g. humidity variations, noise levels)

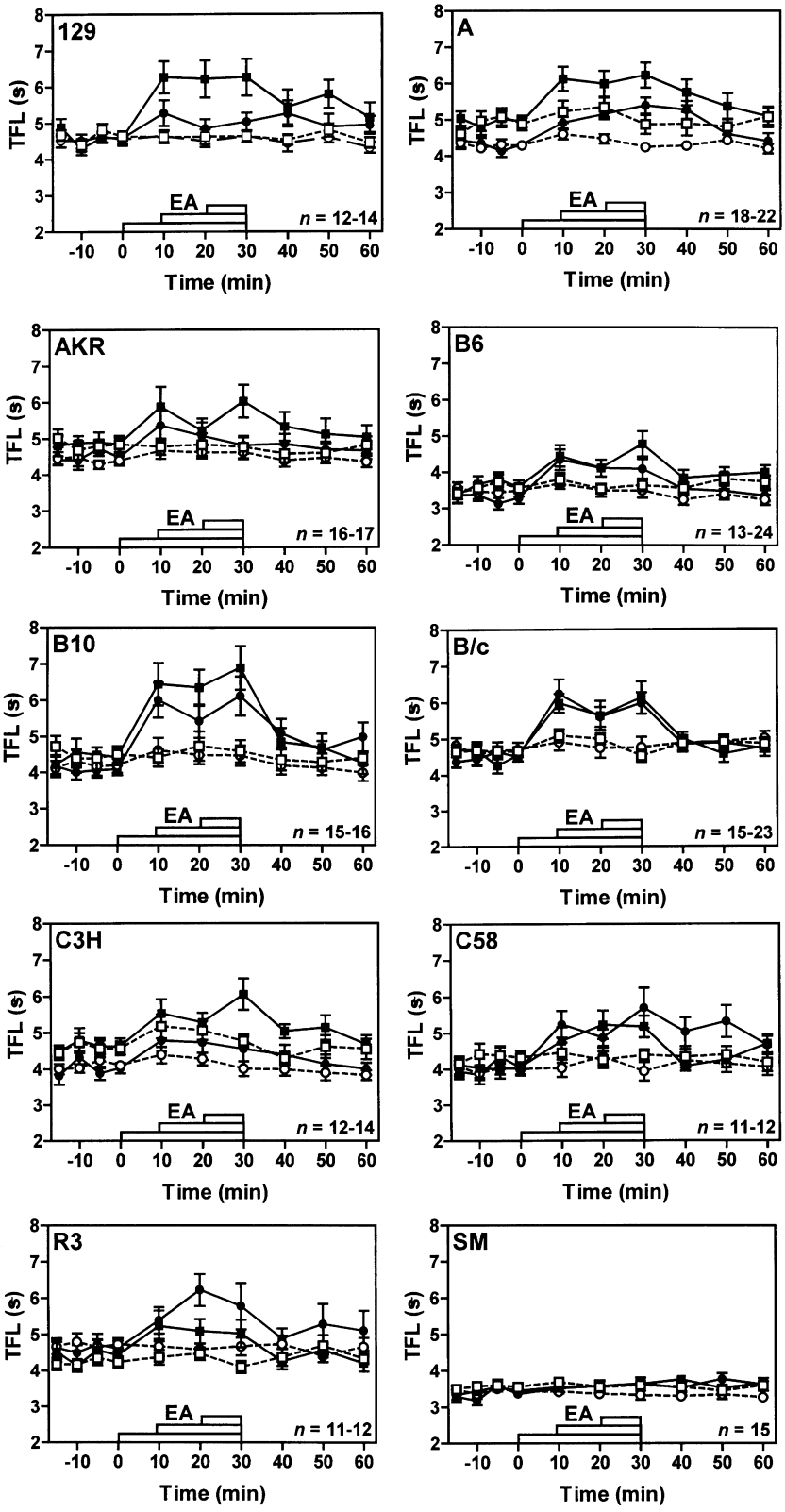

Fig. 1. Sensitivity to acute, thermal nociception before, during and after 2 Hz EA (–■–) or 100 Hz EA (–●–) in 10 inbred mouse strains. Following a 30 min habituation to Plexiglas restrainers (see text), all mice were tested three times for their nociceptive sensitivity on the tail-flick test at 5-min intervals. Five minutes later, EA was applied to the Zusanli (ST 36) and Sanyinjiao (SP 6) acupoints, for 10 min at 1.0 mA, 10 min at 2.0 mA, and 10 min at 3.0 mA. At the end of each 10-min period of stimulation, and at three post-EA time points separated by 10 min, tail-flick latency (TFL) was reassessed. A separate group of Control mice (- -□- -, - -○- -) were tested equivalently and concurrently to their corresponding EA group, but received no electrical stimulation. Symbols represent mean (±SEM) TFLs of 11–24 mice per group.

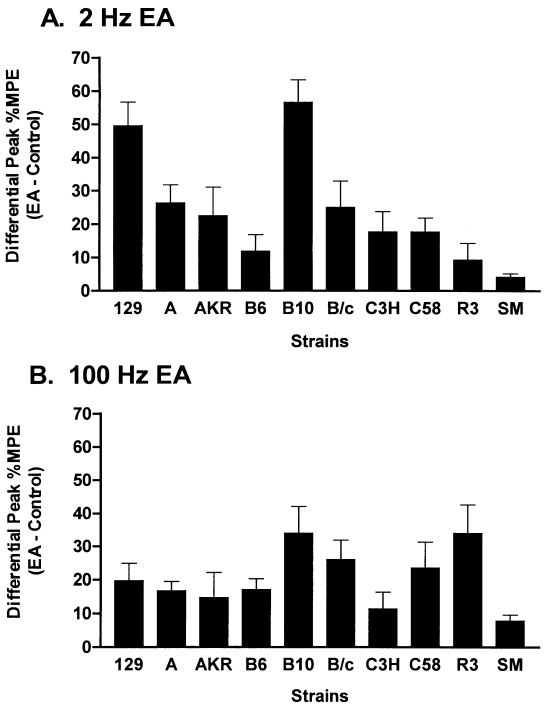

Fig. 2. Strain-dependent magnitude of 2 and 100 Hz EAA. Bars represent mean differential peak %MPE (±SEM), a transformation of the raw data in Fig. 1 expressing the peak increase in analgesic magnitude (relative to basal latencies) in EA mice versus Control mice of the same strain (see text for details).

and experimental error, and such artifacts will tend to lower heritability estimates.

It has been hypothesized that acupuncture may work more effectively on the Chinese versus other groups, conceivably due to differences in allele frequencies at relevant genes between racial groups. However, Knox et al. (1977) observed no racial differences in acupuncture analgesia, and concluded that if acupuncture does work better for the Chinese than for other racial groups, the likely cause is a more refined patient selection procedure rather than an inherent difference in response to acupuncture. Whereas these human data do not in any way rule out a role for genetic variation in acupuncture sensitivity, they also reinforce the important role of non-genetic factors.

4.2. Influence of stress-induced analgesia

A genetic-environmental interaction quite relevant to the present study is sensitivity to stress-induced analgesia (SIA), which is known to vary with genotype (Panocka et al., 1986; see Mogil et al., 1996a,b). As mentioned above, the present experiment involved prolonged restraint, well known to produce SIA (Porro and Carli, 1988), as well as needle insertion and passage of electrical current. Thus, it is possible that the observed strain differences in EAA are partially or completely due to strain differences in SIA. A number of facts are pertinent to this issue.

First, Control group mice of only one strain (C3H), and then at only one EA frequency (2 Hz), exhibited a significant effect of repeated measure (see Fig. 1) on TFLs. That is, TFLs were stable in Control mice of virtually every strain. It is possible, of course, that TFLs remained stable at a high level, indicative of ongoing SIA. To test this possibility, we ran an experiment with outbred CD-1® mice comparing three groups: (1) restraint in Plexiglas tubes only; (2) Plexiglas restraint plus needle insertion (the condition used in the strain survey itself; and (3) brief restraint (only when being tested) in cloth/cardboard 'pockets.' The last method is preferred in this laboratory (see Mogil et al., 2000). Tail-withdrawal latencies (from 48°C water, producing equivalent basal latencies to those using a 12.5 W bulb in this strain) were measured at 0, 5 and 10 min, and every 10 min thereafter for 2 h. The results were very clear (Mogil et al., 2000). Mice restrained in Plexiglas tubes exhibited a transient (0–10 min) sharp increase in latencies relative to brief 'pocket' restraint of about 2 s (5 vs. 3 s, respectively). Following this habituation period, latencies in the Plexiglas group dropped to approximately 4 s from 10 min to at least 120 min, but remained significantly higher than the stable 3-s latencies of the pocket group. Latencies of the Plexiglas plus needle insertion group were equivalent to those of the Plexiglas only group (not shown).

We have shown previously that the SIA produced by intracerebroventricular injection of saline in the lightly anesthetized mouse is strain-dependent (Mogil et al., 1999a), and thus it is possible that the ≈1-s restraint SIA observed in CD-1® mice is larger in some strains. It was a practical impossibility to repeat this control study on every strain in the survey. However, it should be noted that Control group mice were equivalently restrained to EA group mice, and the latter nonetheless displayed significantly higher TFLs after the onset of stimulation. Therefore, we do not believe restraint SIA is a major confound of the present findings.

We are unable at the present time to address the issue of whether the stress and/or pain associated with introducing electrical current – a factor *not* shared by the Control and EA groups – influenced the data in a meaningful way. The perfect control, of course, is one in which needles are inserted into non-acupoint areas but with electric stimulation. Unfortunately, such a control is impractical. The Research Group of Acupuncture Anesthesia, P.M.C. (1973) reported that the specificity of acupoints was relative. They found that acupuncture of the *hoku* and *zusanli* acupoints in human beings elevated pain threshold by 65–95%. But when another non-acupoint (situated midway between the second and third metacarpal bones, outside the 14 classical meridians) was stimulated, the needle sensation and the analgesic effect were the same as those produced at *hoku*. Lynn and Perl (1977) tested the pain sensitivity of 49 healthy volunteers before and during electroacupuncture at two or three widely separated places on the body surface. They demonstrated small, but statistically

highly significant, decreases in pain sensitivity during acupuncture. However, pain sensitivity fell by the same amount at 'acupoint' and 'non-acupoint' areas. In another study, acupuncture, either applied at *Zusanli* or at a non-acupoint (a point very near to *Zusanli*) and noxious thermal stimulation induced similar strong inhibitory effects – i.e. diffuse noxious inhibitory controls (DNIC) – on the C-fiber-evoked responses of trigeminal ganglion convergent neurons (Bing et al., 1990).

However, in a comprehensive study, Takeshige et al. (1992) specifically compared the effects of anatomical (stimulation, lesions) and pharmacological manipulations on acupoint and non-acupoint analgesia in rats. They demonstrate a number of dissociations between the two – e.g. acupoint analgesia is naloxone- but not dexamethasone-reversible, and vice-versa for non-acupoint analgesia – although both are shown to activate an ascending pathway that ultimately synapses in the lateral centromedian nucleus of the thalamus and the posterior hypothalamus. Although not presented in these terms, their data are quite reminiscent of the collateral inhibition between opioid and non-opioid analgesias (Kirchgessner et al., 1982). These authors did, however, distinguish acupoint and non-acupoint analgesia from 'pure' SIA induced by electrical shock (1.5–3.0 mA) through plate electrodes in the hindleg, demonstrating the absence of cross-tolerance between SIA and acupoint/non-acupoint analgesias (Takeshige et al., 1992).

The difficulty in obtaining a non-analgesic control condition for EAA studies is admittedly worrisome. However, it is increasingly apparent to us that SIA accompanying nociceptive testing is a ubiquitous feature of pain research, and a potential confound of studies of pharmacological analgesia as well. Also, if what we have uncovered presently is a genetic basis of electric shock-induced SIA and/or behavioral DNIC, that too is potentially of great scientific value.

4.3. Genetic correlations

Differences between inbred strain means can be taken to reflect the effects of alternate alleles of trait-relevant genes. If those genes are pleiotropic, affecting more than one trait (as virtually all genes are), the possession of a certain allele of that gene will affect each trait accordingly. Thus, subject to certain caveats (Carey, 1988; Crabbe et al., 1990), panels of inbred strains can be used to establish genetic correlations among traits. The existence of genetic correlations in turn suggests common physiological mediation of traits (see Mogil and Adhikari, 1999; Mogil et al., 1999c). It is therefore of heuristic value to consider the genetic correlation of 2 Hz EAA and 100 Hz EAA, and of these analgesias to other pain-related traits tested in common strains.

The genetic correlation between 2 and 100 Hz EAA was found to be an almost significant $r = 0.61$ ($P = 0.06$). Note that the most sensitive strain (B10) and the most resistant strain (SM) are the same for both EA frequencies (see Fig. 2). However, note as well the differential sensitivity of 129 mice to 2 Hz (high) versus 100 Hz (low) EAA, and also of R3 mice to 2 Hz (low) versus 100 Hz (high) EAA. These data suggest both the existence of genes common to both EA frequencies and also of genes specific to each. A major caveat to any conclusion at this time is that following removal of the B10 strain the correlation falls to near zero.

Given the considerable data from other laboratories suggesting commonalties between factors mediating individual differences in sensitivity to EAA and morphine analgesia (Takeshige et al., 1983; Tang et al., 1996; 1997; Tian et al., 1998), one might have predicted a high correlation between these two traits. We were unable to test this hypothesis, however, due to the extreme non-linearity of the obtained regressions. One reason to suspect considerable genetic independence between systemic morphine analgesia and both EAA frequencies is the status of the B10 mouse. Whereas this strain is the most sensitive to both 2 Hz and 100 Hz EAA of the 10 strains tested, it is the *least* sensitive strain to morphine of 12 inbred strains recently tested in our laboratory (unpublished data), exhibiting no detectable analgesia whatsoever from even a 20 mg/kg dose.

4.4. B6 versus B10 strain sensitivities: the possible role of δ-opioid receptors

A surprising and intriguing finding of the present study is the significant difference in 2 Hz EAA magnitude between the B6 and B10 strains. These strains are highly related, both arising from a common mating (Festing, 1996). The sublines were separated prior to 1937, but remain very similar genotypically, differing only at 31 microsatellite markers of 128 tested in a recent analysis, by far the fewest of any strain comparison (Schalkwyk et al., 1999). Of the documented genotypic differences between B6 and B10 strains, the majority are in a particular region of chromosome 4 (McClive et al., 1994; Slingsby et al., 1995). This region contains the *Oprd1* gene (64.8 cM), coding for the δopioid receptor. Given that δ receptors have already been implicated in the mediation of 2 Hz EAA (see Ulett et al., 1998), we submit that the documentation of this strain difference per se suggests the *Oprd1* gene as a 'candidate gene' for 2 Hz EAA. We have sequenced the coding region of this gene in both B6 and B10 strains, and served no differences (unpublished data), but the responsible DNA variant may very well reside in a promotor or enhancer region upstream. We are currently conducting a comprehensive comparison of δ-mediated analgesia and δ receptor levels in these two strains to support this hypothesis. Of note is the fact that 100 Hz EAA, which is thought to be mediated via κ-opioid receptors (see Ulett et al., 1998), is *not* significantly different between these two strains.

4.5. Significance and future directions

The identification of divergent strains provides a powerful initial step in the molecular genetic analysis of EAA, because these strains can be exploited to identify poly-

morphic genes contributing to the behavior-level variability. Thus, a linkage mapping study (see Lander and Schork, 1994) of EAA using B10 and SM mice as progenitors would be of value. The moderate heritability of 2 Hz EAA makes it a viable candidate for genetic dissection; doing the same for 100 Hz EAA would be more problematic and less worthwhile, since the vast majority of variance for this trait is environmental in origin. As mentioned above, the significant strain difference observed between B6 and B10 strains may allow us to focus on the few chromosomal regions known to be polymorphic between them, avoiding the need for a full-genome screen. Ultimately, by some combination of positional cloning and/or candidate gene evaluation strategies (see Collins, 1997), DNA sequence differences representing the differential alleles at EAA-relevant genes can be identified. This may have considerable clinical value for the practice of acupuncture and the treatment of pain, since it could facilitate the identification, prior to any treatment, of people likely to be EAA 'responders' and 'non-responders.'

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