The Surprising Complexity of Pain Testing in the Laboratory Mouse

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Introduction

The phenotyping of mutant mice for pain-related traits (e.g., nociception, drug- and stress-induced antinociception, injury-induced hypersensitivity) is an active pursuit, both for pain researchers and for others studying phenomena in which pain sensitivity may affect results (e.g., learning and memory, tolerance, and dependence). We have recently compiled an interactive database of mutant mice tested for behavioral pain phenotypes: the *Pain Genes Database* (http://paingeneticslab.ca/4105/06_02_pain_genetics_database.asp) (LaCroix-Fralish et al., 2007). As of this writing, 212 null mutants (both transgenic knockouts and spontaneous mutants) display at least one significant difference compared with wild types on one or more pain-related trait, findings that are described in 456 published manuscripts appearing in the literature at a rate of more than 60 papers per year. Largely because of the continuing popularity of the transgenic knockout mouse, *Mus musculus* is rapidly overtaking *Rattus* as the "default" subject of basic pain research (Mogil et al., 2001; Wilson and Mogil, 2001).

Establishing the pain sensitivity of a laboratory mouse is far more difficult than it may first appear, and more an art than a science. We have extensive experience testing not only mutant mice (Rubinstein et al., 1996; Mogil et al., 2000b; Kest et al., 2001; Mogil et al., 2003; Mogil et al., 2005b,c) on pain traits, but also a large set of inbred strains providing the genetic background on which these mutations are placed (Mogil and Belknap, 1997; Mogil et al., 1998; Kest et al., 1999; Mogil and Adhikari, 1999; Mogil et al., 1999a,b; Kest et al., 2002a,b; Lariviere et al., 2002; Chesler et al., 2003; Mogil et al., 2003; Wilson et al., 2003a,b; Mogil et al., 2005a,d; Mogil et al., 2006). We have learned from these experiments that genotype robustly affects pain, but that interindividual variability is affected by a large number of additional organismic and environmental factors. In this syllabus, I present the state of this art, with an introduction to the myriad complexities that attend pain phenotyping in the mouse.

Algesiometry

Acute and tonic pain (seconds to days) is induced by noxious stimuli of three modalities: thermal (hot or cold), mechanical, and chemical (including protons released during inflammatory states, ATP released from damaged cells, and any number of exogenous and endogenous compounds that activate and/or sensitize nociceptors). The etiology of chronic pain (weeks to years) is less clear but can generally be classified as either inflammatory (e.g., arthritis), neuropathic (e.g., postherpetic neuralgia), or idiopathic/functional (e.g., fibromyalgia). Although most research attention, for reasons of practicality, is paid to somatic pain to the trunk and limbs, of equal or greater clinical importance are visceral pain and orofacial pain.

Reflecting the diversity of pain etiologies and characteristics is a panoply of available animal models (Walker et al., 1999; Le Bars et al., 2001; Wilson and Mogil, 2001; Negus et al., 2006). Table 1 provides information on popular models. The general trend over

Table 1. Common algesiometric assays (excluding orofacial models)*

Duration	Modality	Assay	Intensity Range[a]
Acute (seconds)	Heat	Hot plate Radiant heat paw withdrawal Tail-flick/withdrawal	46–56°C not reported 46–56°C
	Cold	Acetone drop Cold plate Cold tail-flick	20 µl 0–5°C –10–0°C
	Mechanical	Paw pressure (Randall-Selitto) Tail clip von Frey	variable 100–500 g 0.1–1.5 g
Tonic[b] (minutes to hours)	Chemical	Acetic acid Bee venom Capsaicin Formalin Magnesium sulfate	0.1–1.0% 0.05–0.5 mg 0.1–50 µg 1–5% 120 mg/kg
Chronic[c] (hours to weeks)	Inflammatory	Carrageenan Complete Freund's Adjuvant Prostaglandins Zymosan	1–5% 50% 10 ng 0.25–1.0 mg
	Neuropathic	Chronic constriction injury Partial sciatic nerve ligation Spared nerve injury Spinal nerve ligation	N.A. N.A. N.A. N.A.

[a]Based on a search of null mutant studies (Lacroix-Fralish et al., 2007).
[b]Typically, the dependent measure in these experiments is the total duration of licking or stretching responses of the affected part, though subsequent hypersensitivity to evoking stimuli can often be demonstrated as well.
[c]Typical dependent measures in these experiments, measured weekly or biweekly, include changes in sensitivity to acute evoking stimuli.
N.A.=not applicable.
*This list is not intended to be exhaustive.

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the past few decades has been favoring the use of chronic-pain models, especially those involving surgical injuries to peripheral nerves, over acute models. However, acute testing paradigms are still highly relevant, because surgical and inflammatory injuries produce robust hypersensitivity to acute pain–evoking stimuli (i.e., radiant heat, cooling stimuli, von Frey fibers), and thus, measuring "chronic pain" in rodents involves, *de facto*, the measurement of injury-induced changes in acute nociceptive sensitivity.

We have argued that the apparent lack of measurable spontaneous pain represents an important limitation of existing models (Mogil and Crager, 2004), given that spontaneous pain is likely the most important symptom of human clinical pain pathology. Others have complained that the reliance of the chronic pain models on purely reflexive, dependent measures ignores the important cognitive and emotional richness of the human pain experience, which might be better modeled in animals using operant techniques; indeed, such techniques are being increasingly adopted (e.g., Sufka et al., 1996; Jabakhanji et al., 2006; Neubert et al., 2006; Pedersen and Blackburn-Munro, 2006; King et al., 2007; Thut et al., 2007).

It is important to note that, although pain research in general is progressing rather quickly toward the use of more sophisticated models, transgenic knockout studies of pain are more often than not performed by scientists who are not pain researchers. As such, these investigations overwhelmingly employ more "simple" (but less clinically relevant) assays like the hot-plate and tail-flick tests (Mogil et al., 2006; LaCroix-Fralish et al., 2007).

Sex Differences

Women are greatly overrepresented as clinical pain sufferers (Unruh, 1996). Meta-analyses of controlled laboratory studies have revealed moderate-to-large (although modality-dependent) sex differences as well (Riley III et al., 1998), with women showing higher pain sensitivity, lower pain tolerance, and greater pain discrimination than men. In general, mice display equivalent sex differences, in that females usually display higher sensitivity across a number of stimulus modalities when differences are reported (Mogil et al., 2000a). The relevance of mice (or rats, for that matter) as a model species to the study of human sex differences in analgesic responses is less clear, since male mice usually display higher analgesic potency than females, though many studies suggest that women are more responsive to opioid analgesics than men (Craft, 2003). Of potentially far greater interest are the repeated demonstrations by us (Mogil et al., 1993; Mogil and Belknap, 1997; Mogil et al., 1997b; Mogil et al., 2003; Sternberg et al., 2004a,b) and other researchers (Liu and Gintzler, 2000; Tershner et al., 2000; Blednov et al., 2003; Mitrovic et al., 2003) of *qualitative* (i.e., genetically and neurochemically distinct) sex differences in the neural processing—possibly including differences in neuroanatomical circuitry itself—of pain modulatory mechanisms.

Although sex-specific pain processing represents a great opportunity for novel drug development, its existence also presents a great challenge to the conclusions of the existing literature. For example, an entire body of literature was amassed documenting the potentiation of morphine analgesia by N-methyl-D-aspartate (NMDA) receptor antagonists (Kozela and Popik, 2002), and the strength of this literature was sufficient to inspire a clinical trial of a morphine-dextromethorphan combination against postoperative pain. However, this clinical trial failed to show efficacy (Caruso, 2000). Virtually the entire preclinical literature used male rats; using male and female mice, we found that noncompetitive NMDA antagonists like dextromethorphan did indeed potentiate morphine analgesia in male mice but were completely ineffective in females (Nemmani et al., 2004).

Although sex differences in murine sensitivity to pain and analgesia are clearly demonstrable and need to be considered seriously, their impact is dwarfed by overall genotypic effects, and sex and genotype interact thoroughly (Mogil, 2003). Importantly, the existence of sex differences is *not* a good reason to avoid using female mice in the study of pain. In fact, the vast majority of basic science studies in the field use male mice only (Mogil and Chanda, 2005) despite the overwhelming epidemiological data suggesting that the modal human pain patient is female. The likely reason for the continued use of male rodents in basic studies of pain, besides sheer inertia, is the belief that estrous cyclicity in female subjects renders their data more variable than those of males. With very large data sets at our disposal, we tested that hypothesis directly, and found that the coefficients of variation in each sex were statistically equal (if anything, the trend was for *male* data to show higher variance) (Mogil and Chanda, 2005). If estrous cyclicity really does affect pain and analgesia (and overall, there appears to be very little evidence that it does) (Mogil et al., 2000a), we suggest that male mice exhibit a source of sex-specific variation as well: within-cage dominance hierarchies. Data from male mice may be affected by the dominance status of the tested subject, and by the time elapsed since there was last a fight in the cage, given that defeat in such encounters produces analgesia (Miczek et al., 1982).

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Genotypic Effects

Pain and analgesia are no different from other biological traits in demonstrating robust variability across a strain. We have concentrated on a set of 12 inbred mouse strains, and performed a systematic "phenome project" by testing pain sensitivity across more than 22 nociceptive assays (Mogil et al., 1999a,b; Lariviere et al., 2002) and analgesic responses to more than 10 different drugs (Chesler et al., 2003; Wilson et al., 2003a,b). Narrow-sense heritabilities ranged from $h^2 = 0.24–0.76$ (median: $h^2 = 0.46$) for nociception and $h^2 = 0.12–0.45$ (median: $h^2 = 0.34$) for drug analgesia.

Beyond the simple observation of robust effects of genotype in every assay considered thus far, and the identification of extreme-responding strains as an entrée to gene mapping efforts via quantitative trait locus (QTL) mapping (see below), our study of pain and analgesic responses across this set of inbred strains has allowed for the examination of genetic correlations across assays that have in turn illuminated some general principles of "pain genetics." For example, 20 of the 22 nociceptive assays "cluster" into five groupings when cross-correlations are analyzed using multi-variate techniques. These five groupings appear to be differentiated largely by the noxious stimulus modality used: (1) thermal assays, (2) chemical assays, (3) assays of mechanical hypersensitivity after injury, (4) assays of thermal hypersensitivity after injury, and (5) assays of thermal hypersensitivity after injury featuring spontaneous pain prior to the development of the hypersensitivity (Lariviere et al., 2002).

Genetic correlations among traits imply common genetic determinants of variability within those traits, and so this finding directly predicts the discovery of pain *symptom*–related genes rather than pain *etiology*–related genes. With respect to analgesia, we obtained compelling evidence that all centrally acting analgesic compounds tested thus far appear to be highly genetically correlated with each other, and furthermore highly correlated with the baseline sensitivity of that strain to the noxious stimulus (Wilson et al., 2003a). This suggests that "master" analgesia genes may be discovered, and that those genes are far more likely to be related to pain circuitry *per se* than related to the binding or metabolism of the drug itself. For peripherally acting ("over-the-counter") analgesics, a different pattern was observed, with two nonsteroidal anti-inflammatory drugs (NSAIDs; aspirin and indomethacin) showing considerable genetic correlation, but acetaminophen yielding a completely different pattern of strain sensitivity (Wilson et al., 2003b). Finally, we (Mogil et al., 1996a; Chesler et al., 2003) and others (Elmer et al., 1998) have observed that the pattern of strain sensitivities to drug inhibition of one noxious stimulus is entirely uncorrelated with the pattern of strain sensitivities of that *same drug* to a different noxious stimulus. Again, surprisingly, analgesia genetics seems to have more to do with the pain being inhibited and less to do with the drug itself.

As a practical matter, the mouse strain chosen for study will have a large impact on the data collected. Since most null mutants are engineered using 129-derived embryonic stem cells and are ultimately placed on a C57BL/6 background, a comparison of the sensitivities of these two strains is of particular importance. Unfortunately for pain researchers, the 129 and C57BL/6 strains diverge greatly on most nociceptive and analgesic phenotypes (Lariviere et al., 2001), rendering most transgenic studies of pain particularly subject to "hitchhiking donor gene" confounds (Gerlai, 1996). It can be argued as well that the behaviorally sensitive strain C57BL/6J, despite being the default biomedical research subject, is not well representative of inbred (or outbred, or wild) mouse strains (Lariviere et al., 2001), and thus the interpretation of knockout data in this strain is greatly affected by epistatic considerations. Although important differences among 129 substrains have been noted (Simpson et al., 1997), we have not observed any major substrain differences in pain or analgesia phenotypes (Mogil and Wilson, 1997).

Using F_2 intercrosses between extreme-responding strains (and recombinant inbred strains derived from such crosses), supplemented more recently by the use of congenic strains and haplotype mapping strategies, we have made considerable progress in the identification of genes responsible for nociceptive and analgesic variability in the mouse (Mogil et al., 1997a,b; Wilson et al., 2002; Mogil et al., 2003; Mogil et al., 2005d; Mogil et al., 2006, unpublished data). Other groups have also performed QTL mapping studies on pain-relevant traits (Seltzer et al., 2001; Furuse et al., 2003; Liang et al., 2006a,b). As a single example, we have provided compelling evidence that the gene primarily responsible for variability in thermal (heat) nociception across the entire set of 12 inbred strains is *Calca*, encoding the calcitonin gene-related polypeptide, α-subunit (CGRP) (Mogil et al., 2005d). QTL mapping in two separate crosses localized the gene to mid-chromosome 7, and an available congenic strain (A.B6-Tyr$^+$/J) confirmed the QTL. Electrophysiological recordings from primary afferent neurons from behaviorally sensitive (C57BL/6J) and resistant (AKR/J) strains suggested that the relevant genetic difference was contained within the nociceptor itself, and we went on to demonstrate strain

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differences in *Calca* expression in the dorsal root ganglion, differential CGRP content there, and differential release of CGRP upon noxious thermal stimulation. Pharmacological and antisense knockdown experiments confirmed that the strain difference could be "rescued" in each strain by mimicking the *Calca* expression level of the other. Finally, we showed the generalizability of this explanation by completely (albeit temporarily) abolishing strain differences in noxious thermal sensitivity by administering CGRP injections into the hindpaw of all 12 strains (Mogil et al., 2005d).

We have also demonstrated that genetic findings gleaned from QTL mapping studies in the mouse can be successfully "translated" in humans. Such studies have revealed the important role the *Mc1r* gene plays in opioid analgesia and nociception in the mouse, and in each case the prediction was confirmed in a counterpart human (MC1R) association study (Mogil et al., 2003; Mogil et al., 2005c).

Parametric Factors

Although genetic factors are robust in their modulation of pain and analgesic sensitivity in the mouse, much interindividual variability is left to explain. From one lab to another, it is likely that parametric differences in the precise application of these assays are primarily responsible, and that the most obvious varying factor is noxious stimulus intensity. The intensity of the noxious stimulus used has obvious effects on baseline nociceptive sensitivity; what is less well appreciated is that the stimulus has very important effects on the measurement of both analgesia and hypersensitivity (after inflammatory or neuropathic injury). Simply put, the more noxious a stimulus is, the harder it is to change responses to it, and by their very definition, analgesia and hypersensitivity represent changes in nociceptive responses. For example, if a hot plate is very hot, and baseline latencies to display nocifensive behaviors (e.g., hindpaw licking, hindpaw shaking, jumping) are very low, only high doses of opioids will produce measurable analgesia, and NSAIDs will be entirely ineffective. Furthermore, any attempts to demonstrate hypersensitivity will likely be foiled by a "floor effect." Lowering the stimulus intensity will solve some of these problems, but only to potentially replace them with others, such as "ceiling effects" (i.e., arbitrary cutoff latencies or pressures imposed by ethical constraints) and nonspecific behavioral and stress effects (see below). Another unappreciated problem is that of "Lord's paradox" (the "law of initial values"), which states that the analysis of correlations between baseline values and change values calculated using the baselines is inherently problematic (Harris, 1963). As mentioned above, there's no way around this problem in research on analgesia and hypersensitivity.

Another parametric factor related to many pain studies surrounds the use of intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections. In the rat, administration of drugs via these routes is often preceded by the surgical installation of indwelling catheters or cannulae. In the mouse, by contrast, both intracerebroventricular and intrathecal injections are given acutely, under light gas anesthesia. Nonetheless, the procedure is very likely stressful, and acute stressors are well known to produce pain inhibition, a phenomenon known as stress-induced analgesia (SIA) (Terman et al., 1984; Yamada and Nabeshima, 1995). Failure to appreciate the possible existence of SIA can lead to misinterpretation of the results of pain studies. In a well-cited example, both groups that had originally isolated the endogenous ligand of the "orphan" ORL1 receptor initially investigated its possible biological role by intracerebroventricular injection into mice; both groups reported that the peptide, orphanin FQ/nociceptin (OFQ/N), produced hyperalgesia (Meunier et al., 1995; Reinscheid et al., 1995). We failed to replicate this finding, instead noting that OFQ/N was a functional anti-opioid (Mogil et al., 1996b). The original groups erred by not including a no-injection control; had they done so, they would have noted that the intracerebroventricular injection itself (i.e., the vehicle control) produced opioid-mediated SIA, which was simply being reversed by the OFQ/N (Mogil et al., 1996b; Suaudeau et al., 1998).

Environmental Factors

Generally speaking, within a laboratory, parametric factors are held constant. Even so, much variability remains. We investigated the possible sources of this environmental variability by compiling and analyzing a large set of 49°C tail-withdrawal baseline latency data from 8 years worth of experiments (Chesler et al., 2002a,b). This archival data set contained measurements from more than 8000 mice of 40 different genotypes, and featured a heritability of $h^2 = 0.24$. Besides strain (genotype) and sex, the following factors related to both husbandry and testing environment were both varied and recorded on the original data sheets: cage density, experimenter, humidity, season, time of day, and within-cage order of testing. Many other factors affect pain (e.g., age, light/sound levels), of course, but these were either strictly controlled in our data set or no records were available, and thus their influence could not be studied. Using classification and regression tree (CART) analysis followed by linear modeling of a data subset,

and confirmed by a fully balanced and crossed experiment performed on a single day, we were able to rank the relative importance of these factors in contributing to variability in the archival data set. We found that genotype was only the *second* most important factor, behind experimenter. This should serve to remind us of the importance of the experimenter-subject interaction, something that can never be exactly reproduced from one lab to another (Crabbe et al., 1999). The tail-withdrawal test is particularly affected by experimenter-specific factors, since the mouse is being actively restrained by the experimenter while being tested. (Restraining the mouse in Plexiglas may solve this particular problem, but it introduces another, since the prolonged restraint required yields significant SIA; Mogil et al., 2001.) We've gone on to demonstrate, however, that other nociceptive assays are similarly affected by experimenter, even those not featuring direct handling of mice during or immediately prior to data collection itself (Mogil et al., 2006).

Effect of Behavioral State

A potentially important factor modulating pain sensitivity in animals that has been entirely ignored in the literature concerns what the animal was *doing* immediate before and during the application of the noxious stimulus. This omission disregards the fact that attentional level can strongly modulate pain perception in humans (Bushnell et al., 2004), and a sleeping animal would traditionally be thought of as having reduced sensitivity to all external stimulation (Dement, 1965). In a study just completed (BL Callahan, ASC Gil, A Levesque, JS Mogil, submitted), we compiled an "ethogram" of mouse behavior within novel Plexiglas cubicles atop a glass or wire grid floor for several hours during the middle of their quiescent phase, which is essentially the modal testing situation in modern murine pain research. Mice are not testable in most assays while moving, and it generally requires several hours for them to reduce their exploration of the cubicle to acceptably low levels. Once this habituation has occurred, we find that CD-1 mice spend approximately 25% of their time grooming, 25% of their time resting or in light sleep, 25% of their time in deep sleep, but <15% of their time fully alert (Fig. 1A). The relevance of this breakdown is that if they are tested during grooming behavior, they are significantly less sensitive to noxious thermal stimuli (Fig. 1B) and profoundly less sensitive to mechanical stimuli (Fig. 1C). For mechanical stimuli, highest sensitivities are seen in resting and lightly sleeping mice (Fig. 1C). In general, C57BL/6 and 129 mice display similar patterns, except that C57BL/6 mice

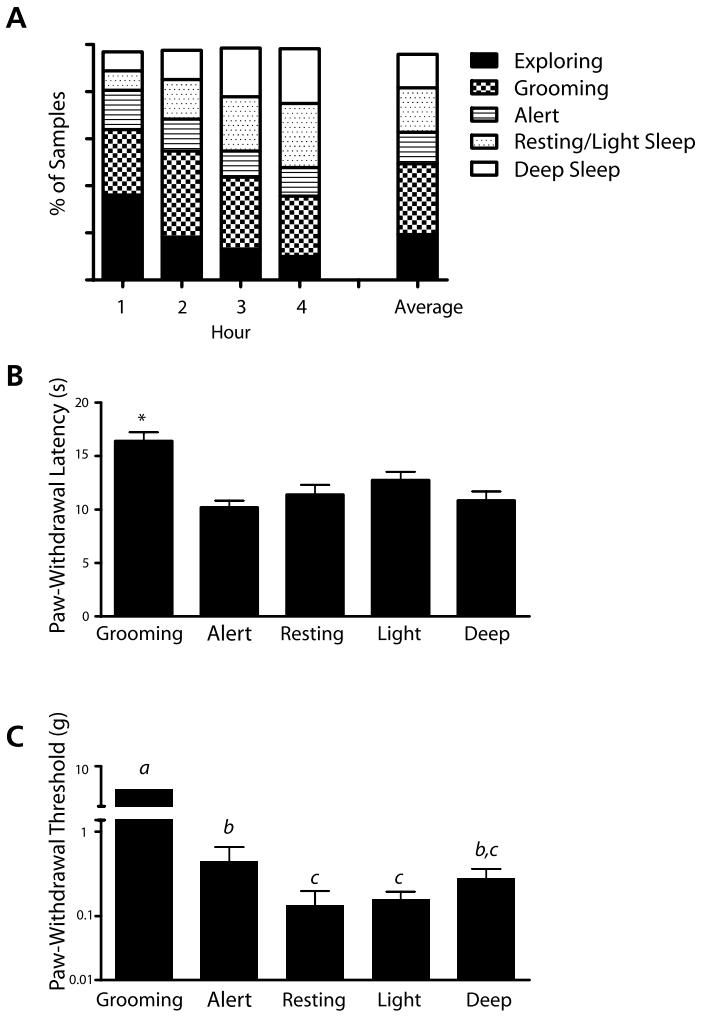

Figure. 1. ***A***, An ethogram of the behavior of naive, adult outbred CD-1 mice of both sexes in Plexiglas observation chambers (5 cm wide × 8.5 cm long × 6 cm high) atop a glass floor. Normally, we commence behavioral testing after 2 hours of habituation. Mice (n = 48) were videotaped and scored later by sampling (5 s of every minute) for the following behavioral states: Exploring (active locomotion), Grooming, Alert (standing on all four paws with eyes fully open, with active behaviors but no locomotion), Resting/Light Sleep (eyes half-open or closed), Deep Sleep (eyes closed, in a curled or hunched position). ***B***, Influence of behavioral state (A) on latency to withdraw hindpaw from noxious radiant heat from below (IITC Model 336; ≈45 W). Bars represent mean ± SEM withdrawal latency. *$p<0.05$ compared with all other groups. ***C***, Influence of behavioral state (A) on threshold to withdraw hindpaw from mechanical stimuli (von Frey fibers). The standard up-down psychophysical method was used (Mogil et al., 2006). Bars represent mean ± SEM withdrawal thresholds estimated using linear regression. Mice did not withdraw from any von Frey fibers while grooming; the threshold in this group is thus >2 g. Letters in italics indicate significantly different groupings.

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alone appear to be robustly analgesic during deep sleep (data not shown).

These observations have considerable implications. Not only do they likely represent a mouse model of attentional analgesia, but they suggest that null mutations that appear to affect pain sensitivity may actually do so by altering gross activity patterns, such that data are more or less likely to be collected in a particular behavioral state.

Social Context

The fact that within-cage order of testing had any measurable impact on hot water withdrawal latencies (Chesler et al., 2002a,b; see above) was a great surprise to us. We found that this effect could be abolished by not returning each mouse to its cage immediately after testing (Chesler et al., 2002b). This, of course, implied social communication among mice in the cage as the mediator of the effect: decreasing withdrawal latencies with each subsequently tested mouse. A serious difficulty in interpreting this and many other testing-environment variables is the demonstrated existence of both SIA (see Parametric Factors, above) and stress-induced hyperalgesia (SIH) (Imbe et al., 2006) as well as our very limited understanding of stress parameters leading to one or the other. Thus, the decreased nociceptive sensitivity displayed by later-tested cage members might represent either increasing SIA or decreasing (habituating) SIH. That is, mice returning to the cage might be saying to their neighbors, "Oh my God, that was horrible!", but they might also be saying, "No, really, I'm fine." An explanation of this phenomenon is not yet at hand, but our interest was piqued by the possibility of social communication among mice affecting their pain responses.

We ended up developing a number of paradigms in which to study this issue. In one such paradigm, the pain behavior of mice tested in dyads was compared with those tested in isolation. Two dyadic conditions were employed: (1) one mouse in pain/one mouse not in pain, and (2) both mice in pain. In the latter condition, of course, each subject was not only in pain (using the acetic acid writhing and formalin tests; Table 1) but observing another mouse in pain. Was this observation of pain enough to alter the observer's pain sensitivity? In fact, when the two mice in the dyad were familiar to each other (i.e., cage mates, for at least 14–21 days), a significant pain hypersensitivity was observed in both mice (Langford et al., 2006). Not only did we observe increased pain behavior, but we also observed a significant synchronization in the timing of the pain behavior of both mice in the dyad. These facts led us to conclude that mice were exhibiting "emotional contagion" of each other's pain, a rudimentary form of empathy (Preston and de Waal, 2002). In this particular case, stress was eliminated as a mediating factor, since stress levels (measured behaviorally and via corticosterone radioimmunoassay) were higher in stranger dyads than in cage-mate dyads, but only cage-mate dyads exhibited the emotional contagion. To our great surprise, the sensory modality implicated in this social communication was vision, since only a visual blockade abolished the hypersensitivity and synchrony. In another paradigm, we demonstrated that mere observation of writhing behavior in a cage mate led to hypersensitivity to withdrawal from radiant heat applied to the hindpaw (Langford et al., 2006). This latter finding is important not only because it eliminates mere imitation as the mediator, but because it suggests that social factors have the ability to sensitize pain circuitry in a general sense.

An intriguing finding from this same study was that, compared with mice tested in isolation, male (but not female) mice tested in a dyad with an unaffected stranger exhibited significantly *decreased* pain behavior (Langford et al., 2006). This result might represent a form of SIA, or might actually be evidence of a conscious decision to inhibit signs of vulnerability in the presence of a strange male mouse.

One way or another, the practical implication for pain testing is quite obvious: To avoid social confounds, mice should always be tested for pain behavior in visual isolation from all other mice. Of course, to completely abolish social confounds would require isolation housing, but this itself is a considerable stressor repeatedly found to alter pain sensitivity and analgesic response (e.g., Katz and Steinberg, 1970; Panksepp, 1980). The powerful effects of housing on pain behavior were elegantly demonstrated by Raber and Devor (2002), who observed that the extreme "autotomy" (self-mutilation after limb denervation) phenotypes of rats selectively bred over many generations for high autonomy (HA) and low autonomy (LA) levels could be dramatically altered simply by housing HA and LA rats together. This simple social manipulation, apparently mediated olfactorily, decreased autotomy behavior in HA rats and increased it in LA rats such that no phenotypic difference remained between the lines.

Our findings and those of Raber and Devor (2002) suggested to us the possibility that long-term exposure to cage mates in pain might itself produce hypersensitivity to pain. To test this theory, we subjected two mice per cage of four to either sham surgery or spared nerve injury (SNI), producing both thermal

and mechanical hypersensitivity (and, presumably, spontaneous pain, although this is difficult to confirm or quantify). Before surgery and on day 14 postsurgery, we tested all mice (in isolation) for thermal and mechanical sensitivity. A separate group of mice was also tested on day 15 using the acetic acid writhing test. As shown in Figure 2, our prediction was very clearly refuted; unoperated "neighbor" mice were unaltered by their constant observation of SNI-related pain behaviors. Although it is possible that our experimental paradigm was simply insufficiently supple to demonstrate the effect, we believe that social factors can likely modulate pain only in *real time*. This is no doubt good news for those trying to control the confounding influence of social factors.

Conclusions

Compared with other fields of inquiry within neuroscience (e.g., depression, schizophrenia), pain is generally regarded as relatively straightforward, and indeed our field has the advantage of possessing at least some models with clear face validity (i.e., I too will withdraw my finger from hot water within a certain number of seconds). Like many issues in biology, however, pain is far more complicated than it seems at first glance, and the animal models are far more subtle than we might suspect. Molecular and cellular techniques are far better funded, and thus more respected, than behavioral techniques in pain research (as in every other field), but ultimately drug development will not proceed without positive and trustworthy behavioral pharmacology data. Sir William Osler said in 1892: "If it were not for the great variability among individuals, medicine might as well be a science and not an art" (Roses, 2000). He was talking about humans, of course, but if we are to understand variability among humans, we must first understand variability in animal models of humans. For pain, this effort has just begun.

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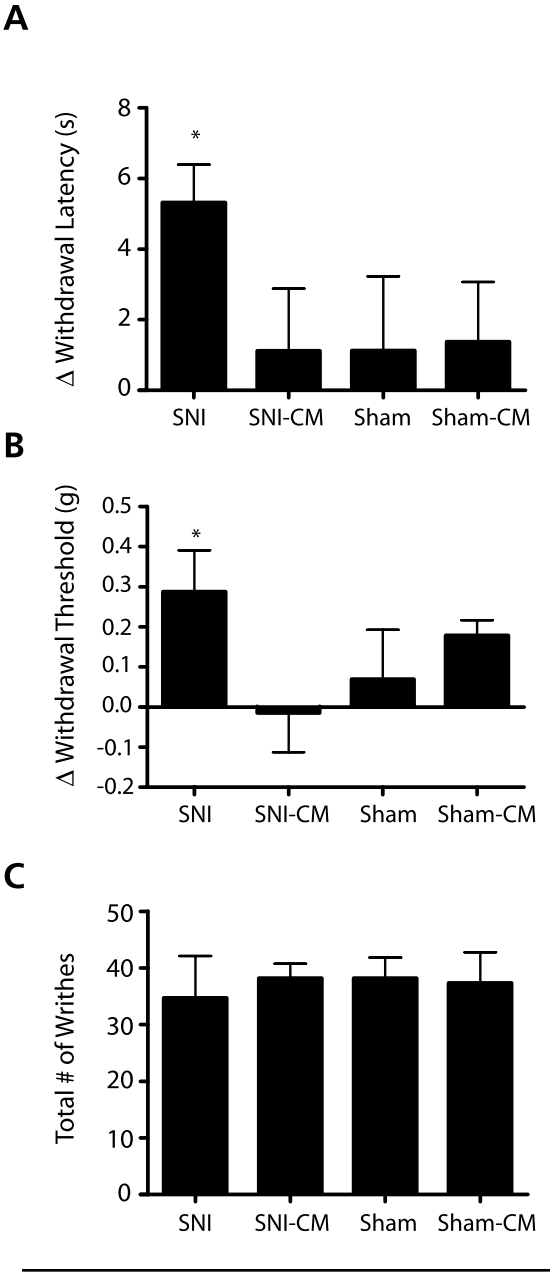

Figure. 2. Two-week co-housing with mice experiencing chronic pain does not produce hypersensitivity to pain in unoperated "neighbor" mice. Mice received either a spared nerve injury (SNI) or sham surgery, or were cage mates of SNI-operated mice (SNI-CM) or sham-operated mice (Sham-CM). Bars in ***A*** represent changes (mean ± SEM) in latency to withdraw from noxious radiant heat (Fig. 1*A* legend) on postoperative day 14 relative to baseline latencies; positive values indicate hypersensitivity. Bars in ***B*** represent changes (mean ± SEM) in threshold to withdraw from mechanical stimuli (Fig. 1*B* legend) on postoperative day 14 relative to baseline thresholds; positive values indicate hypersensitivity. Bars in ***C*** represent mean ± SEM total number of "writhes" in 30 minutes following intraperitoneal injection of 0.9% acetic acid on postoperative day 15. **p*<0.05 compared with zero (i.e., significant hypersensitivity observed).

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