Anesthesiology 2005; 103:751–8

Influence of Nociception and Stress-induced Antinociception on Genetic Variation in Isoflurane Anesthetic Potency among Mouse Strains

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Background: **Genetic background influences anesthetic potency to suppress motor response to noxious stimulation (minimum alveolar concentration [MAC]) as well as nociceptive sensitivity in unmedicated animals. However, the influence on MAC of baseline sensitivity to the noxious stimuli used to assess MAC has virtually never been studied. The authors assessed room air nociceptive sensitivity and isoflurane MAC in multiple mouse strains. Isoflurane requirement for loss of righting response (MAC_{LORR}) was also measured.**

Methods: **One outbred and 10 inbred mouse strains were tested for latency to respond (in room air) to a tail clip (either 500 g or 2,000 g). Naive mice of the same 11 strains were tested for isoflurane MAC and MAC_{LORR}. To assess the role of opioid-mediated stress-induced antinociception, mice were also tested for nociceptive sensitivity after injection of naloxone (10 mg/kg) or saline.**

Results: **Robust strain differences were observed for all measures. The authors found that tail-clip latency (using a 500-g or 2,000-g clip, respectively) correlated significantly with MAC ($r = -0.76$ and -0.58, respectively) but not MAC_{LORR} ($r = -0.10$ and -0.26). Naloxone produced strain-dependent reductions in open air tail-clip latencies, and these reductions were also strongly correlated with MAC ($r = -0.67$ and -0.71).**

Conclusions: **The authors suggest that genetic variability in isoflurane MAC (but not MAC_{LORR}) may reflect genetic variability in the underlying sensitivity to the noxious stimulus being used to measure MAC. This variable sensitivity to nociception in the awake state is at least partially mediated by endogenous antinociceptive mechanisms activated by the tail-clip stimulus itself.**

UNCONSCIOUSNESS and blockade of somatic motor response to noxious stimulation are the most important goals of general anesthesia.[1] Most basic science investigations of anesthetic action have focused on blockade of motor response to a noxious stimulus. The concept of minimum alveolar concentration (MAC) of anesthetic required to block movement to pain in 50% of subjects dates back 40 yr.[2] MAC is a clinically useful operational definition, allowing easy comparisons between the potency of different anesthetic agents and featuring impressive stability.[3] It remains unclear, however, to what extent MAC is determined by activity in afferent pathways relevant to pain perception *versus* efferent (*i.e.*, motor) pathways.

Despite the stability of MAC, a number of factors can influence it nonetheless, including genetic background. A number of transgenic knockout mice have been determined to have altered MAC compared with wild types,[4] and both rats[5] and mice[6,7] display genotype-dependent MAC to a variety of anesthetics. Different strains of rats and mice display strain-dependent sensitivity on every assay of nociception tested thus far,[8–11] including the tail-clip test,[9,12] which uses a noxious stimulus similar (or identical) to that used in many MAC determinations. Furthermore, robust differences among strains have been observed in the potency and efficacy of a number of analgesic drugs[13–16] and (to a less systematic degree) in the activation of endogenous antinociceptive systems by stress (so-called stress-induced antinociception [SIA]).[17–19]

The genetic determinants of individual variability in MAC are largely uncharacterized. It is as yet unknown whether anesthetics act in pathways that are also used for transmission or modulation of nociceptive information. To this end, we assessed baseline (room air) nociceptive sensitivity in mice of 10 inbred strains and 1 outbred strain using the tail-clip test with two noxious pressure intensities. The effect of naloxone injection on awake tail-clip latencies was also measured to investigate the possible role of endogenous opioid antinociception. We measured the isoflurane MAC loss of righting reflex (MAC_{LORR}) and MAC inhibiting nociceptive responses to examine relations between nociception and the hypnotic and motor-inhibiting actions of isoflurane.

This article is accompanied by an Editorial View. Please see: Antognini JF, Carstens E: Measuring minimum alveolar concentration: More than meets the tail. ANESTHESIOLOGY 2005; 103:679–80.

Materials and Methods

Experimental Animals

All experiments were approved by the local animal care and use committee (Faculty of Science, McGill Uni-

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Received from the Department of Psychology and Centre for Research on Pain, Mcgill University, Montreal, Quebec, Canada. Submitted for publication July 19, 2004. Accepted for publication April 29, 2005. Supported by grant No. DA15191 from the U.S. National Institutes of Health, Bethesda, Maryland; the Canada Research Chairs and Canada Foundation for Innovation programs, Ottawa, Ontario, Canada (to Dr. Mogil); and Candian Institutes of Health Research grant No. MOP-49583 Ottawa, Ontario, Canada (to Dr. Plourde).

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versity, Montreal, Quebec, Canada). Naive, adult mice (6–12 weeks old) of both sexes were used. They included outbred CD-1:Crl mice and inbred (*i.e.*, genetically identical) mice of the following 10 strains: 129P3, A, AKR, BALB/c, C3H/He, C57BL/6, C57BL/10, CBA, DBA/2, and RIIIS (all J substrains). All mice were bred in our vivarium at McGill University for no more than three generations from breeding pairs obtained from Charles River Canada (Boucherville, Quebec, Canada) or The Jackson Laboratory (Bar Harbor, ME). Mice were housed in standard polycarbonate shoebox cages (2–4 mice/cage), in a temperature-controlled (21° ± 2°C) environment and with *ad libitum* access to food (Harlan Teklad 8604, Indianapolis, IN) and tap water. Sample sizes were $n = 8$–33/strain in each experiment for inbred strains and $n = 8$–65 in each experiment for CD-1 mice, with both sexes equally represented. Sample sizes in drug-treated (saline *vs.* naloxone) mice were $n = 4$–11/strain/drug.

Tail-clip Testing

The tail-clip test of Takagi *et al.*[12] was used, which is itself a modification of the tail-pinch test of Haffner.[20] In one experiment, naive mice of all strains were injected subcutaneously with either naloxone (10 mg/kg; Sigma, St. Louis, MO) or saline (10 ml/kg volume) and returned to their home cage for 20 min before testing. This high naloxone dose was chosen for its demonstrated ability to block opioid receptors of all classic types (μ, δ, and κ). Each mouse was lightly restrained in a cloth/cardboard holder, and an artery clip (exerting approximately 500 g of force) was applied to the tail 1–2 cm from the base. The mouse was immediately removed from the holder onto a table top (in room air) and the latency to lick, bite, or grab the clip (*i.e.*, the latency to attempt to remove the clip) or even bring the nose to within 1 cm of the clip was measured with a stopwatch to the nearest 0.1 s, after which the clip was immediately removed. A maximum cutoff latency of 120 s was imposed, at which time the clip was removed to prevent injury.

The results obtained with the tail clip described above might be specific to a weaker nociceptive stimulus. To evaluate this possibility, all experiments were repeated in naive mice using a stronger clip, exerting approximately 2,000 g of force. The cutoff latency in these experiments was 60 s.

Isoflurane Exposure

Studies were performed in an airtight Plexiglas chamber (75 cm × 28 cm × 20 cm) equipped with two rubber flap iris diaphragm air seals to allow the experimenter to slide his or her arm inside. Mice were tested in groups of 10–20. Isoflurane in oxygen was introduced into the chamber (3 l/min) at one end. The concentration of isoflurane was measured with a gas analyzer (Datex Capnomac Ultima; Datex-Ohmeda, Helsinki, Finland) calibrated before use with gas samples provided by the manufacturer. Gas was sampled from two sites at the cage floor level close to the animals and away from the gas inflow site. Rectal temperature was measured intermittently and was maintained at 36.0°–38.0°C using an air heater/fan. The fan served to achieve adequate mixing of gas within the chamber. Mice from multiple strains and both sexes were tested simultaneously to counterbalance strains and sex across sessions.

Starting at 0.62% atm, isoflurane partial pressure was increased in the following sequence: 0.62, 0.75, 0.88, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, and 1.8% atm. To more rapidly achieve the next desired concentration, we transiently increased the oxygen flow (up to 10 l/min) and vaporizer dial setting (up to 5%). The concentration at each level was kept constant for at least 20 min before behavioral testing to permit equilibration of anesthetic concentration in the mice. Pilot studies revealed that 30- and 40-min equilibration periods yielded equivalent data in CD-1 mice. Testing of individual mice was discontinued after two consecutive concentrations eliciting no response. Testing mice at all these concentrations took 6–8 h/day. However, pilot experiments (data not shown) revealed that using descending or up–down sequences of concentrations yielded highly similar strain means, suggesting that our data were not strongly influenced by either the direction of anesthetic presentation or timing factors.

MAC_{LORR} Testing

MAC_{LORR} was tested at isoflurane partial pressures ranging from 0.62 to 1.1%.

At each partial pressure, mice were placed in a supine position and observed for 30 s for their ability or inability to fully right themselves (with all four hind paws on the floor).

MAC Testing

Minimum alveolar concentration was tested at isoflurane partial pressures ranging from 0.75 to 1.8%. The starting partial pressure for each mouse was determined by that mouse's MAC_{LORR} already obtained, *i.e.*, mice were not tested for tail-clip responses at partial pressures below their individual MAC_{LORR}. At each partial pressure, the tail clip used in the baseline nociceptive assay (either 500 g or 2,000 g) was applied to the mouse's tail, again approximately 1–2 cm from the base, and mice were observed for 30 s for gross purposeful movement of the head, body, or extremities. We used only one tail-clip intensity (500 g or 2,000 g) per session.

Statistics

Strain and sex differences in tail-clip response latencies in air were evaluated by analysis of variance, followed by *t* tests, Tukey's *post hoc* test, or both, as appropriate. MAC_{LORR} and MAC data were analyzed both by logistic

Table 1. Sensitivity to Mechanical Nociception and Isoflurane Anesthesia in 11 Mouse Strains

Strain	Latency (500-g Clip) (Room Air), s	Latency (2,000-g Clip) (Room Air), s	MAC_{LORR}, % atm	MAC (500-g Clip), % atm	MAC (2,000-g Clip), % atm
129P3	44.9 (9.3)	31.4 (7.1)	0.71 (0.02)	0.86 (0.03)	0.99 (0.03)
A	54.7 (9.7)	13.8 (3.6)	0.71 (0.02)	0.89 (0.03)	1.23 (0.03)
AKR	10.1 (2.2)	6.9 (1.5)	0.91 (0.02)	1.14 (0.03)	1.54 (0.02)
BALB/c	58.4 (15.0)	21.5 (6.7)	0.86 (0.03)	0.99 (0.04)	1.38 (0.04)
C3H/He	19.4 (4.8)	6.3 (0.7)	0.78 (0.02)	0.98 (0.04)	1.39 (0.04)
C57BL/6	13.1 (2.3)	9.1 (2.5)	0.82 (0.02)	0.98 (0.03)	1.34 (0.03)
C57BL/10	6.6 (1.6)	3.4 (0.4)	0.77 (0.02)	1.10 (0.03)	1.39 (0.03)
CBA	2.7 (1.0)	15.2 (3.5)	0.85 (0.02)	1.20 (0.03)	1.59 (0.04)
CD-1	14.7 (2.4)	16.9 (5.8)	0.78 (0.01)	1.05 (0.02)	1.10 (0.04)
DBA/2	1.0 (0)*	4.5 (0.6)	0.73 (0.02)	1.10 (0.04)	1.42 (0.05)
RIIIS	34.5 (5.0)	9.0 (1.8)	0.88 (0.03)	1.05 (0.03)	1.19 (0.04)

Values for minimum alveolar concentration producing loss of righting response (MAC_{LORR}) and minimum alveolar concentration inhibiting responding to tail clip (MAC) are strain means as derived by the bracketing method described by Sonner[21]; values in parentheses are SEMs. Strain means and errors derived by logistic regression were virtually identical (not shown).

* All DBA/2 mice tested responded to the application of the tail clip with immediate vocalization and vigorous efforts to remove it.

regression and by the bracketing technique described by Sonner,[21] as the average of the last response/first no-response partial pressures. A criterion α level of 0.05 was used in all cases.

Inbred strains are virtually isogenic within strain, and thus narrow-sense heritability (h^2) can be estimated from the between-strain genetic variation (V_a) and the within-strain (error) variation (V_e) using the formula $h^2 = V_a/(V_a + V_e)$,[22] which is an estimate of the population intraclass correlation coefficient. Because strains were chosen randomly with respect to the trait, these values are likely accurate estimates of the true trait heritabilities.[23]

The extent to which one can infer the influence of a common set of genes on two traits can be estimated from the correlation of strain means, assuming that these strains are housed and tested equivalently,[23] as was the case here. Genetic correlations among (the normally distributed) strain means were assessed using the Pearson product–moment correlation coefficient (r). Use of the Spearman rank correlation coefficient (not shown) yielded qualitatively similar conclusions.

Results

There were no significant main effects of sex or significant strain × sex interactions for any dependent measure in the strain survey. Therefore, data from both sexes were collapsed for all further analyses. It should be noted, however, that the main effect of sex for both MAC_{LORR} and 500-g tail-clip MAC approached significance ($F_{1,197} = 2.1$, $P = 0.14$ and $F_{1,216} = 3.0$, $P = 0.09$, respectively), with male mice showing a tendency toward increased anesthetic sensitivity in both cases. For all dependent measures, strain sensitivities were unimodally distributed, with distributions not deviating statistically from normality (not shown).

Tail-clip Latency

Latency to attempted removal of the 500-g tail clip was robustly strain dependent ($F_{10,177} = 11.4$, $P < 0.001$), as shown in table 1 and as we have previously reported.[9] The heritability of this trait was estimated as $h^2 = 0.39$. These responses are very similar to those reported previously in 10 of the same 11 strains using the same 500-g tail clip,[9] with a correlation between the strain means in the two experiments of $r = 0.75$ ($P < 0.05$). In that study, we also showed that there was no correlation whatsoever between tail diameter and response latency.[9]

Latency to attempted removal of the 2,000-g tail clip was also strain-dependent ($F_{10,129} = 4.4$, $P < 0.001$), as shown in table 1. The heritability of this trait was estimated as $h^2 = 0.25$. As might be expected, removal latencies for this stronger clip were generally shorter (grand mean, 13.0 s) than removal latencies for the lighter, 500-g clip (grand mean, 22.6 s), except for CD-1 mice that showed no change and CBA and DBA/2 mice, which had inexplicably longer latencies. Because of these outliers, the genetic correlation between 500-g and 2,000-g clip latencies in all 11 strains was just significant ($r = 0.60$, $P = 0.05$).

MAC_{LORR}

The mouse strains displayed highly strain-dependent MAC_{LORR} sensitivity ($F_{10,208} = 11.1$, $P < 0.001$), as shown in figure 1A and table 1. The heritability of this trait was estimated as $h^2 = 0.35$.

Tail-clip MAC

The mouse strains displayed highly strain-dependent 500-g tail-clip MAC means ($F_{10,227} = 10.5$, $P < 0.001$), as shown in figure 1B and table 1. The heritability of this trait was estimated as $h^2 = 0.32$.

Similarly, highly strain-dependent 2,000-g tail-clip MAC

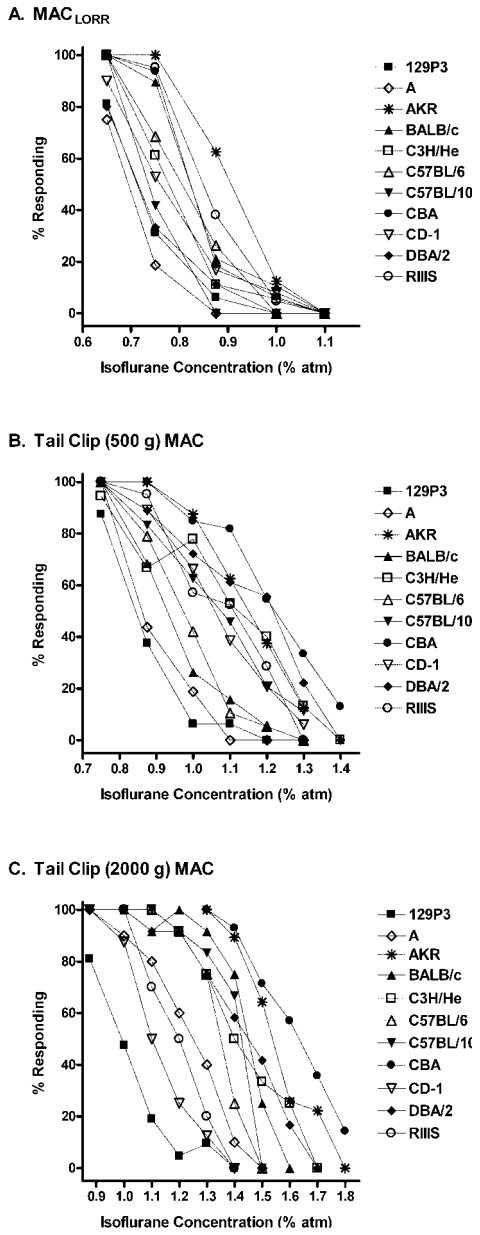

Fig. 1. Genetic variability in isoflurane potency among inbred mouse strains. (*A*) Sensitivity to isoflurane minimum alveolar concentration producing loss of righting response (MAC_{LORR}) in 11 mouse strains. *Symbols* represent mean percentage of mice responding (*i.e.*, able to right themselves) at each isoflurane concentration; error bars are omitted for clarity. (*B*) Sensitivity to isoflurane minimum alveolar concentration (MAC) against tail-clip nociception in 11 mouse strains, using a 500-g tail-clip stimulus. *Symbols* represent mean percentage of mice responding (*i.e.*, attempting to remove the tail clip); error bars are omitted for clarity. (*C*) Sensitivity to isoflurane MAC against nociception in 11 mouse strains, using a 2,000-g tail-clip stimulus. *Symbols* represent mean percentage of mice responding (*i.e.*, attempting to remove the tail clip); error bars are omitted for clarity.

means were observed ($F_{10,150} = 34.5$, $P < 0.001$), as shown in figure 1C and table 1. The heritability of this trait was estimated as $h^2 = 0.70$. As would be expected, MAC values for this stronger clip were higher, in every strain, than the corresponding values using the weaker

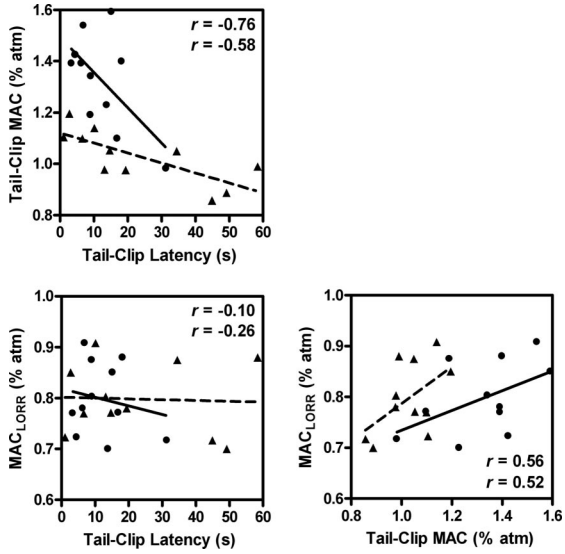

Fig. 2. Genetic correlations among strain means. ▲ = Strain means using the 500-g tail clip (regression indicated by *dotted line*); ● = strain means using the 2,000-g tail clip (regression indicated by *solid line*). In all graphs, the top *r* value is for the 500-g tail clip, and the bottom *r* value is for the 2,000-g tail clip. MAC = minimum alveolar concentration; MAC_{LORR} = minimum alveolar concentration producing loss of righting response.

clip. The genetic correlation between 500-g and 2,000-g clip MAC means in all 11 strains was highly significant ($r = 0.72$, $P < 0.05$).

Genetic Correlations

Correlations between awake state (uninjected) tail-clip latencies, MAC, and MAC_{LORR} are shown in figure 2. Because what are being correlated are strain means, not individual values, these are genetic correlations (with some contamination by nonisogenic CD-1 mice, in which variability may be both genetic and nongenetic) in which high values imply similar genetic determination of correlated traits. A robust and significant (even after false discovery rate correction[24]) negative genetic correlation ($r = -0.76$, $P < 0.01$) was obtained between 500-g tail-clip latency in room air and isoflurane 500-g tail-clip MAC, such that mouse strains showing lower sensitivity (*i.e.*, longer latencies to attack the clip) in air showed higher sensitivity to isoflurane (*i.e.*, lower concentrations required to abolish responding). Similarly, a significant negative genetic correlation ($r = -0.58$, $P < 0.05$) was obtained between 2,000-g tail-clip latency in room air and isoflurane 2,000-g tail-clip MAC. By contrast, tail-clip latency and isoflurane MAC_{LORR} were uncorrelated genetically ($r = -0.10$ for 500-g clip; $r = -0.26$ for 2,000-g clip). MAC_{LORR} and MAC to isoflurane exhibited a modest ($r = 0.56$ for 500-g clip; $r = 0.52$ for 2,000-g clip) but nonsignificant positive correlation with each other.

Effect of Naloxone

Strain surveys of room air tail-clip latencies were performed as before, but with saline or naloxone pretreat-

ment. For both 500-g and 2,000-g clips, analysis of variance revealed significant main effects of strain, drug, and a strain × drug interaction (all P values < 0.001). Saline injection itself produced no appreciable effects on response latencies in most strains; correlations with latencies of uninjected mice (table 1) were $r = 0.69$ and $r = 0.86$ for the 500-g and 2,000-g clips, respectively. In virtually all strains, mice injected with 10 mg/kg naloxone showed reduced tail-clip response latencies compared with saline-treated mice (figs. 3A and B). However, naloxone treatment did not appreciably alter the *relative* sensitivities among strains, as shown by the high correlations between saline- and naloxone-treated strain means ($r = 0.90$ and $r = 0.73$ for 500-g and 2,000-g clips, respectively). The amount of tail clip–related SIA blocked by naloxone administration was estimated as the difference between the saline- and naloxone-treated latencies. Quantifying SIA as the percent change relative to saline yielded qualitatively similar results (data not shown). The magnitude of SIA itself correlated highly with uninjected, saline-injected, and naloxone-injected latencies (all r values > 0.62). Because of this, the genetic correlations between SIA magnitude, MAC, and MAC_{LORR} were very similar to those described above (SIA *vs.* MAC: $r = -0.67$ and $r = -0.71$ for 500-g and 2,000-g clips, respectively; SIA *vs.* MAC_{LORR}: $r = -0.21$ and $r = -0.48$ for 500-g and 2,000-g clips, respectively). The correlations between SIA and MAC (fig. 3C) were highly significant even after false discovery rate correction.[24]

Discussion

Significant between-strain differences were demonstrated in tail-clip nociception, tail clip–induced SIA, isoflurane MAC_{LORR}, and isoflurane tail-clip MAC, representing *prima facie* evidence for genetic determination of variability in these traits. Strain means in every case were normally distributed, suggestive of multigenic inheritance. The data suggest that strain differences in isoflurane MAC derive to a considerable extent from strain differences in the underlying sensitivity to the noxious mechanical stimulus and/or are related to the magnitude of SIA produced by stimulus application. Strain differences in isoflurane MAC_{LORR} show no such codetermination with nociception or SIA and are likely mediated by separate genetic factors. The possible genetic relation between MAC and MAC_{LORR} remains unclear, because we obtained suggestive but nonsignificant correlations.

Genetic Mediation of Anesthetic Potency in Mice and Its Relation to Nociception and/or SIA

Mouse strain differences in tail-clip MAC have been investigated previously.[6,7] These studies featured eight

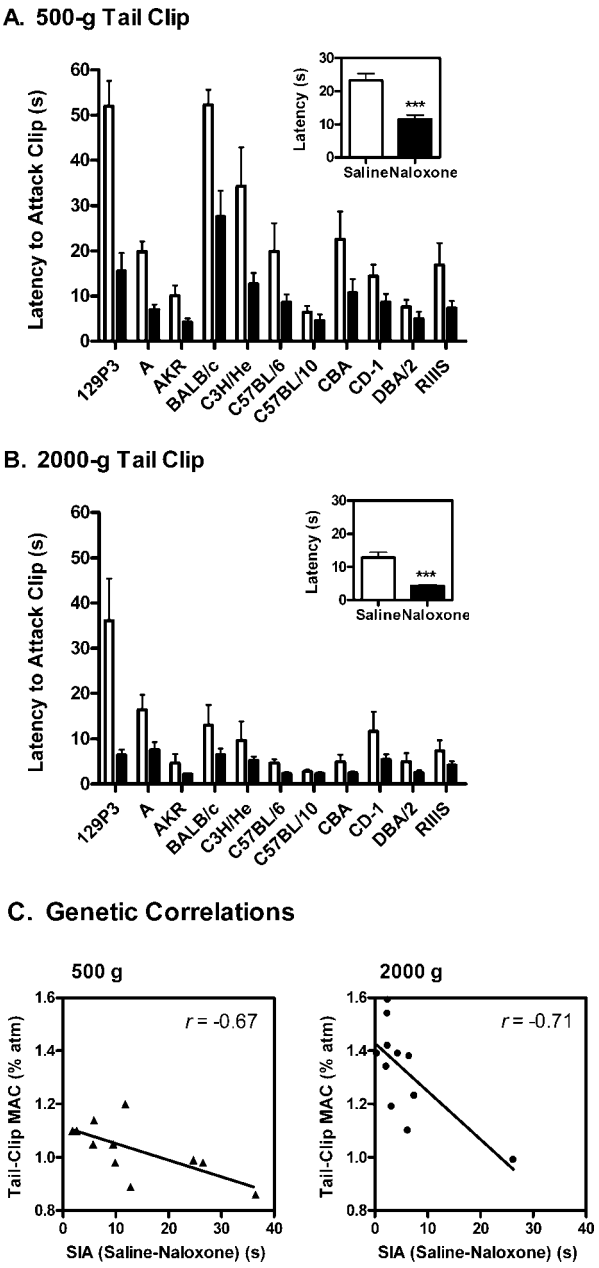

Fig. 3. Opioid-mediated (naloxone-reversible) stress-induced antinociception (SIA) produced by tail-clip application in room air, and correlation of strain-dependent SIA magnitude with minimum alveolar concentration to inhibit response to tail clip (MAC). (*A*) Latency to attack (*i.e.*, attempt to remove) a clip exerting 500 g of force to the tail in mice of 11 strains pretreated with saline (*open bars*) or 10 mg/kg naloxone (*filled bars*). *Bars* represent mean ± SEM latencies (s). *Inset* shows the main effect of naloxone in all mice, collapsed across strain (* $P < 0.001$). (*B*) Latency to attack (*i.e.*, attempt to remove) a clip exerting 2,000 g of force to the tail in mice of 11 strains pretreated with saline or 10 mg/kg naloxone. *Bars* represent mean ± SEM latencies (s). *Inset* shows the main effect of naloxone in all mice, collapsed across strain (*** $P < 0.001$). (*C*) Genetic correlation between SIA magnitude (*i.e.*, saline-treated strain mean − naloxone-treated strain mean) and MAC using a 500-g clip (*left*) or a 2,000-g clip (*right*). *Symbols* and *regression lines* are as in figure 2.**

strains in common to those tested here (assuming that the CH3/J strain referred to, a designation unknown to us, is indeed the C3H/HeJ strain used here). Mean isoflurane MAC values in these eight strains ranged from 1.30 to 1.66 (median, 1.40). Although the force exerted by the clip used in those investigations was not stated, it might be inferred that their clip was also approximately 2,000 g, because we obtained MAC values in these eight strains using the 2,000-g clip ranging from 0.98 to 1.54 (median, 1.36). Importantly, the two data sets show an almost significant genetic correlation ($r = 0.65$, $P = 0.08$). Although our study did not investigate any other inhalant anesthetic besides isoflurane, Sonner *et al.*[6] demonstrated that strain mean MAC values for desflurane correlated significantly with those for isoflurane. In turn, significant correlations between desflurane and nitrous oxide MAC among rat strains have also been demonstrated.[5] To our knowledge, there are no published reports of inbred strain sensitivity to isoflurane-induced MAC_{LORR} to compare to the current data.

The very strong correlation between our 500-g and 2,000-g tail-clip MAC values ($r = 0.72$) suggests that the genetic variation in MAC is not specific to stimulus strength. It is commonly assumed that variability in MAC should decrease as stimulus strength increases, with a "supramaximal" stimulus yielding MAC values that no longer depend on stimulus intensity.[3,25] Ethical constraints prevented us from testing this hypothesis directly by using a yet-stronger stimulus, because the 2,000-g clip already was producing obvious (but modest) tissue damage. However, although the absolute differences between strains in their room air latencies to remove the 2,000-g clip were smaller than those using the 500-g clip, the isoflurane MAC strain differences using the 2,000-g clip were actually larger than those using the 500-g clip, covering a range of 0.60 and 0.34% atm, respectively (table 1). More to the point, the latencies of uninjected, fully conscious mice to remove the 2,000-g tail clip were obviously far from immediate in all but a few strains.

The decidedly nonzero latencies to remove tail clips in the awake state are at odds with the idea that these are supramaximal stimuli. Naloxone was very effective at reducing these latencies, especially at 2,000 g, suggesting that opioid-mediated SIA produced by application of the clip itself is partly responsible for the noninstantaneous response to the clip. Although easily explainable, the ability of an acute pain stimulus to induce SIA *to itself* has actually been rarely if ever directly reported in the pain literature. In the vast SIA literature,[26] nonpainful stressors (*e.g.*, forced swim, restraint) are usually employed, and when a painful stimulus such as foot shock is used as the stressor, the nociceptive measurement is generally made at the tail. It has long been known that "pain inhibits pain," and an animal model of this phenomenon has been developed, called *diffuse noxious inhibitory controls*.[27,28] However, in existing behavioral diffuse noxious inhibitory control paradigms, a pain stimulus in one part of the body is demonstrated to inhibit pain in *another* part.[29,30] We should note that most modern studies examining the effect of naloxone treatment on "basal" nociceptive sensitivity in humans and animals have yielded negative findings,[31,32] and we have found no effect of 10 mg/kg naloxone on 49°C tail-withdrawal test latencies (unpublished data, Shad B. Smith, B.S., and Jeffrey S. Mogil, Ph.D., McGill University, Montreal, Quebec, Canada, November 2001). Incidentally, our unpublished data rule out the possibility that restraint is producing the SIA in question, because mice in that study were restrained for far longer (approximately 1 min) than was the case here (< 10 s), with no evidence of SIA.

Although it is unmistakable that both tail clips produced strong SIA, it should be recognized that even in the presence of naloxone blockade of this SIA, significant (and at the 500-g clip, robust) strain differences in awake nociceptive responsiveness persisted, and those (naloxone-treated) latencies correlated equally highly with tail-clip MAC. Nonopioid forms of SIA are well known,[33,34] and it is possible that the "residual" nonzero latencies might be due to SIA unaffected by opioid receptor blockade with naloxone. However, because multiple neurochemical systems have been implicated in nonopioid SIA,[35] this is not a trivial matter to test.

Genetic variability in baseline nociception is highly correlated with genetic variability in SIA; we have noted this correlation before using thermal nociceptive stimuli and drug antinociception.[13] In turn, both nociception and SIA are genetically correlated with MAC. To the extent that one believes that awake state tail-clip latencies are truly noninstantaneous, our data suggest that either something is wrong with the concept of the "supramaximal" stimulus in basic science studies of anesthesiology, or the stimuli being used in such studies are not actually supramaximal. In the majority of existing studies, the force being exerted is not reported, not known, or both.[36] Conversely, if one assumes that in the absence of SIA (as would surely be the case during anesthesia) responses to the tail clips would be instantaneous, we have still demonstrated herein that the magnitude of this "unrealized" SIA can predict the variable potency of isoflurane. As such, the genetic factors underlying isoflurane potency have little to do with the anesthetic itself (*e.g.*, genetic alterations at the anesthetic's molecular target) but instead are related to the operation of brain mechanisms of pain modulation. That is, although it is doubtful that SIA mechanisms are active in unconscious mice, it seems that the analgesic components of both isoflurane anesthesia and the stress response are mediated by similar pathways. This is somewhat surprising, because previous studies of naloxone administration during inhalant anesthesia indicate no

effect on MAC.[37] However, antinociception from electrical stimulation of the periaqueductal gray matter—a phenomenon thought to be mediated by the same mechanisms as SIA[38]— can decrease anesthetic requirements in humans.[39] Also, isoflurane at clinically relevant concentrations has been demonstrated to interact in a complex fashion with descending supraspinal mechanisms of pain modulation[40] in addition to its actions within the spinal cord.

Implications and Future Directions

The current findings may have implications for existing and future studies of anesthetic mechanisms in animals, because any number of genes/proteins implicated in anesthetic MAC by pharmacologic and/or transgenic studies[4] also have demonstrated effects on nociception and/or antinociception *per se*.[41,42] We recommend that investigators consider incorporating room air testing of noxious stimulus responsivity into studies of inhalant MAC. To the extent that isoflurane MAC really is a function of nociceptive sensitivity to the stimulus itself (or to SIA that is itself correlated with nociceptive sensitivity), many isoflurane sensitivity genes will also be nociceptive sensitivity genes. We have conducted a systematic study during the past few years of the genetics of nociception in the mouse.[43–45] These experiments have shown that the modality of the noxious stimulus is the inherited unit, *i.e.*, different sets of genes are responsible for variable sensitivity to thermal nociception, chemical nociception, mechanical allodynia (increased sensitivity to evoking mechanical stimuli after injury), and thermal hyperalgesia (increased sensitivity to evoking thermal stimuli after injury).[8,9,46] The status of acute mechanical nociception within this scheme has yet to be clarified, because tail-clip latencies showed very high genetic correlations with most thermal assays, but von Frey filament withdrawal thresholds did not.[9] We have identified a number of quantitative trait loci containing genes responsible for variability in nociceptive and analgesic sensitivity[47–50] and, in some cases, have provided evidence in support of particular candidate genes within the genetically linked (*i.e.*, coinherited) regions.[47,50–52] A quantitative trait locus mapping study of tail-clip mechanical nociception is ongoing, using an F_2 hybrid intercross between the A/J and C3H/HeJ mouse strains. As has been pointed out, currently identified genes underlying differences in the sensitivity to some inhalant anesthetic effects in *Caenorhabditis elegans* may not be relevant in vertebrates.[6]

The discovery of genes relevant to anesthetic potency may have considerable implications for basic scientific research into physiologic mechanisms of anesthesia and ultimately for clinical anesthesia.

The authors thank Sarah Harbottle, B.S. (Department of Psychology, McGill University, Montreal, Quebec, Canada), for preliminary data collection and Samson A. Sotocinal, Ph.D. (Department of Bioresource Engineering, McGill University), for construction of the induction chamber.

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