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Identification of a Genetic Region in Mice that Specifies Sensitivity to Propofol

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Background: **Long-sleep (LS) and short-sleep (SS) mice, initially selected for differential sensitivity to ethanol, also exhibit differential sensitivity to propofol. By interbreeding LS and SS mice to obtain progeny whose chromosomes are a patchwork of the LS and SS chromosomes, the authors determined whether differential propofol sensitivity cosegregates with any particular chromosomal region(s). Such cosegregation is the essence of genetic linkage mapping and a first step toward isolating a gene that can modulate propofol sensitivity in mammals. A gene underlying a quantitative trait such as anesthetic sensitivity is commonly called a quantitative trait locus (QTL).**

Methods: **The propofol dose was 20 mg/kg injected retroorbitally. Sensitivity was measured as the duration of the loss of righting reflex (LORR). The LORR and propofol brain levels at awakening were determined for 24 LSXSS recombinant-inbred (RI) strains, derived by intercrossing LS and SS for two generations followed by >20 generations of inbreeding. A genetic linkage between LORR and an albino mutation on chromosome 7 was investigated further using 164 second-generation progeny (F_2s) from intercrossing inbred LS and inbred SS mice, similar to the LSXSS RIs except F_2s are not inbred. The linkage between propofol sensitivity and the albino locus also was investigated using additional genetic markers on chromosome 7. Statistical significance was assessed by interval mapping using a regression method for RIs and Mapmaker/QTL (Whitehead Institute, Cambridge, MA) for F_2s.**

Results: **Genetic mapping in the LSXSS RIs revealed a QTL tightly linked to the *Tyr* (albino) locus that accounts for nearly all of the genetic difference in propofol sensitivity between LS and SS mice. Analysis of propofol brain levels at awakening indicated that this QTL results from differential neurosensitivity. Mapping in F_2s confirmed the genetic linkage to *Tyr*. Mice (ISS $c/c \times$ C57BL/6 c^{2J}/C) that differed only by an albino mutation at *Tyr* were not differentially sensitive to propofol.**

Conclusions: **A single QTL, called *Lorp1*, underlies most of the genetic difference in propofol neurosensitivity between LS and SS mice. Although this QTL is tightly linked to *Tyr*, propofol sensitivity is not modulated by albinism. For mapping this QTL, the LSXSS RIs proved to be an especially powerful resource, localizing the candidate-gene region to a 99% confidence interval of only 2.5 centimorgans. (Key words: Albino locus; genetic mapping; QTL mapping; recombinant inbreds.)**

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ELUCIDATING the actions of general anesthetic agents at the molecular level is difficult because of the seemingly endless number of targets with which these agents are associated.[1] One promising approach for identifying molecular pathways directly relevant to anesthetic action is to identify and isolate genes that can modulate anesthetic sensitivity. Because anesthetic sensitivity is measured quantitatively and is likely to be influenced by multiple genes, these genes are also called quantitative trait loci or QTLs; presumably, each QTL corre-

sponds to a single gene.[2] Here we characterize a QTL that modulates central nervous system (CNS) sensitivity to the commonly used anesthetic agent propofol (2,6-diisopropylphenol).

The long-sleep (LS) and short-sleep (SS) mice used in this study were selectively bred by McClearn and Kakihana[3] for high or low (respectively) sensitivity to ethanol. Ethanol sensitivity was measured as the duration of the loss of righting reflex (*i.e.*, the inability of a mouse to turn over after being placed on its back, abbreviated LORR) and differs markedly between LS and SS. Given a dose of 4.1 g/kg ethanol, the LORR for LS mice is about 2 h, whereas the LORR for SS mice is only 15 min. The blood ethanol concentration at the time of regaining righting response also differs markedly,[4,5] but ethanol metabolism does not,[6] thus indicating that LS and SS differ in their neurosensitivity. This differential neurosensitivity is also reflected by differences between LS and SS in their response to ethanol at the level of Purkinje cells,[7,8] intraocular transplants,[9] and γ-aminobutyric acid$_A$ receptors.[10] The LS and SS lines, therefore, are well suited for studying CNS sensitivity, and no model of ethanol-induced hypnosis has been more intensively analyzed.[11,12]

In addition to ethanol, the LS and SS mice have been found to be differentially sensitive to various CNS depressants. These agents include chloral hydrate, halogenated ethanols, paraldehyde, urethane, flurazepam, enflurane, etomidate, isoflurane, ketamine,[13-16] and midazolam (V. J. Simpson, unpublished data). The diverse chemical structures represented by these agents suggest that LS and SS have been selected, at least in part, for genes that can modulate sensitivity at a point in a molecular pathway where the actions of multiple classes of agents converge. However, LS and SS do not differ in sensitivity to ether or halothane,[17] indicating that there has not been indiscriminate selection for a developmental difference such as differential motor skills. Whether those agents that cause differential sensitivity between LS and SS also have differential effects similar to ethanol on the Purkinje cells has not been determined.

More recently, LS and SS were shown to be differentially sensitive to propofol-induced LORR.[18] When given the same 20 mg/kg dose retroorbitally, SS mice had an LORR of about 3.5 min (SD = 1.01, SEM = 0.4), and the LORR for LS mice was about 2.2 min longer, with no difference between males and females (table 1, section 1). The SS mice also regained the righting response at a significantly higher propofol brain level, indicating that resistance is neurologic rather than pharmacokinetic.[18] However, LS and SS did not exhibit differences in γ-aminobutyric acid$_A$-activated chloride channels, assayed in brain microsac preparations, even though propofol has a potent effect on γ-aminobutyric acid$_A$ receptors.[19] To further elucidate the difference in propofol sensitivity between LS and SS, we are pursuing a positional cloning strategy.

Positional cloning involves first positioning or "mapping" the gene(s) to be cloned to a small chromosomal region. Mapping of propofol sensitivity is greatly facilitated[11,12,16] by the availability of LSXSS recombinant-inbred (RI) strains of mice.[20] These mice were derived by

Table 1. Comparison of Propofol-induced Loss of Righting Reflex for LS, SS, and Mice Derived from ILS and ISS*

Population	Total N	Mean ± SEM (min)	Variance (min)
LS and SS *versus* ILS and ISS			
LS	19	5.7 ± 0.3	1.8
ILS	19	7.4 ± 0.4	2.7
SS	16	3.5 ± 0.2	0.88
ISS	37	3.8 ± 0.2	2.2
ILS by ISS F_1s	25	6.1 ± 0.4	3.7
ILS × ISS	17	6.5 ± 0.5	3.8
ISS × ILS	8	5.3 ± 0.7	3.4
ILS by ISS F_2s	194	6.5 ± 0.2	6.9
Pigmented	135	6.8 ± 0.2	7.1
Albino	59	5.8 ± 0.3	6.5
ILS/ISS × ILS/ISS			
Pigmented	32	6.7 ± 0.5	9.6
Albino	18	6.1 ± 0.6	6.7
ILS/ISS × ISS/ILS			
Pigmented	35	6.8 ± 0.4	7.0
Albino	8	4.3 ± 0.8	5.3
ISS/ILS × ILS/ISS			
Pigmented	36	6.7 ± 0.3	2.9
Albino	18	6.2 ± 0.7	8.0
ISS/ILS × ISS/ILS			
Pigmented	32	7.1 ± 0.6	9.8
Albino	15	5.7 ± 0.6	4.6
ILS by ISS N_2s			
ILS × ILS/ISS	27	6.6 ± 0.4	4.9
ISS/ILS × ILS	28	6.0 ± 0.5	6.1
ISS × ILS/ISS	25	6.0 ± 0.5	6.5
Pigmented	19	6.2 ± 0.6	7.8
Albino	6	5.2 ± 0.6	2.2
ILS/ISS × ISS	29	7.1 ± 0.4	4.1
Pigmented	15	7.6 ± 0.6	4.7
Albino	14	6.7 ± 0.5	3.2

* LS and SS data are from Reference 18. F_1s are progeny from crossing ILS and ISS. F_2s are progeny from crossing two F_1s. N_2s are progeny from backcrossing an F_1 with either ILS or ISS. For each cross, the female parent is listed first.

crossing LS and SS and then intercrossing their progeny (F_1s). Chromosomal recombination in the F_1 gametes resulted in the next generation (F_2) having chromosomes that were a random patchwork of the LS and SS chromosomes. F_2 progeny were then inbred by brother-sister matings for more than 20 generations, resulting in chromosome pairs that are identical (homozygous) at virtually all loci.[21] Strain distribution patterns of genetic markers throughout the genome indicate which chromosomal regions of each RI strain are derived from LS or SS. The strain distribution patterns for 133 marker loci are published[15,22] and available online (at http://ibgwww.colorado.edu/~shale/ris.html). Because RI strains are genetically stable, any newly discovered phenotype difference between LS and SS (such as sensitivity to anesthetics) can be mapped without further genotyping by using these markers to look for allelic associations greater than expected by chance (*i.e.*, genetic linkage).[23]

A disadvantage of the RIs is that there are so few strains available that often QTLs cannot be mapped with high statistical certainty.[24] This is especially true of traits specified by multiple genes or that are only weakly heritable; therefore, most QTLs discovered using RIs are only provisional, meaning that they must be confirmed by testing additional mice. Such provisional QTLs have been identified for differential LORR sensitivity to sedative hypnotics[25] and ethanol[26] using the LSXSS RIs. Several, but not all, of the provisional QTLs for ethanol-induced LORR have been confirmed.[27,28]

Confirmation of a QTL typically involves testing a large population of F_2 mice. Like the RIs, F_2 mice represent a chromosomal patchwork of the parental strains; however, there is no subsequent inbreeding. In this study, an F_2 population was constructed using ILS and ISS strains, which are inbred strains derived from LS and SS.[15,20] The ILS and ISS strains still carry all of the differential sensitivity to ethanol of their respective parental lines[29,30] and also appear to carry all of the differential sensitivity to propofol as shown in this study (table 1, section 1). However, because ILS and ISS are inbred, they are genetically stable and greatly simplify the search for genetic linkage.

Materials and Methods

Origin of Mice

ILS, ISS, and LSXSS RI mice were produced in the Specific Pathogen Free facility at the Institute for Behavioral Genetics, University of Colorado at Boulder. ILS and ISS were derived from LS and SS by 20 rounds of brother-sister mating in the absence of any behavioral selection.[15] The LSXSS RIs were similarly derived without selection by reciprocal intercrosses between LS and SS followed by >20 generations of brother-sister mating.[15,20] Although the RI inbreeding began with 40 sibling pairs, only 25 strains were extant at the time of this study. Animals were weaned at about 25 days of age and housed one to four per cage with like-sex littermates on a 12-h light/dark cycle. Experimental protocols were approved by animal care and use committees at the University of Colorado at Boulder and University of Colorado Health Sciences Center at Denver. For the LSXSS RIs, 5-15 mice of each sex were tested per strain. Because mice of the same RI strain are genetically identical, the difference between testing 5 or 15 mice was minor and was accounted for by differences in the SEM. For the ILS by ISS F_2s, 194 mice were phenotyped, but 30 mice were inadvertently discarded without being genotyped; therefore the F_2 variance of table 1 is based on 194 mice, but only 164 mice were analyzed for genetic mapping. C57BL/6J coisogenic mice having the spontaneous albino mutation c^{2j} were obtained from Jackson Laboratory, Bar Harbor, Maine.

Phenotypic Assessments

LS and SS have been shown to be differentially sensitive to propofol at all doses tested (ranging from 15-35 mg/kg), and all doses had about equal efficacy in bringing about differential sensitivity.[18] In this study, a dose of 20 mg/kg was selected for its reliability in producing LORR without causing death. Propofol (10 mg/ml in Intralipid, Stuart Chemicals, Wilmington, DE) injections were performed when mice were 60 to 94 days old. F_2s had a mean age of 73.9 days (SD, 5) and a mean weight of 22.4 g (SD, 3). Dosages of 20 mg/kg (volumes ranging from 30-60 μl) were administered by injection into the retroorbital venous sinus using a 26-gauge, $^3/_8$-inch needle and Hamilton syringe. Room temperature was 23-26°C. Propofol sensitivity was measured as LORR.[18] At injection, mice immediately lost consciousness and were placed on their backs in V-shaped plexiglass troughs until recovery of the righting reflex. Animals were judged to have regained righting reflex when they turned over three times in less than 1 min. Full recovery after regaining consciousness was usually immediate. The RI and F_2 mice were naive to any previous injections and were injected only once with propofol. The LORRs of the (ISS c/c × C57BL/6

c^{2j}/C) F_1 mice represent the mean of two injections spaced 7 days apart. The mean of the second injection was not significantly different from the mean of the first injection ($P = 0.36$; analysis of variance, two-tailed significance). The influence of tyrosine brain levels on LORR was tested by intraperitoneal injection of tyrosine (200 mg/kg) or saline (control) immediately before or 30 min before the propofol injections. Brain levels of propofol at awakening (BLA) for the RIs were determined by gas chromatography of pooled samples of brain cortex, as previously described by Simpson and Blednov.[18]

Marker Assessments

DNA was extracted and purified from spleen, kidney, or liver using a Super-Quik Gene DNA Isolation Kit (AGTC Research, Denver, CO). *Mit* simple-sequence length polymorphism (SSLP) primers were obtained from Research Genetics (Huntsville, AL) and the polymerase chain reaction was carried out as previously described.[22] These primers are genetic markers that detect alleles at specific chromosomal locations differing in their polymerase chain reaction product length due to different numbers of cytosine and adenine (CA) dinucleotide repeats.[31-33] Coat color was scored by eye on living mice; pigmented RI mice were homozygous *C/C* and albino RI mice were homozygous c/c[34] (the *c* locus was renamed *Tyr* because it encodes for tyrosinase[35,36]).

Statistical Analyses

Broad-sense heritability measures dominant, additive, and between-gene interactions, whereas narrow-sense heritability measures only additive interactions.[21] Broad-sense heritability was estimated as $100\% \times (V_{F2} - V_{F1})/V_{F2}$, where V_{F2} is the variance of the F_2 population and V_{F1} is the weighted average of the variances of ILS, ISS, and ILS by ISS F_1s.[21] Narrow-sense heritability was estimated as $100\% \times 0.5\ \sigma^2_B/(\sigma^2_w + 0.5\sigma^2_B)$, in which σ^2_B is the between-strain variance (the variance of the strain means minus the weighted average of the within-strain variances, which is then divided by the number of strains) and σ^2_w is the weighted average of the within-strain variances.[37] This formula assumes that interactions between genes are negligible. Within-strain variance (σ^2_w) is equivalent to environmental variance because the RI strains had undergone >20 generations of inbreeding and thus genetic variability within strains would be expected to average <1.4%.[21] In contrast to F_2s, in which one half the mice are homozygous and the other half are heterozygous at any given locus, all of the RIs are homozygous at all loci; therefore, the between-strain variance (σ^2_B) of the RIs is multiplied by one half to obtain a standardized genetic variance.

Although the heritability estimates were made from RI and F_2 mice derived from different parental strains (noninbred LS and SS *vs.* inbred ILS and ISS, respectively), the parental strains are closely related. This inference is based on the amount of inbreeding (>60%) at the time of RI derivation.[20] This inbreeding is confirmed by the predominance of ISS and ILS alleles in the RI strain distribution pattern of SSLP markers throughout the genome.[15] It also can be seen from table 1 (section 1) that LS and ILS, SS and ISS have very similar LORRs in response to propofol.

The QTL maps were anchored using a map position for *c* and *D7Mit31* of 44 centimorgans (cM), which is based on the Mouse Genome Database (MGD, World Wide Web URL: http://www.informatics.jax.org/mgd.html). The positions of other SSLP markers were placed relative to *c* and *D7Mit31* based on the Whitehead Institute/MIT Center for Genome Research map (WI/MIT/CGR, World Wide Web URL: http://www.genome.wi.mit.edu), which is the more recently updated and more accurate map regarding the relative position of the SSLP markers.[38]

The RI map was constructed using an interval-mapping procedure previously described by Markel *et al.*[26] The F_2 linkage map was constructed using Mapmaker 3.0 and QTL mapping was done using Mapmaker/QTL Version 1.1. For the F_2s, two mice were excluded because LORR was not induced, and four mice were excluded because of LORRs greater than 2 SDs from the mean (15.9, 16.8, 17, and 44.7 min). Including these mice had no effect on the LOD (logarithm of the odds of linkage) profile, but the peaks were reduced by about 0.5 LOD.

Results

Heritability of Propofol Sensitivity

Heritability is an estimate of the relative importance of heredity *versus* the environment.[21] We estimated heritability for the propofol sensitivity difference between LS and SS using two approaches. The first estimate was based on the variance of LORR for the ILS by ISS F_2 mice (table 1, section 3). The phenotypic variance of F_2 mice is due to heredity and environment. However, the variance of ILS, ISS, or ILS by ISS F_1 mice (table 1, sections 1 and 2) is due only to environment, because

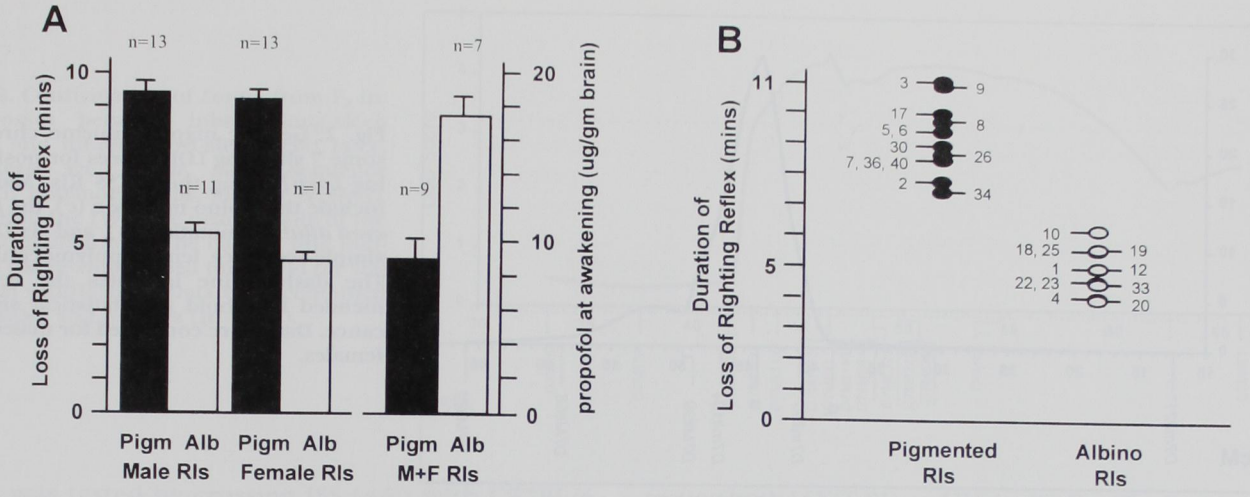

Fig. 1. Duration of loss of righting reflex (LORR) and brain levels of propofol at awakening (BLA) in pigmented and albino LSXSS RI strains. (*A*) The difference in LORR between males and females was not significant, whereas the difference between pigmented (black bars, 4.8 ± SEM) and albino RI strains (*white bars,* 9.3 ± SEM) was highly significant for males and females ($P < 0.0001$, two-tailed test). *n* refers to the number of RI strains. The LORR for each strain was determined from the mean of 5–15 animals of each sex, and the BLA for each strain was determined from the mean of at least three samples. The difference in propofol BLA between pigmented (8.4 ± 1.2 μg/gm) and albino (17.2 ± 1.9 μg/gm) strains is highly significant ($P < 0.0001$, two-tailed test). The pigmented RI strains (black bars) used for propofol BLA determination were 2, 3, 6, 7, 8, 9, 17, 30 and 36; and the albino RI strains (*white bars*) used were 4, 10, 18, 20, 23, 25, and 33. Male and female data are combined for propofol BLA. The correlation coefficient *r* between propofol BLA and LORR for the 16 RI strains for which both measures could be obtained was −0.85 ($P < 0.001$). Error bars indicate standard error of the mean. (*B*) The LORR means for the pigmented RI strains are indicated by the filled ovals, and the means for the albino RI strains are indicated by the open ovals. The numbers next to the ovals indicate the RI strain(s) having that mean. RI strain 32 had a mean LORR of 4.4 min (SEM, 0.7) for females and 6.6 min (SEM, 1.1) for males, but it was not included in this analysis because at that time it was still segregating for the albino mutation.

the mice within each group are genetically identical. Therefore, the F_2 variance (heredity and environment) minus the average of the variance of the ILS, ISS, and F_1 mice (environment) provides an estimate of genetic variance. The ratio of the genetic variance to the total variance (F_2 variance) is heritability. In this manner, the propofol sensitivity difference between LS and SS was found to be about 55% heritable. In a similar manner, the LSXSS RI strains yielded a heritability estimate of 32% (see Materials and Methods).

We also examined the nonheritable portion of the difference between LS and SS to determine if there might be a maternal effect. Table 1 (sections 2 and 4) shows that the F_1 and N_2 (F_1s backcrossed with ILS or ISS) offspring of ILS mothers had a mean LORR about 0.7 min longer than the mean LORR of the offspring of ISS mothers. The probability that this difference was due to chance alone was less than 0.1 ($P < 0.1$, one-tailed test), thereby suggesting that a difference between ILS and ISS mothers could be having a small effect on LORR. The LORR for RIs and F_2s did not covary with age, weight, sex, or time of day of injection.

Location of a Quantitative Trait Locus for Propofol Sensitivity

The LS and SS selected lines are completely differentiated at the albino locus (*Tyr*), with all SS mice being albino and all LS mice being pigmented. When we examined the LSXSS RIs we found that the 11 albino RI strains had a mean LORR of 4.8 min (SEM, 0.3) in response to propofol, whereas the 13 pigmented strains had a significantly ($P < 0.0001$, two-tailed test) longer mean LORR of 9.3 min (SEM, 0.4), with no difference between males and females (fig. 1A). (RI 32 was excluded from this analysis because it was still segregating for the albino mutation at that time.) Furthermore, the LORRs of the albino strains did not overlap with the LORRs of the pigmented strains (fig. 1B), suggesting a major QTL. The albino strains also had a significantly higher BLA ($P < 0.0001$; fig. 1A), suggesting the the difference in LORR was due to differential CNS sensitivity and not pharmacokinetic differences. Plotting BLA *versus* LORR for the RI strains (not shown) confirmed a strong negative correlation (Pearson *r* correlation coefficient, −0.85) that was highly significant ($P < 0.001$).

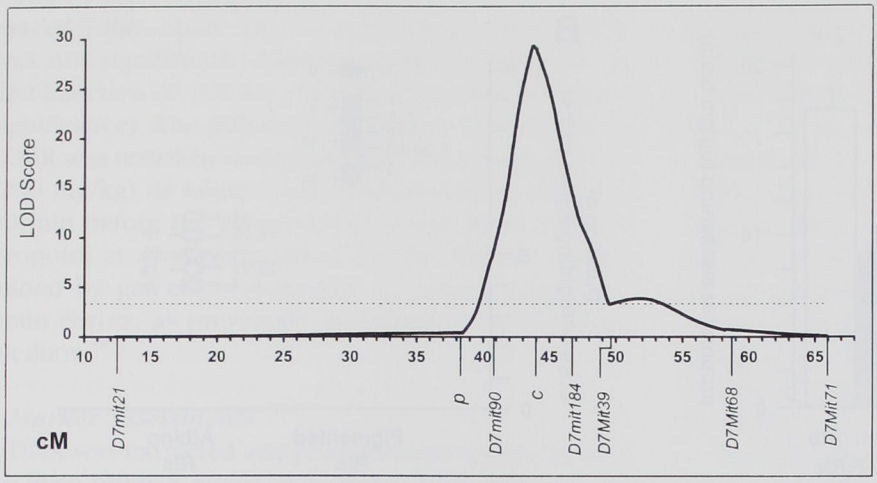

Fig. 2. Genetic map of murine chromosome 7 showing LOD scores for positioning *Lorp1* using the LSXSS RIs. Markers include the albino mutation (*c*), the *pink-eyed dilution* mutation (*p*), and six *D7Mit* simple-sequence length polymorphisms. The dashed line indicates the recommended threshold for statistical significance. Data were combined for males and females.

The region around *Tyr* was genetically mapped using the regression approach described by Haley and Knott,[39] which we previously used in mapping QTLs for alcohol sensitivity.[26] We assessed the genotypes of seven SSLP markers spanning 50 cM of chromosome 7, obtaining peak LOD scores of 19 and 21 for males and females, respectively. The combined LOD score for males and females was about 28 (fig. 2), far exceeding the value of 3.3 recommended for significant linkage in a whole-genome scan.[40] This assignment was highly specific and positioned the QTL to a 2.5-cM region encompassing *Tyr* with 2-LOD support, indicating that the probability that the QTL being within this region is greater than 0.99. The LOD peak turned out to be at *Tyr* itself. This QTL, which we have called *Lorp1* (Loss of righting due to propofol), explained about 80% of the genetic variance between LS-like and SS-like RI strains.

Confirmation of Linkage

We also were interested to see if F_2 mice derived by intercrossing the ILS and ISS strains would have the same QTL indicated by the LSXSS RIs. A panel of 164 F_2s was genotyped with 16 SSLP markers from murine chromosome 7 that included all of the informative markers within 10 cM of *Tyr*.[38] A peak LOD score of 4.4 was obtained between marker *D7Mit31* within *Tyr* and *D7Mit123* less than 2 cM distal to *Tyr* (fig. 3), thus confirming *Lorp1*. F_2 mice homozygous for the SS alleles of *D7Mit123* had an LORR of 5.7 min (SEM, = 0.3), heterozygotes had an LORR of 6.5 min (SEM, 0.3), and LS homozygotes an LORR of 8 min (SEM, 0.4). The 2.3-min difference in LORR between mice homozygous for the S allele and those homozygous for the L allele matches the 2.2-min difference previously observed between LS and SS.[18] This difference is consistent with all of the genetic difference between LS and SS for propofol sensitivity having been captured in the ILS and ISS strains. This difference also suggests that most or all of the genetic difference between ILS and ISS is due to *Lorp1*. *Lorp1* explained about 23% of the F_2 genetic variance.

Mapping of the RI BLA data also supported a QTL linked to *Tyr*, with a statistically significant peak LOD score of 3.3 occurring at the same location as *Lorp1* (not shown). RIs homozygous for the L allele (long LORR) of *Lorp1* had a propofol BLA of 8.4 (g/g brain), whereas those homozygous for the S allele (short LORR) had a BLA of 17.2 (g/g brain), a highly significant difference ($P = 0.001$, one-tailed test assuming equal variances) previously seen in the LS and SS lines.[18] That mice with the S allele had a significantly higher BLA indicated that their CNSs were less sensitive to propofol and not that they were better able to metabolize propofol or sequester propofol outside the CNS (as in fat tissue). F_2s could not be used to confirm BLA due to the need to pool samples from several mice to meet the minimum sensitivity requirements of the propofol assay (another advantage of RIs).

Test of Involvement of the Albino Phenotype in Determining Propofol Sensitivity

Considering the tight linkage between *Lorp1* and *Tyr*, we asked whether the resistance of SS mice to propofol might result from the albino phenotype.

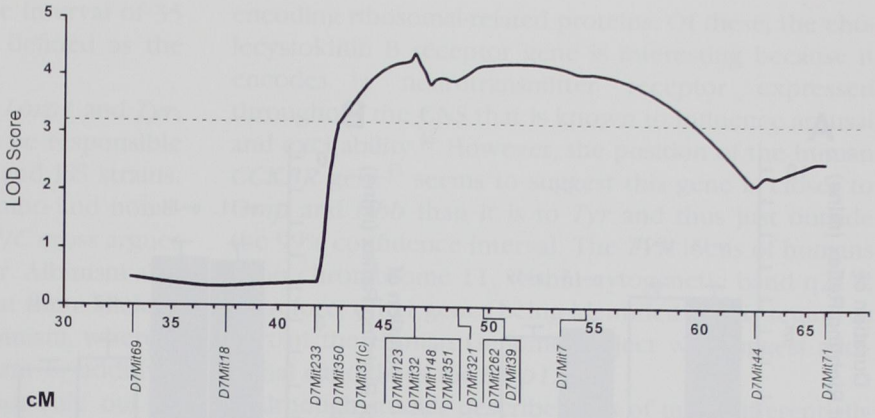

Fig. 3. Confirmation of *Lorp1* from F_2 intercrosses between inbred long-sleep (ILS) and inbred short-sleep (ISS) mice. Markers are *D7Mit* simple-sequence length polymorphisms. Marker *D7Mit31* is within the *Tyr* gene near the site of the albino mutation, *c*. The dashed line indicates the recommended threshold for statistical significance. Data were combined for males and females (n = 158).

This was tested by crossing ISS (*c/c*) with C57BL/6 mice from the Jackson Laboratory that were completely inbred but heterozygous for a spontaneous albino mutation (c^{2j}/C). The F_1 progeny were thus coisogenic, identical at all loci except *Tyr*; all F_1 progeny derive a *c* allele from the ISS parent and either a c^{2j} or *C* allele from the other parent. Thus one half of the F_1 mice are albino (c/c^{2j}) and the half are normally pigmented (*c/C*). If propofol resistance were due to albinism, then albinos would be expected to have a mean LORR about 0.8 min less than their pigmented littermates based on the ILS by ISS F_2 results. However, the albino LORR was not significantly less, and a 0.8-min difference was ruled out at the 95% confidence level after accounting for sex (fig. 4A).

Because *Tyr* encodes for the enzyme tyrosinase, we also tried to determine whether tyrosine injections might alter LORR. The connection between *Tyr* and tyrosine levels was appealing because tyrosine is converted to L-dopa in the rate-limiting step of catecholamine synthesis, and tyrosine shares structural similarities with propofol. It also was intriguing that tyrosine availability was previously shown to alter the ethanol-induced LORRs of LS and SS,[41] and a QTL (*Lore7*) for ethanol-induced LORR has been found to be linked to *Tyr*.[28] It was found, however, that coinjecting the same dose of tyrosine that influenced ethanol-induced LORR had no influence on propofol-induced LORR for either ILS or ISS mice (fig. 4B).

Discussion

Differential sensitivity to propofol between LS and SS has a substantial genetic component, as indicated by testing both LSXSS RIs and ILS by ISS F_2s. The F_2 estimate of heritability was 55%, which is considerably greater than the more limited estimate of 32% from the RIs, which detects only one type of genetic effect (additive). The difference between the two estimates suggests that in the F_2s there are additional dominance or between-gene interactions, or both. Part of the difference could be due to the RIs having been derived from noninbred LS and SS mice, whereas the F_2s were derived from inbred ILS and ISS mice. However, the difference between LS and ILS and between SS and ISS is small (see Materials and Methods) and thus unlikely to be a major factor.

A QTL, *Lorp1*, is tightly linked to *Tyr* in the middle of murine chromosome 7. This QTL accounts for about 80% of the genetic variance in the RIs, indicating that this is the major locus specifying differential propofol sensitivity between LS and SS. The LORR difference of 2.3 min observed between ILS by ISS F_2 mice homozygous for the S allele of *Lorp1* compared with those homozygous for the L allele also is sufficient to account for all of the LORR difference observed between LS and SS. Although the involvement of other genes is suggested by the heritability estimates, their effects were not detected in the LSXSS RIs, and no whole-genome map of F_2s has yet been generated.

The LORR of F_2s heterozygous at *Lorp1* (6.5 min) was more similar to that of S allele homozygotes (5.7 min) than L homozygotes (8 min). Therefore the S allele appears to be partially dominant over the L allele, although a strictly additive model cannot be ruled out. It is curious, however, that heterozygotes and S allele homozygotes were more similar in LORR to their ILS progenitor (7.4 min) rather than ISS (3.8 min). This could be due to a difference in gene interactions, but it also could

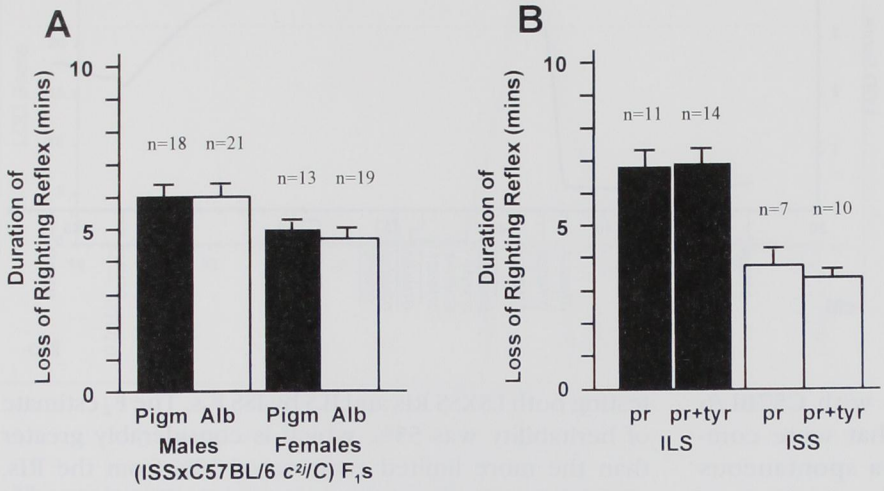

Fig. 4. Propofol loss of righting reflex for (inbred short-sleep [ISS] $c/c \times$ C57BL/6 c^{2j}/C) F_1s and for inbred long-sleep (ILS) and ISS mice coinjected with tyrosine. (*A*) ISS mice have the classical albino mutation, whereas the c^{2j} albino mutation arose spontaneously in C57BL/6 at Jackson Laboratory. Therefore, F_1 mice are identical at all loci except the albino locus, pigmented mice (*black bars*) are c/C, and albino mice (*white bars*) are c/c^{2j}. LORRs of females are significantly shorter than those of males by 1.3 min ($P = 0.002$, analysis of variance, two-tailed test). However, LORRs of albinos are not significantly shorter than those of pigmented mice ($P = 0.35$, analysis of variance, one-tailed test). (*B*) ILS (*black bars*) and ISS (*white bars*) mice coinjected with tyrosine (indicated as pr + tyr) also did not exhibit significant differences in LORR ($P = 0.78$, analysis of variance, two-tailed test) from controls at a concentration of tyrosine (200 mg/kg) previously shown to differentially alter ethanol-induced LORRs in LS and SS.[41] *n* indicates the number of mice (males and females) tested. Error bars indicate the SEM.

be due to environmental differences (*e.g.*, room temperature, noise level, injection proficiency) because the F_2s were not tested at the same time as ISS and ILS. In this regard, we have observed that the mean LORR of ILS or ISS can vary from day to day by 1–2 min (in our experiments, however, ILS and ISS were almost always tested together on the same day).

Two methods of mapping were used to position *Lorp1*: RI interval mapping and F_2 mapping. Unlike the usual situation, the RI mapping proved to be extremely powerful, resulting in an LOD score of 28. Therefore, the identification of *Lorp1* was not merely provisional, but rather highly significant. Further, the RI mapping localized *Lorp1* to a 99% confidence interval of just 2.5 cM region. By comparison, large-scale F_2 mapping of QTLs for ethanol-induced LORR resulted in 99% confidence intervals that were an order of magnitude larger.[28]

Given the RI mapping result, it was surprising that the F_2 result did not approach the same significance or sharpness of LOD peak. This probably occurred for several reasons. First, the number of F_2s used (164) is relatively small, much smaller than the number used (approximately 1,000) to confirm ethanol sensitivity QTLs.[28] Second, *Lorp1* is a major QTL nearly equivalent to a single-gene effect. Therefore, the ability to test an RI strain repeatedly to reduce environmental variance can drive even a single recombination event between the QTL and a tightly linked marker to very high statistical significance, thus excluding the region beyond that marker. Such independent, repeated testing cannot be done with an F_2 population. Third, the QTL effect size (the amount of the genetic effect resulting from variation at a particular locus) between SS-like and LS-like RIs was 4.5 min compared with only 2.3 min between F_2 homozygotes. This suggests additional gene interactions in the F_2s that attenuated the influence of *Lorp1*; however, it also could reflect environmental differences given that RIs and F_2s were not tested at the same time.

The LOD score for BLA peaked at the same location as *Lorp1*, suggesting that *Lorp1* affects neurosensitivity. However, the LOD for BLA was much lower at 3.3 than the LOD for *Lorp1* at 28. This is most likely due to the smaller number of BLA measurements, a higher coefficient of variation (SD/mean) for BLA, and not including RI strain 1 having a recombination close to *Tyr*. The difference also could suggest that the LORR difference is not entirely determined by BLA, although variation in BLA did explain about 72% of the variation in LORR.

Provisional QTLs for LORR induced by ethanol, propanol, and chloral hydrate also have been linked to *Tyr* in LS and SS mice.[25,26] The ethanol QTL, confirmed by Markel and Corley,[27] is called *Lore7*.[28] It is possible that *Lore7* and *Lorp1* are the same; however, the map

position of *Lore7* has a 90% confidence interval of 35 cM and thus is not nearly as sharply defined as the position of *Lorp1*.

Given the very tight linkage between *Lorp1* and *Tyr*, we considered whether albinism could be responsible for the decreased sensitivity of the SS and ISS strains. However, comparison of coisogenic albino and nonalbino F_1 mice from the ISS × C57BL/6 c^{2j}/C cross argues that albinism *per se* is not a causal factor. Albinism also seems to be an unlikely factor given that the *c* allele is completely recessive with respect to albinism, whereas the S allele of *Lorp1* is partially dominant or additive. Although the coisogenic result does not rule out an effect unrelated to albinism and unique to the *c* allele, such an effect seems unlikely given that the *c* and c^{2j} alleles abolish tyrosinase activity[42–45] and thus would appear to be functionally equivalent in all aspects.

The coisogenic experiments also do not exclude the possibility that *Tyr* affects LORR due to the *C* allele of LS. If the *C* allele is responsible, a simple hypothesis is that this allele leads to an increase in tyrosinase activity that reduces the availability of tyrosine in the brain for other purposes such as synthesizing catecholamines. If it does, then increasing tyrosine levels might alleviate this competition and cause LS mice to have a shorter LORR similar to SS mice. This is exactly the result obtained when LS mice were coinjected with tyrosine and measured for ethanol-induced LORR (SS mice were unaffected).[41] In our study, however, coinjecting the same dose of tyrosine as that used in the ethanol study had no effect on propofol-induced LORR in the LS or SS mice. Although these results still do not rule out unusual effects of *Tyr*, they argue against *Tyr*'s involvement due to modulating tyrosine levels. These results also imply that, even though propofol and tyrosine are structurally similar, propofol does not act through competing with tyrosine.

Many other genes besides *Tyr* fall within the 99% confidence interval of 2.5 cM for *Lorp1*. Assuming an average gene density, 2.5 cM would be expected to harbor about 150 genes. So far, however, only about ten genes have been either physically or genetically mapped to this region with better than 1-cM resolution (MGD map). These genes are *Cckbr*, encoding the cholecystokinin B receptor; *Cht*, a coat color gene; *eed*, essential for embryonic ectoderm development; *fit1*, essential for normal growth and fertility; *jdf*, essential for normal juvenile development and fertility; *mod2*, encoding mitochondrial malic enzyme; *mod2r*, encoding a regulator of *mod2*, and *Rps17-rs6* and *Rps4-rs2*, encoding ribosomal-related proteins. Of these, the cholecystokinin B receptor gene is interesting because it encodes a neurotransmitter receptor expressed throughout the CNS that is known to influence arousal and excitability.[46] However, the position of the human *CCKBR* gene[47] seems to suggest this gene is closer to *Omp* and *Hbb* than it is to *Tyr* and thus just outside the 99% confidence interval. The *TYR* locus of humans is on chromosome 11, within cytogenetic band q21[48]; therefore, other genes being identified in this region as part of the Human Genome Project will suggest additional candidates for *Lorp1*.

Although studies describe lines of mice differentially sensitive to nitrous oxide,[49] diazepam,[50] and opiates,[51] no loci specifying these differences have been mapped. Therefore, *Lorp1* is the first locus specifying differential sensitivity to a clinically relevant general anesthetic agent to have been mapped in a mammalian species. In nonmammalian species, single-gene mutants in the nematode *Caenorhabditis elegans* and in the fruit fly *Drosophila melanogaster* have been found to be differentially sensitive to the anesthetic effects of halothane,[52–56] and QTL studies in *C. elegans*[57] have revealed additional loci underlying halothane sensitivity. There is optimism, therefore, that identifying these gene products will pinpoint molecular pathways directly relevant to the action of general anesthetics.

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