Online Methods

Mice

Subjects in all experiments were naïve, adult (6–12 weeks old) mice of both sexes. Genotypes tested included A/J, C57BL/6J (B6), AcB/BcA-set recombinant congenic (RC) strains (AcB63, AcB64, BcA70, BcA72, BcA79, BcA81, BcA86) [22], and congenic lines to be described below. Haplotype mapping was performed by phenotyping 14 other inbred strains (all "J" substrains). Upon weaning (at 18–21 d) or immediately after arrival, mice were housed in standard shoebox cages of 2–4 with same-sex littermates in a temperature-controlled (20±1°C) environment (14 h:10 h light/dark cycle; lights on at 07:00 h), and with *ad lib* access to food (Harlan Teklad 8604) and tap water. Mice were habituated to the laboratory for at least one week before any behavioral testing commenced.

We used the AcB64 and BcA72 strains to construct speed congenics (A.AcB64^{Nociq2} and B6.BcA72^{Nociq2} lines, respectively); these were generated with marker-assisted selection of breeding males [33]. The purity of the congenic strains was confirmed by genotyping 20 informative microsatellites (CEQ 8000 Genetic Analysis System; Beckman-Coulter) chosen at random throughout the genome.

We used one of the A.AcB64^{Nociq2} congenic lines (line 64C1; see Fig. S2) to generate two interval-specific subcongenic lines. We backcrossed the congenic line to A/J, and searched for crossovers via the observation of heterozygosity at markers distal to *D10Mit233* (114.0 Mb). We found one female mouse with heterozygosity at *D10Mit297* and *D10Mit103*, indicating a crossover somewhere between 121.7–124.4 Mb (A/J alleles distal to the crossover). This mouse was backcrossed to A/J and then intercrossed, to form the 64SCA (AcB*64*-derived *s*ub*c*ongenic

line with *A*/J alleles at distal chromosome 10) line (see Fig. S3). We also found a male mouse with heterozygosity at *D10Mit14* and *D10Mit237*, and used it to reduce the B6-derived region of A.AcB64^{Nociq2}, forming the 64SCB (AcB*64*-derived *s*ub*c*ongenic line with *B*6 alleles at distal chromosome 10) line (see Fig. S3).

Avpr1a knockout mice (–/–) and wildtype controls (+/+), bred congenic onto a C57BL/6 background for >10 generations, were obtained from the National Institute of Mental Health (Bethesda, MD) [34]. Lines were maintained as heterozygote (HET) x HET breeding colonies, and all offspring were genotyped by custom polymerase chain reaction (PCR) analysis of DNA isolated from tail tissue.

qPCR

We quantified basal mRNA expression of *Avpr1a* in A/J and B6 mice (compared to the housekeeping gene, *Gapdh*), in lumbar (L4–L6) dorsal root ganglion (DRG), the dorsal horn of the spinal cord, periaqueductal gray, hypothalamus, whole brain (minus cerebellum), and liver. Experiments in all tissues were conducted twice. Tissue was dissected and harvested, and total RNA was isolated and reverse transcribed using standard protocols. Gene expression was quantified using the 7700 Sequence Detector (TaqMan) and the SYBR Green PCR Core Reagent Kit, as described in the manufacturer's manual (Applied Biosystems).

Unpublished microarray gene expression profiling data from our laboratory, using these two strains, suggest that formalin is not a sufficient stimulus to regulate a large number of genes. It is also questionable as to whether strain differences, which are obvious even at the beginning of the late phase of the formalin test, could be secondary to formalin-induced gene expression changes.

Thus, we feel comfortable in asserting that basal strain differences in gene expression are likely responsible for phenotypic strain differences in this trait.

Habituation

In most experiments, mice were not habituated to either the testing room or the Plexiglas testing enclosures prior to the day of testing. A 30 min habituation was provided to *all* subjects immediately prior to testing. Those groups designated as "habituated" were, in addition, placed in the testing room within testing enclosures identical to those to be used in the experiment for 60 min on each of 6 days preceding the experiment, and then returned to the vivarium.

Formalin Test

Mice were habituated for 30 min in individual transparent Plexiglas cylinders (15 cm diameter; 22.5 cm high) placed atop a glass surface suspended over high-resolution video cameras. All subjects were then given a subcutaneous injection of 0.5%–5% formalin (Sigma) into the plantar right hindpaw (20 μl volume), and digitally videotaped for 60 min following the formalin injection. Video files were later sampled for 5 sec at 1-min intervals, and the presence or absence of right hind paw licking/biting in that 5-sec period was scored (Noldus Observer™). The early (acute) phase of the formalin test (F_{early}) was defined as 0-10 min post-injection, and the late (tonic) phase (F_{late}) as 10-60 min post-injection. Data are presented as the percentage of samples in which licking/biting was detected (percentage samples licking).

Hindpaw edema was quantified as the difference in the weight of the injected versus uninjected paw, as a percentage of total body weight, as previously described [20].

Mouse Capsaicin Test

Mice were habituated for 30 min in individual transparent Plexiglas cylinders (15 cm diameter; 22.5 cm high) placed atop a glass surface above high-resolution video cameras. All subjects were then given a subcutaneous injection of 2.5 μg capsaicin (Sigma) into the plantar right hindpaw (20 μl volume), and digitally videotaped for 10 min following the formalin injection. Video files were later scored for the total duration (s) of licking/biting of the right hind paw. Sampling was not used in the capsaicin test because of the short duration of behavioral responses.

Hot-Plate Test

Mice were gently placed on a metal surface maintained at 56°C (IITC Model PE34MHC; Stoelting) within a transparent Plexiglas cylinder (15 cm diameter; 22.5 cm high). The latency to either lick or shake either hind paw was measured with a stop watch as a nocifensive endpoint.

Mouse Stress Measures

Stress was estimated in three different ways: fecal boli deposition, plasma corticosterone and plasma AVP. The number of fecal boli deposited at the end of the 30-min habituation period (i.e., immediately before formalin or capsaicin injection) was counted. In separate subjects, trunk blood was extracted from mice euthanized at the end of the 30-min habituation period. Plasma was extracted from trunk blood by cold (4°C) centrifugation (15 min at 13,000 rpm) and immediately frozen at –20°C. Samples were assayed for corticosterone (CORT) and AVP by enzyme immunoassay kits, according to manufacturer's instructions (Cayman Chemical: CORT kit #500655; AVP kit #583951).

Swim Stress-Induced Analgesia

Immediately following the measurement of baseline latencies on the 56 ºC hot-plate test, mice were gently placed in a 2-L cylindrical container approximately two-thirds filled with water maintained at 32±1 ºC. Escape being impossible, mice swam for 3 min, and then were removed, dried off, and left in a towel-lined cage for 2 min. They were then retested on the hot plate. Analgesia was quantified as the percentage of the maximum possible analgesia using a cut-off latency (not reached by any subject) of 45 s: [(post-swim latency – baseline latency)/(45 – baseline latency)] x 100.

Mouse Drug Administration

Arginine vasopressin (AVP; Sigma) was administered at a dose of 0.1 mg/kg (s.c.) in a volume of 10 ml/kg. This dose was chosen based on our recent experience with the compound in multiple genotypes [23]. Naloxone (Sigma) was administered at a dose of 10 mg/kg (s.c.) in a volume of 10 ml/kg. Control groups received physiological saline at the same volume.

Capsaicin Pain Association Study

The sample consisted of 104 healthy adults (51.9% female; mean age = 28.6 [±8.6]; self-reported ethnicity: 50.9% Caucasian, 35.2% African American; 13.9% Asian; 94.4% of non-Hispanic origin). Whole blood samples or saliva was obtained for genetic analysis, and stored until DNA was extracted. Participants rated their current stress level on a 0 to 10 scale (0 = no stress, 10 = extreme stress) immediately before capsaicin administration. Capsaicin application methods were similar to procedures published previously [35]. A topical cream

consisting of 0.35–0.5 g of 10% capsaicin was applied to a 6.25-cm^2 area on the dorsal aspect of the non-dominant hand, and was evenly spread on the skin. Pain rating data were collected over a set interval, up to 90 min. Participants rated their pain continuously on a computerized 0–100 visual analog scale (VAS; 0 = no pain, 100 = most intense pain imaginable).

We selected two SNP markers in the *AVPR1A* gene for genotyping, based on relatively high minor allele frequencies and physical location within potentially functional sites: 1) rs1042615, a silent substitution in exon 1 (A/G; TTT to TTC (Phe/Phe) at amino acid 136; could affect RNA stability or splicing); and 2) rs10877969, in the promoter region (could affect gene expression). These markers are validated, have minor allele frequencies greater than 10% in Caucasians and other ethnic groups, and they are 3 kb apart (covering most of the gene yet displaying fairly good linkage disequilibrium for haplotyping). The primers and probes for genotyping were from ABI. Genomic DNA was purified using QIAampTM 96 DNA Blood Kit (Qiagen). Genotyping was performed by the 5' nuclease method [36] using fluorogenic allele-specific probes. Allele-specific signals were distinguished by measuring endpoint 6-FAM or VIC fluorescence intensities at 508 nm and 560 nm, respectively, and genotypes were generated using Sequence Detection System Software (ABI). Genotyping error rate was directly determined by re-genotyping 25% of the samples, randomly chosen, for each locus. The overall error rate was <0.005. Genotype completion rate was 0.98. Allele frequencies were calculated in each sample, and Hardy-Weinberg Equilibrium (HWE) testing using Fisher's exact test were performed by PLINK [37]. Single-marker analyses were conducted for the associations between *AVPR1A* markers and capsaicin-induced pain scores.

The current study also applied an established dynamic mathematical model that has successfully parsed the marked individual variability in the response to capsaicin stimuli, and

with which we have detected three common dynamic (i.e., temporal) response patterns among human subjects [24,38]. See Fig. S5 for details.

Desmopressin Analgesia Study

Forty (20 female, 20 male) healthy participants between the ages of 18 and 45 were recruited via advertisements and word of mouth, and one female participant failed to complete the study due to medical exclusion. Each participated in two testing sessions—one involving desmopressin (Sanofi Aventis) and the other involving saline—administered intranasally in randomly counterbalanced and double-blind fashion. At the outset of each session, tension (i.e., stress) ratings were obtained using a visual analog mood scale (VAMS) [39]. Two participants failed to complete the VAMS correctly and were excluded from analyses involving these data.

Intranasal desmopressin (1-desamino-8-D-arginine vasopressin; DDAVP; a vasopressin analog without the pressor effects of AVP) at a concentration of 100 μg/ml of saline was administered using a Pfeiffer SAP#77061 metered spray pump, as a series of five 100-μl sprays, for a total dose of 50 μg. Saline was administered in identical fashion.

In each session, heat pain threshold and tolerance and pressure pain threshold were assessed immediately before and 10 and 50 min after drug administration, using standard methods [40]. Twenty-five min after drug administration, capsaicin cream (8%) was applied topically to a 3-cm^2 area of the right ventral forearm. Participants provided pain ratings using a 0 (no pain) to 100 (most intense pain imaginable) numerical rating scale (NRS) every 10 min for 50 min after capsaicin application.

Genomic DNA was purified from saliva samples collected with the Oragene system (DNA Genotek) using the manufacturer's recommended procedure. Genotyping was performed using

ABI TaqMan SNP assays for rs10877969 and rs1042615), using the Applied Biosystems 7900HT SNP platform. Genotyping for rs10877969 failed in two subjects, and thus 36 subjects were ultimately available for genotype-phenotype correlation analysis. The G allele frequency was in Hardy-Weinberg equilibrium and was somewhat closer to that reported in dbSNP than to that observed in the capsaicin pain sample set.

For most statistical analyses the mean NRS score over the 50-min testing period was used as a dependent measure; broadly similar results were obtained when substituting other possible dependent measures.

Statistics

Data from were analyzed using ANOVA followed by Student's *t*-tests as appropriate, using SYSTAT v. 13 (SPSS Inc.). A criterion $\alpha = 0.05$ was adopted to establish significance.