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Identifying genetic determinants of inflammatory pain in mice using a large-scale gene-targeted screen

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Abstract

Identifying the genetic determinants of pain is a scientific imperative given the magnitude of the global health burden that pain causes. Here, we report a genetic screen for nociception, performed under the auspices of the International Mouse Phenotyping Consortium. A biased set of 110 single-gene knockout mouse strains was screened for 1 or more nociception and hypersensitivity assays, including chemical nociception (formalin) and mechanical and thermal nociception (von Frey filaments and Hargreaves tests, respectively), with or without an inflammatory agent (complete Freund's adjuvant). We identified 13 single-gene knockout strains with altered nocifensive behavior in 1 or more assays. All these novel mouse models are openly available to the scientific community to study gene function. Two of the 13 genes (*Gria1* and *Htr3a*) have been previously reported with nociception-related phenotypes in genetically engineered mouse strains and represent useful benchmarking standards. One of the 13 genes (*Cnrip1*) is known from human studies to play a role in pain modulation and the knockout mouse reported herein can be used to explore this function further. The remaining 10 genes (*Abhd13*, *Alg6*, *BC048562*, *Cgnl1*, *Cp*, *Mmp16*, *Oxa1l*, *Tecpr2*, *Trim14*, and *Trim2*) reveal novel pathways involved in nociception and may provide new knowledge to better understand genetic mechanisms of inflammatory pain and to serve as models for therapeutic target validation and drug development.

Keywords: Pain, Nociception, Nocifensive behavior, Sensitization, Formalin, Hargreaves, von Frey, Complete Freund's adjuvant, Single-gene knockout mouse, Screen, IMPC, Comorbidity, Autism

1. Introduction

Pain is a huge unresolved health burden with approximately 20% of adults worldwide reported to suffer from chronic pain, defined as lasting 12 or more weeks.[25,26] An estimated 50 million Americans suffer from chronic pain,[13,60] which leads to increased health costs, loss of productivity, and perceived lower quality of life. Pain can present as the primary condition or as a comorbidity of conditions as diverse as cancer, multiple sclerosis, human immunodeficiency virus

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

A full list of consortium members appears in the Acknowledgements section.

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infection,[18] and neuropsychiatric conditions.[8] Clinical pain perception is complex, involving physical, cognitive, contextual, and emotional components. Despite this complexity, the heritability of pain-related traits in mammals is well accepted.[49]

Nociception is defined as "the neural process of encoding noxious stimuli."[35] Although animal models cannot capture all components of clinical pain, the mouse has been used extensively as a genetic model of nociception. For example, PainGenesdb[41] reports 430 genes with published pain-related phenotypes (queried March 11, 2021). Analysis of the correlation of common nociception parameters in inbred mouse strains reveals at least 5 genetically dissociable categories of nociception and hypersensitivity.[42,48,51,76] For example, assays assessing baseline thermal nociception, such as Hargreaves and tail flick, cluster together but are under genetic control mechanisms distinct from assays assessing spontaneous responses to noxious chemical stimuli, such as formalin and capsaicin.[42]

The International Mouse Phenotyping Consortium (IMPC) is a global consortium tasked with identifying the function of every protein-coding gene in the mammalian genome through the creation and phenotypic characterization of single-gene knockout mouse strains.[20] As of data release 15.0, phenotyping data were available for 7824 genes (www.mousephenotype.org). The IMPC phenotyping pipeline is a hypothesis-generating screen designed to identify gross abnormalities in neurobehavior, cardiac function, metabolism, body composition, skeletal structure, vision, hearing, and blood composition. This broad screen is not designed to explore mechanistic underpinnings but is an invaluable first step in the characterization of genetic models of human disease. The IMPC pipeline does not include any screen for nociception. In the current study, 5 IMPC centers assessed the feasibility of including such a screen. Assays were selected from the genetically distinct classifications mentioned above[48] and include (1) subacute/late phase formalin, a tonic nociceptive test that induces an organized (licking) nocifensive response; (2) von Frey and (3) Hargreaves, which are evoked mechanical and thermal stimuli, respectively, that induce a reflexive withdrawal response; and (4) complete Freund's adjuvant, a chronic inflammatory agent that when coupled with von Frey and Hargreaves can assess mechanical and thermal hypersensitivity, respectively. Each center piloted the assays available to them on strains from their active IMPC mouse colonies. Gene selection criteria fell into 3 categories: (1) nominations from domain experts, (2) functional evidence highlighted on GeneWeaver,[2] and (3) unbiased selection. This resulted in 110 single-gene knockout mouse strains being screened for 1 or more nociception and hypersensitivity assays. The results expand our understanding of genes underlying nocifensive responses and may provide novel targets to accelerate or refine the development of analgesics.

2. Methods

2.1. Ethical Approval

All institutes that generate, breed, and phenotype mice are guided by local ethical review panels and national licensing and accreditation bodies. Details of ethical review bodies and licenses for the 5 contributing centers are as follows: Baylor College of Medicine's (BCM) Institutional Animal Care and Use Committee approved license AN-5896; Medical Research Council Harwell's (HAR) Animal Welfare and Ethical Review Body approved licenses 70/8015 and 30/3384; The Center for Phenogenomics (TCP) Animal Care Committee approved animal use protocols 0153, 0275, 0277, and 0279; and The Jackson Laboratory's (JAX) Institutional Animal Care and Use Committee approved licenses 14004, 11005, and 99066. JAX AAALAC accreditation number was 000096, NIH Office of Laboratory Animal Welfare assurance number was D16-00170, and the University of California, Davis's (UCD) Institutional Animal Care and Use Committee approved animal care and use protocol number was 19075. UCD AAALAC accreditation number is 000029, and the NIH Office of Laboratory Animal Welfare assurance number is D16-00272 # (A3433-01). Animal welfare was assessed routinely for all mice involved.

2.2. Animals

Mice carrying knockout alleles were generated from the KOMP/EUCOMM-targeted embryonic stem cell resource or through CRISPR/Cas9 mutagenesis using standard techniques.[6,20] Mutant strain production details can be found at www.mousephenotype.org by searching for the gene symbol. Cumulatively across 5 contributing centers, 110 unique single-gene (listed in **Table 1**) knockout mouse strains were screened for their nocifensive behavior using up to 3 phenotyping assays. All knockout mouse strains were produced and maintained on a C57BL/6N genetic background of substrains C57BL/6NJ (BCM and most JAX strains, stock number JR005304), C57BL/6NTac (HAR strains), C57BL/6NCrl (TCP and UCD strains), and B6N(Cg) (B6N Complex Genomics designation, 3 JAX strains).

Homozygous animals were tested when viable and available (76 knockout strains), including 3 X-linked genes for which the zygosity is reported as homozygous (females) and hemizygous (males). Heterozygous animals were tested for the remaining 34 knockout strains, primarily because they were either homozygous lethal or subviable (classed as less than 50% of the expected number of homozygous progeny resulting from intercrossing parents heterozygous for the allele of interest) (32 strains) or there was a lack of available homozygous animals (2 strains). A full list of zygosity is presented in **Table 1**. Target group size ranged from 8 to 12 mice per sex per strain depending on the contributing center, and the minimum group size was defined as 5 mice per sex per strain, below which data were excluded from the analysis.

Husbandry practices vary between centers and are detailed as follows. All centers were specific pathogen-free barriers using a 12-hour light–dark schedule, and experiments were conducted in the light phase. Mice had ad libitum access to water and food. BCM used a housing density of 1 to 5 animals per cage in individually ventilated cages {Tecniplast Sealsafe Plus (overall dimensions of caging: [L × W × H]: 199 × 391 × 160 mm)}. Envigo (formally Harlan) Teklad 2920X diet, quarter-inch corn cob bedding substrate, and environmental enrichment of a nestlet were provided. Room temperature was 20 to 21˚C, and humidity was regulated at 30% to 70%. HAR used a housing density of 4 to 5 animals per cage in individually ventilated cages {Tecniplast IVC Sealsafe Blue line U temp 1284L (overall dimensions of caging: [L × W × H]: 365 × 207 × 140 mm)}. Special Diet Services RM3 diet, Eco-Pure Aspen chips2 (Datesand, United Kingdom) bedding substrate, and environmental enrichment of FDA Paper shavings–single bale nesting material (Datesand, United Kingdom) and cardboard tunnels (small 75 × 38.1 × 1.25 mm; Datesand, United Kingdom) were provided. Room temperature was 19 to 21˚C, and humidity was regulated at 45% to 65%. The Center for Phenogenomics used a housing density of 2 to 5 animals per cage in individually ventilated cages {Tecniplast Sealsafe Plus (overall dimensions of caging: [L × W × H]: 199 × 391 × 160 mm)}. Envigo Teklad 2918X diet, quarter-inch corn cob bedding substrate, and environmental enrichment of shredded paper (EnviroPak) were provided. Room temperature was 21 to 22˚C, and humidity was regulated at 30% to 55%. JAX

used a housing density of 1 to 5 animals per cage in individually ventilated cages {Thoren Duplex II Mouse Cage #11 and Thoren Maxi-Miser PIV System (overall dimensions of caging: [L × W × H]: 308 × 308 × 162 mm)}. LabDiet 5K52 diet, aspen shavings bedding substrate, and a cardboard tunnel for individually housed animals were provided. Room temperature was 20 to 22°C, and humidity was regulated at 44% to 60%. UCD used a housing density of 1 to 3 males or 1 to 5 females in individually ventilated cages {Animal Care Systems OptiMice (overall dimensions of caging: [L × W × H]: 343 × 292 × 155 mm)}. Envigo Teklad 2018 diet, quarter-inch corn cob bedding substrate, and environmental enrichment of a nestlet and Enviro Dry were provided. Room temperature was 20 to 26°C, and ambient environmental humidity was not regulated (typically between 25% and 40%).

2.3. Gene selection

The 110 single-gene knockout mouse strains included in this study originated from the active mouse colonies within the IMPC. Before phenotypic testing, GeneWeaver.org (RRID: SCR_003009) was used independently at the 5 contributing centers to identify genes within their active IMPC mouse colonies that had functional genomics evidence supporting a role in pain. After the completion of the study, a centralized post hoc analysis was run using the current GeneWeaver database [database queried October 31, 2020] to identify the most up-to-date functional annotations to pain-related evidence for the 110 genes selected. The GeneWeaver database was queried for nociception or pain-related gene sets, and the query results added to a project in GeneWeaver. The project contained 145 gene sets including 90 gene sets corresponding to gene expression correlated with pain phenotypes in mouse models, 31 lists of quantitative trait loci (QTL) positional candidates from mouse and rat, PainGenesdb database hits,[41] 3 gene sets of Mammalian Phenotype Ontology term association to pain-related terms, 6 gene sets indexed in PubMed to Medical Subject Headings related to pain, 5 gene sets derived from genome-wide association studies, 3 pain-related Online Mendelian Inheritance in Man gene sets, and 6 literature-based studies. This GeneWeaver project of gene sets was hand curated to remove gene sets that were duplicate database entries or had been wrongly associated with nociception (eg, where pain-related text in the abstract was not relevant to the content of the gene set). Full GeneWeaver results are available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]).

2.4. Formalin testing

Three contributing centers used formalin to study nocifensive behavior in a total of 75 knockout mouse strains (18 strains at BCM, 27 strains at HAR, and 30 strains at JAX). The number of mice tested per sex per mutant strain ranged from 5 to 16. Mice were acclimated to the testing room for at least 30 minutes before testing. To optimize the consistency of injections in both volume and site and to minimize stress, the mice were anesthetized for formalin administration. Routinely, mice recovered consciousness from gas anesthesia within 1 minute of being placed in the testing arena and were completely ambulatory within 3 minutes. For this reason, the first 5 minutes of video collected after the mouse was placed in the testing arena was excluded from the analysis. The formalin test elicits 2 phases of nocifensive behavior: phase 1, which occurs between 0 and 5 minutes after formalin administration, is directly linked to peripheral sensitization through the stimulation of primary sensory neurons and phase 2, which occurs between 10 and 60 minutes after formalin administration, is triggered by inflammation and involves central sensitization.[45] The use of gas anesthesia can mask the phase 1 response. For this reason, only the phase 2 centralized sensitization results are reported herein. Although the standard formalin procedure was executed at each center, subtle differences in the method are described below.

At HAR, mice were anesthetized with inhaled sevoflurane (5% flow; Zoetis, United Kingdom)[45] followed by subcutaneous injection of formalin (20 μL of 5% formalin solution; Sigma; United Kingdom, Product number: 252549) into the plantar surface of the right hind paw. The mouse was then placed in an acrylic glass testing arena ([L × W × H]: 400 × 360 × 130 mm) consisting of 3 mirrored and 1 transparent wall (built in-house). The arena was placed in the Home Cage Analyzer system (Actual Analytics, Edinburgh[1]), with the transparent wall facing the camera. Video was recorded for 60 minutes after a mouse entered the arena, after which the animals were humanely euthanized. Experimenters who were blinded to the genotype manually annotated the videos using Simple Video Coder.[3] The following nocifensive behaviors were scored: duration and number of bouts of licking or biting behavior as well as duration and number of bouts of dragging or limping behavior. For statistical analysis, the duration data were summed between 10 and 60 minutes after formalin administration.

BCM adopted the same protocol as HAR with the exception that inhaled isoflurane (3% flow; Henry Schein IsoThesia, Melville, NY; Product number: 1169567762) was used to anesthetize the mice, and the left hind paw was injected. In addition to licking or biting and dragging or limping behavior, BCM manually scored duration and number of bouts of flinching behavior. Manual scoring was performed using Behavioral Observation Research Interactive Software (BORIS[24]).

At JAX, mice were anesthetized with inhaled isoflurane (4% flow; Henry Schein IsoThesia; Product number: 1169567762) followed by subcutaneous injection of formalin [30 μL of 2.5% formalin solution, formaldehyde solution (Sigma-Aldrich, Burlington, MA; Product number: 15512), and sterile saline solution (Henry Schein; Product number: 002477)] into the plantar surface of the right hind paw. The testing arena was a clear acrylic animal enclosure ([L × W × H]: 220 × 216 × 127 mm; IITC Life Science, Woodland Hills, CA, Product number 433) containing 4 separate arenas divided by opaque black walls. This tetrad was placed atop a clear glass surface, and a video camera (black and white camera, Bosch, Dinion; Noldus media recorder v4 software, Noldus) was positioned 16 cm below the glass surface directly underneath the tetrad to provide a clear view of the paws. Four such tetrads were set up allowing 16 mice to be tested simultaneously. After formalin injection, mice were placed individually into a testing arena and video recorded for 90 minutes, after which the animals were humanely euthanized. Video recording was extended beyond the typical 60-minute experimental duration to capture strain differences in the timing of peak behavior. However, we observed consistently that the peak response was captured within the first 60 minutes after formalin administration and licking or biting behavior was observed to level off from 60 to 90 minutes postinjection. For this reason, we restricted behavioral analysis to the period of 10 to 60 minutes postinjection, as is standard in the field. A machine learning algorithm developed in-house and published independently[73] was used to automatically score the duration and number of bouts of licking or biting behavior. For statistical analysis, the duration data were summed between 10 and 60 minutes after formalin administration.

As licking or biting was the common behavior scored by all contributing centers and is reported to be the most important and reliable measure of nocifensive behavior,[33,50] data analysis focused specifically on licking or biting. All unprocessed data are available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]).

2.5. Complete Freund's adjuvant inflammatory administration

Three contributing centers used complete Freund's adjuvant (CFA) to induce a delayed thermal and mechanical hyperalgesia response in a total of 43 knockout mouse strains (9 strains at JAX, 13 strains at TCP, and 21 strains at UCD). Complete Freund's adjuvant was administered while the mice were under anesthesia to maximize the consistency of both the injection site and the volume delivered and to reduce stress for the mice. Mice were provided supplementary heat (26.6-38°C) to support recovery from anesthesia. Typically, mice regained consciousness from anesthesia within 1 minute of CFA injection and were fully ambulatory within 3 minutes. Although the standard CFA protocol was executed at each center, subtle differences in the method are described below.

At JAX, mice were anesthetized with inhaled isoflurane (4% flow; Henry Schein IsoThesia; Product number: 1169567762) followed by subcutaneous injection of CFA (30 μL of undiluted CFA [InvivoGen, San Diego, CA; Product number: vac-cfa-60]) into the plantar surface of the right hind paw.

At TCP and UCD, mice were anesthetized with inhaled isoflurane (5% flow; CDMV; Product number: USP 108737) followed by subcutaneous injection of CFA (20 μL of undiluted CFA [Sigma Aldrich; Product number: F5881]) into the plantar surface of the right hind paw.

All strains were tested for mechanical (von Frey test) and thermal (Hargreaves test) hyperalgesia before CFA injection to establish baseline sensitivity, then at 2 time points after CFA administration.

2.6. von Frey testing

Three contributing centers assessed CFA-induced mechanical hypersensitivity by performing the von Frey test on a total of 43 knockout mouse strains (9 strains at JAX, 13 strains at TCP, and 21 strains at UCD). A fourth center, HAR, performed the von Frey test on 28 knockout mouse strains in the absence of CFA or any other sensitizing agents. The number of mice tested per sex per mutant strain ranged from 5 to 13. While JAX followed the von Frey method first described by Chaplan,[14] all other centers used the simplified up-down (SUDO) method.[7] Center-specific details are described below.

JAX used a base plate attached to a self-standing perforated metal platform (Ugo Basile, Italy; Product codes 37000-003 and 37450-005) on which clear acrylic animal enclosures were placed (Ugo Basile, Italy; Product code 37000-006), each divided by a gray acrylic inset to create 4 testing arenas ([L × W × H]: 100 × 100 × 127 mm). Three such enclosures could be used simultaneously allowing 12 mice to be tested per session. The range of von Frey filaments (Stoelting, Wood Dale, IL; Touch-Test Sensory Evaluator, kit of 12, Product number: 58011) used was 0.02 to 1.4 target force in grams (equivalent to filament number 2 through 9 and handle value 2.36-4.17). Mice were acclimated to the testing arena for 60 minutes before testing. When all 4 feet were touching the platform, the von Frey filament was pressed against the plantar surface of the right hind paw with enough force to cause the filament to bend, then held in place for up to 3 seconds. A positive response was recorded if the animal withdrew from the stimulus, including sharp withdrawal, licking, flicking, or flinching responses. After a positive response, the subsequent trial was conducted with the next smaller size of filament in the series. In the absence of a paw withdrawal response, the subsequent trial was conducted with the next larger size of filament in the series. Testing began with the filament giving a target force of 0.4 g (filament number 6 and handle value 3.61), and a minimum of 6 consecutive trials were conducted with an intertrial interval of at least 2 minutes. Testing completion required at least 2 changes in response. Paw withdrawal threshold (PWT) was calculated using the equation $X_f + kd$,[14] where X_f is the starting filament handle value, k is taken from the lookup tables[14,21] and is based on the pattern of positive and negative responses observed for the mouse, and d is a constant defined as the mean difference between the range of filaments used in the test ($d = 0.289$ when using filament number 2 through 9). Log base 10 of the mean PWT in grams was used in analysis for each time point. Baseline measurements were collected 24 hours before CFA administration, and mice were retested at 24 and 48 hours after CFA administration.

TCP used a mesh stand with hexagonal perforations measuring 8 mm corner to corner (IITC Life Science; Product number 410) on which clear acrylic animal enclosures were placed (IITC Life Science, Product number 433), each divided by a white opaque acrylic inset to create 4 testing arenas ([L × W × H]: 100 × 100 × 125 mm). Three such enclosures could be used simultaneously allowing testing of 12 mice per session. The range of von Frey filaments (IITC Life Science; Aesthesio, Precise Tactile Sensory Evaluator 20-piece Kit, Product number: 514000-20C) used was 0.02 to 1.4 (target force in grams; equivalent to filament number 2 through 9 and handle value 2.36-4.17). The test was conducted as described above for JAX except that testing began with the filament giving a target force of 0.16 g (filament number 5 and handle value 3.22). Five consecutive trials, with an intertrial interval of at least 2 minutes, were conducted to complete 1 run. Two runs were performed for each time point. The average PWT for each time point was calculated using the SUDO method approach.[7] In brief, the equation $log_{10}(PWT) = x^{*}SUDO\ score + B$ was used, where x and B are constants derived specifically for the mouse filament set ($x = 0.24$, $B = -2.00$) and *SUDO score* is the number of the final presented filament corrected using a ±0.5 adjustment factor (positive adjustment for no response and negative adjustment for paw withdrawal). Log base 10 of the mean PWT in grams was used in analysis for each time point. Baseline measurements were collected 2 hours before CFA administration, and mice were retested at 24 and 144 hours (6 days) after CFA administration.

UCD adopted the same protocol as TCP with the exception that only 1 run of 5 consecutive trials was performed at each time point to calculate PWT.

HAR used a mesh stand with square perforations measuring 5 × 5 mm on which clear acrylic animal enclosures were placed, each divided by an opaque acrylic inset to create 4 testing arenas ([in-house] [L × W × H]: 80 × 60 × 100 mm). Three such enclosures could be used simultaneously allowing 12 mice to be tested per session. The range of von Frey filaments (Stoelting; Touch-Test Sensory Evaluator Kit of 12, Product number: 58011) used was 0.04 to 4.0 target force in grams (equivalent to filament number 3 through 11 and handle value 2.44-4.56). The test was conducted as described above for JAX except that testing began with the filament giving a target force of 0.6 g (filament number 7 and handle value 3.84). Five consecutive trials, with an intertrial interval of at least 2 minutes, were conducted to complete 1 run. In the absence of administration of a sensitizing substance, both hind paws were tested at each time point, left hind paw first, followed by the right hind paw in a separate run. The average PWT was calculated using the SUDO method approach as described above; however, the

Table 1

Metadata for 110 genes screened for nociception or hypersensitivity.

Abhd13 (3), HOM; VH	*Cntnap2* (3), HOM; FV^{o}	*Lgals4* (2), HOM; F	*Oxa1l* (1), HET; F	*Slc9a7* (0), HOM/HEMI; V^{o}
Acod1 (0), HOM; FV	*Col20a1* (1), HOM; VH	*Lrrc55* (1), HOM; F	*Pah* (3), HET; VH	*Slc9a9* (2), HOM; F
Acox3 (1), HOM; F	*Col9a3* (3), HOM; VH	*Maged1* (4), HOM/HEMI; V	*Pdcd6ip* (3), HOM; V	*Stk36* (0), HET; FVH
Adamtsl3 (3), HOM; VH	*Cp* (2), HOM; FVH	*Mdh1* (3), HET; F	*Pex14* (3), HET; F	*Taf13* (1), HET; F
Agbl1 (2), HOM; F	*Dnmt3b* (1), HET; VH	*Med27* (1), HET; F	*Piezo2* (1), HET; FVH	*Tecpr2* (0), HOM; FVH
Akr1b3 (2), HOM; F	*Dusp16* (1), HOM; VH	*Mkrn3* (0), HOM; VH	*Pink1* (4), HOM; FV^{o}	*Tedc1* (0), HET; VH
Alad (1), HET; VH	*Eif2d* (5), HOM; V	*Mme* (2), HOM; V	*Pip4k2c* (4), HOM; F	*Timp1* (1), HOM/HEMI; FV^{o}
Alg6 (1), HET; VH	*Emp1* (3), HOM; V	*Mmp16* (1), HOM; FV^{o}	*Polr1d* (1), HET; F	*Trak2* (2), HOM; V
Aqp1 (1), HOM; FV^{o}	*Esd* (1), HOM; F	*Mrps12* (3), HET; F	*Ppp2r5c* (1), HOM; V	*Trappc1* (3), HET; V
Atf3 (2), HOM; FV^{o}	*Exoc2* (3), HET; F	*Mtg2* (4), HET; F	*Ptprk* (5), HOM; F	*Trim14* (3), HOM; VH
AU040320 (3), HOM; F	*Ficd* (0), HOM; FV^{o}	*Myh10* (2), HET; FVH	*Rex1bd* (1), HOM; F	*Trim2* (2), HOM; F
Avpr1a (4), HOM; FVH	*Foxn3* (2), HET; F	*Myom2* (3), HOM; F	*Rnpepl1* (3), HOM; V	*Trpc1* (3), HOM; FV^{o}
BC048562 (1), HOM; FV^{o}	*Gabra2* (0), HOM; FV^{o}	*Nars* (0), HET; F	*Rps20* (5), HET; F	*Trpm3* (3), HOM; FVH
Bdkrb1 (8), HOM; FV^{o}	*Gapvd1* (5), HET; VH	*Nav2* (2), HOM; VH	*Rsad2* (2), HOM; FV^{o}	*Tspan17* (2), HOM; FV^{o}
Bloc1s6 (4), HOM; V	*Gria1* (7), HOM; FV^{o}	*Ndufa6* (1), HET; F	*Scrn2* (1), HOM; F	*Tspoap1* (4), HOM; FV^{o}
C4b (3), HOM; FV^{o}	*Grik1* (3), HOM; FV^{o}	*Nrxn2* (4), HOM; FV^{o}	*Sez6l* (1), HOM; FV^{o}	*Tubb6* (1), HOM; FV^{o}
Cacna2d4 (0), HOM; F	*Grm1* (2), HET; FV^{o}	*Nsmce2* (2), HET; F	*Shank3* (3), HOM; FV^{o}	*Unc13c* (4), HOM; FV^{o}
Cd2ap (2), HET; F	*Hmgb4* (2), HOM; F	*Nt5dc2* (1), HOM; F	*Shisa6* (4), HOM; VH	*Utp4* (1), HET; F
Cdk2ap2 (2), HOM; F	*Htr3a* (9), HOM; FV	*Nup155* (4), HET; V	*Slc17a8* (4), HOM; V	*Ypel2* (4), HOM; VH
Cenpt (1), HET; F	*Ipo9* (2), HET; F	*Ola1* (3), HET; F	*Slc24a4* (1), HOM; FV^{o}	*Zfp236* (3), HET; V
Cgnl1 (5), HOM; VH	*Lamb3* (0), HOM; FV^{o}	*Olfr1006* (1), HOM; V	*Slc30a4* (1), HOM; VH	*Zfp597* (1), HOM; F
Cnrip1 (2), HOM; VH	*Lats1* (1), HOM; FV^{o}	*Otud7a* (2), HET; F	*Slc7a14* (0), HOM; FV^{o}	*Zfp91* (2), HET; VH

Knockout mice for 110 genes were screened for nociception or hypersensitivity. The number of nociception or pain-related GeneWeaver associations is provided in parenthesis for each gene symbol. The zygosity of the knockout animals screened is annotated as homozygous (HOM), heterozygous (HET), or homozygous/hemizygous (HOM/HEMI) for X-linked genes. The phenotyping assays used to screen each mutant mouse line are summarized as formalin (F), von Frey with (V) and without (V^{o}) CFA administration, and Hargreaves with CFA administration (H). For example, the first gene listed, *Abhd13* is annotated as "(3), HOM; VH" indicating that it has 3 pain-related GeneWeaver associations [(3),], homozygous animals were screened [HOM], and both von Frey and Hargreaves with CFA administration were assessed [VH].

range of filaments used was different which resulted in different x and B constants ($x = 0.24$, $B = -2.03$). Measurements were collected at time 0 (habituation), 24, and 48 hours.

2.7. Hargreaves testing

Immediately after the von Frey testing described above, using the same mice on the same testing days, 3 contributing centers went on to assess thermal nociception by performing the Hargreaves[28,30] test on a total of 27 knockout mouse strains (7 strains at JAX, 13 strains at TCP, and 7 strains at UCD). The number of mice tested per sex per mutant strain ranged from 5 to 13. All 3 centers used the Plantar Test equipment from IITC Life Science, including the Model 400 Heated Base with an elevated heated glass platform (Product number 400 G), the 390 Plantar Test head (light and heat source; Product number 300 TH), and

Table 2

Nociception result summary for 13 statistically significant single-gene knockout mouse strains.

	Chemical nociception (Formalin)		Mechanical nociception (von Frey)						Thermal nociception (Hargreaves)					
	Sum (10-60 min)		Baseline		Delta 1		Delta 2		Baseline		Delta 1		Delta 2	
Gene	genotype	sex × genotype	genotype	sex × genotype	genotype	sex × genotype	genotype	sex × genotype	genotype	sex × genotype	genotype	sex × genotype	genotype	sex × genotype
Abhd13			1.21E-02		1.89E-03		1.73E-04		9.14E-02		5.38E-01		2.60E-01	
Alg6			5.98E-01		2.90E-01		2.25E-01		4.21E-02		5.03E-01		5.67E-04	8.00E-04
BC048562	4.08E-01		4.04E-01		5.78E-02	6.08E-02	1.18E-04	2.00E-04						
Cgnl1			3.63E-03		6.08E-05		3.43E-04		5.50E-01		2.01E-01		1.61E-01	
Cnrip1			1.80E-02	2.07E-02	9.41E-05	1.00E-04	7.77E-01		2.23E-01		2.51E-02		3.80E-01	
Cp	6.71E-01		1.22E-02		3.40E-01		6.08E-05		4.50E-01		1.68E-01		7.11E-04	8.00E-04
Gria1	4.80E-06		7.47E-05		7.16E-01		4.62E-01							
Htr3a	1.51E-01		6.01E-01		3.89E-01		4.10E-06							
Mmp16	1.48E-04	1.25E-05	9.72E-04		1.68E-01		1.71E-02	1.35E-02						
Oxa1l	4.54E-05													
Tecpr2	3.29E-01		3.33E-04		9.77E-01		2.84E-04		6.19E-05		1.93E-02		1.85E-02	
Trim14			2.85E-01		8.89E-01		7.18E-01		2.54E-04		2.24E-01		8.18E-02	
Trim2	1.61E-05													

Results of statistical comparisons for 13 genes designated as hits, with significant *P* values ($P < 0.001$) highlighted in yellow. Gray cells indicate that the assay was not performed. White cells indicate that the assay was performed but no interaction term was observed. Note that the equivalent table for all 110 genes tested is available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]).

the testing arena composed of a clear acrylic animal enclosure ([L × W × H]: 220 × 216 × 127 mm; IITC Life Science, Product number 433) containing 4 separate arenas. Three such enclosures could be used simultaneously allowing 12 mice to be tested per session. The heated glass platform was set to measure 29 to 32 ˚C, and mice were acclimated to the testing arena for 30 to 60 minutes before testing. The right hind paw was tested 3 times at each time point, with a minimum of 2 minutes between stimulus presentations, and the average latency to respond was used for statistical analysis. Although the standard Hargreaves procedure was executed at each center, subtle differences in the method are described below.

JAX used a white opaque acrylic inset to create 4 testing arenas in the animal enclosure. The 390 Plantar Test head was set at 2% idle intensity and 25% test intensity. Latency to respond was measured in seconds up to a maximum exposure time of 30 seconds, at which time the heat was stopped. Baseline measurements were collected 1 day before CFA administration, and mice were retested at 24 and 48 hours after CFA administration.

TCP used a white opaque acrylic inset to create 4 testing arenas in the animal enclosure. The 390 Plantar test head was set at 3% to 4% idle intensity and 30% test intensity. Latency to respond was measured in seconds up to a maximum exposure time of 20 seconds, at which time the heat was stopped. Baseline measurements were collected 2 hours before CFA administration, and mice were retested at 24 and 144 hours (6 days) after CFA administration.

UCD used a clear acrylic inset to create 4 testing arenas in the animal enclosure. The 390 Plantar Test head was set at 10% idle intensity and 100% test intensity. This is a high heat administration intensity relative to the other contributing centers which resulted in very short (sub 3 seconds) response times even pre-CFA administration. This is atypical for Hargreaves which is classically configured to yield a response latency of 10 to 12 seconds in naive mice to detect hyposensitivity and hypersensitivity.[19] The detection of thermal stimuli depends on activation of heat-gated ion channels with threshold temperatures ranging from warm (TRPV3, TRPV4, TRPM4, and TRPM5) to very warm (TRPV1) to extremely hot (TRPV2).[11,12,37,57,67,68] These channels open once their threshold is exceeded, so the rapid response latency detected by UCD simply represents the speed with which thermal thresholds are exceeded for multiple channel types when using a high-intensity administration. Latency to respond was measured in seconds up to a maximum exposure time of 30 seconds, at which time the heat was stopped. Baseline measurements were collected 30 to 60 minutes before CFA administration, and mice were retested at 24 and 144 hours (6 days) after CFA administration. Using this relatively high heat intensity, significant sensitization was detected 24 hours after CFA administration. For both sexes, sexual dimorphism was detected because females presented with increased sensitivity,

and response was trending back to baseline levels by 6 days after CFA administration.

2.8. Open Field testing

A separate cohort of mice for each of the 110 single-gene knockout strains reported herein was phenotyped by the IMPC as part of the early adult phenotyping pipeline (http://www.mousephenotype.org/impress). This pipeline included open-field testing, performed on a minimum of 7 male and 7 female mutant mice per strain, aged 8 to 9 weeks at testing. Procedural details are reported on the IMPC web site (available at: https://www.mousephenotype.org/impress/ProcedureInfo?action=list'procID=496'pipeID=7). In brief, animals were acclimated to the open-field procedural room for at least 30 minutes before testing, and the test duration was 20 minutes. The testing arena was illuminated between 150 and 200 lux. Within the arena the peripheral zone was delineated as 8 cm from the arena walls, and the central zone accounted for approximately 40% of the total surface area. As of IMPC data release 15.0, open-field data were available for 103 of the 110 single-gene knockout strains reported herein. Of those 103 strains, 27 were designated as behaving abnormally in open-field testing. Our approach was to mine the Mammalian Phenotyping (MP) ontology terms assigned to the abnormal strains by the IMPC following standardized data analysis using OpenStats.[31] A complete list of all abnormal open-field parameters and the associated MP terms for the 27 abnormal strains is available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]). We broadly categorized all the open-field abnormalities reported for the 27 strains into 2 groups: (1) abnormal locomotor activity using the MP terms "hyperactive," and "hypoactive" and (2) anxiety-like or abnormal exploratory behavior using the MP terms "increased thigmotaxis," "decreased thigmotaxis," "increased vertical activity," "decreased vertical activity," "increased anxiety-related response," "increased exploration in new environment," and the more generic "abnormal behavior." A chi-square test of independence was performed to examine the relation between nocifensive phenotypes and abnormalities in open field, and the nature of the open-field abnormalities was reported for genes with statistically significant abnormalities in nocifensive behavior.

2.9. Data quality control

Each center manually examined data for errors and excluded mice from analysis if technical or experimental errors were indicated. Strains were included if data were complete for 1 or both sexes with sample size of 5 or more.

2.10. Statistical Analysis

The analyses were automated for each assay, and statistical models were optimized using the IMPC tool PhenStat[40] with the significance level of 10^{-3}. The choice of significance level was based on the need to balance sensitivity for hits against the probability of false positives. This level of 10^{-3} has previously been adopted within large screen surveys to assess specific parameters, as for example, determining lethality threshold[20] or circadian phenotypic deviance.[81] Typically, screens of thousands of genes aim to maintain a false discovery rate below 5% and use a significance level of 10^{-4}. This is the strategy adopted by the IMPC (www.mousephenotype.org). By contrast, experiments that compare only a few strains may use 10^{-2} or greater as the statistical decision threshold level. This study of 110 genes falls between these extremes, and our choice of significance level was made accordingly. To assess the risk of false positive rate of discovery, the resultant P values of all the statistical tests were converted to false discovery rate q values using the Benjamini–Hochberg[5] method (R software[61]). The chosen significance level ($P < 0.001$) corresponded to less than a 2% risk of false discovery. The results are shown in figures as hits ($P < 0.001$), genes of potential interest ($0.001 \leq P < 0.01$), or not significant ($P \geq 0.01$).

2.10.1. Formalin statistical analysis

Data were summed to produce the total number of seconds of licking or biting behavior within the period of 10 to 60 minutes after formalin injection. PhenStat was used to optimize the mixed model analyses of variance by including the initial fixed effects of gene (wild type control, mutant), sex, age, and body weight (when available) and the random effect of batch (day of experiment). The results of the final optimized model were retained, and effect sizes were expressed as percentage changes based on the ratio of genotype effect to wild type effect.[39,40] If sexual dimorphism was reported in the model, then effect sizes were reported for each sex separately. Three strains were analyzed as single-sex models (knockouts for *Avpr1a, Sez6l,* and *Trpc1*).

2.10.2. von Frey and Hargreaves statistical analysis

PhenStat models were used to assess 3 dependent variables: nocifensive behavior in the absence of CFA administration (baseline measure) and the change in response (compared with baseline) for the 2 subsequent test days (delta1 and delta2). The models were optimized starting with the initial fixed effects of gene (wild type control, mutant), sex, age, and body weight (when available) and the random effect of batch (day of baseline measure). The results of the final optimized model were retained, and effect sizes were expressed as percentage change based on the ratio of genotype effect to wild type effect. If sexual dimorphism was reported in the model, then effect sizes were reported for each sex separately.

The von Frey PWT data (grams) were log10 transformed before analysis as recommended by Mills et al.,[47] and the variables tested were log10_baseline, delta1 (log10_baseline − log10_test1), and delta2 (log10_baseline − log10_test2). The subtraction of logarithms effectively normalizes the change to the baseline. Four strains were analyzed as single-sex models (knockouts for *Eif2d*, *Olfr1006*, *Pdcd6ip*, and *Ppp2r5c*).

The analyzed Hargreaves variables were baseline latency, delta1 (baseline − test1), and delta2 (baseline − test2). The changes from baseline were not normalized. Two strains were analyzed as single-sex models (knockouts for *Cgnl1* and *Col20a1*). JAX and UCD initially tried lower stimulus intensities, but the response variability was very high and both centers shifted and stayed with higher intensity stimuli (25% test intensity for JAX; 100% test intensity for UCD). The early measures were excluded as incomplete data.

2.11. Data availability

All mutant mice reported here are available through public mouse repositories for further investigation. All data sets and analyses are available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015,[74]).

3. Results

3.1. Assessing nocifensive behaviors

Five contributing centers piloted nociception assays that were available to them. From the final list of 110 genes and 3 possible

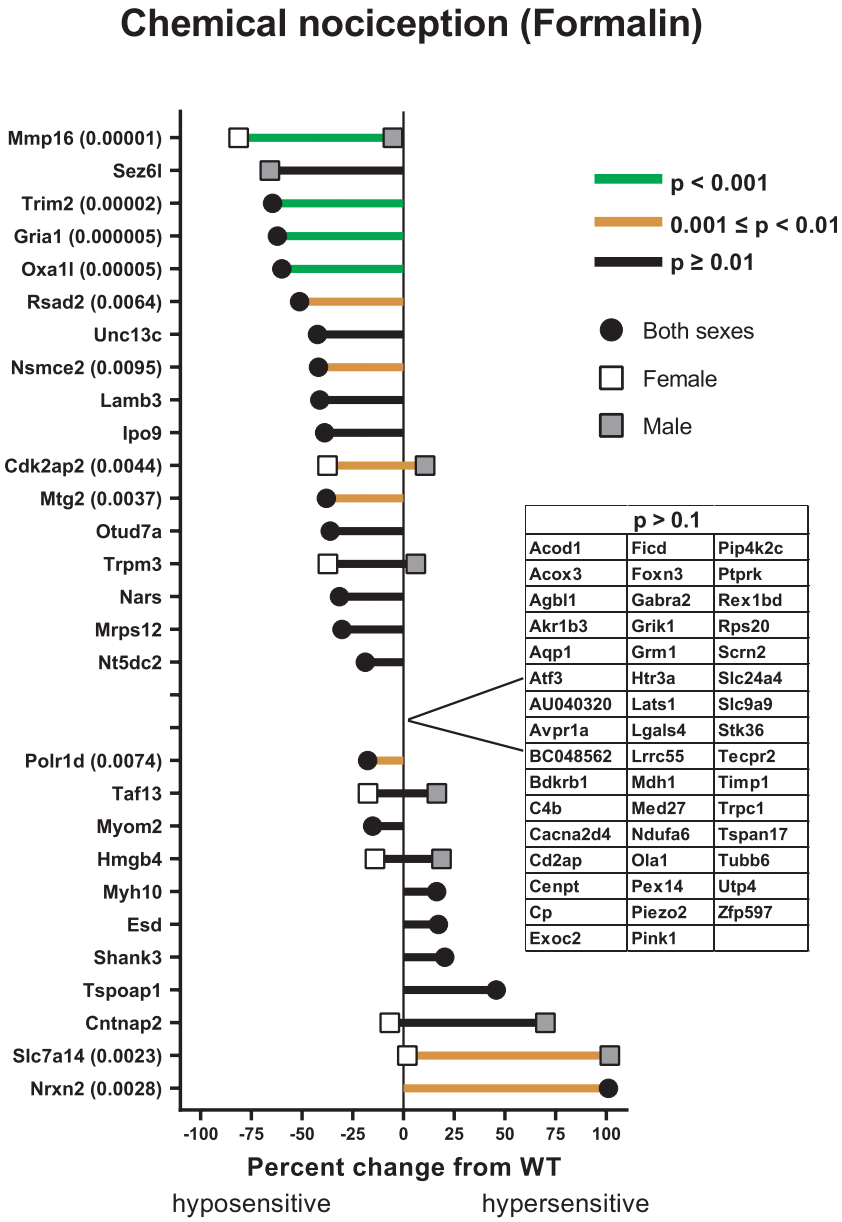

Figure 1. Relative effect size of mutant compared with wild type (WT) mice for chemical nociception. The subacute or late phase response to formalin was measured using the sum of the time spent licking or biting between 10 and 60 minutes after formalin was administered. The percentage effect size of genotype (mutant—WT control) on late phase response to formalin is plotted by gene symbol. The unadjusted *P* value is inserted after the gene symbol for all significant gene effects and genes of potential interest, reporting the effect of genotype of the null model hypothesis analysis where both sexes are affected or from the sex × genotype interaction where sexual dimorphism is present. A positive effect represents an increase in licking or biting by mutant relative to control (hypersensitive) and a negative effect a decrease in licking or biting (hyposensitive). Only genes with *P* values less than or equal to 0.1 are plotted, and all other genes with a *P* value greater than 0.1 are named in the text box. A brown bar indicates that the *P* value was less than 0.01 but not considered significant, and a green bar indicates that the *P* value for this test was considered significant with a *P* value below 0.001. Bars with a single symbol (black circle) represent both sexes. Where sexual dimorphism was detected, the male value is indicated by a grey square and female by a white square.

nociception assays (formalin, von Frey, and Hargreaves), results from 173 unique gene×assay combinations (**Table 1**) are reported herein.

Wild type control data are available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]) and reveal that the testing protocols established by all 5 contributing centers permitted detection of hypersensitive and hyposensitive genetically altered mouse strains. Although trends in the data were concordant between contributing centers, for example, peak thermal sensitivity was detected 24 hours after CFA administration and began to resolve at later time points, variability in absolute values (eg, latency to withdraw) was seen across contributing centers. This is consistent with published literature and serves to highlight the need for detailed documentation of the experimental design and execution and the likely genotype×environment interactions[38] involved in the complex behavioral phenotype of nociception. Recognizing that the primary goal of this study was identification of novel nociception gene associations delivered through large-scale screening of single-gene knockout mouse strains, we adopted a center-specific control strategy that ensured wild type and mutant comparisons were made only for data collected within the same contributing center. We also focused our attention on strains presenting with large and highly significant effects.

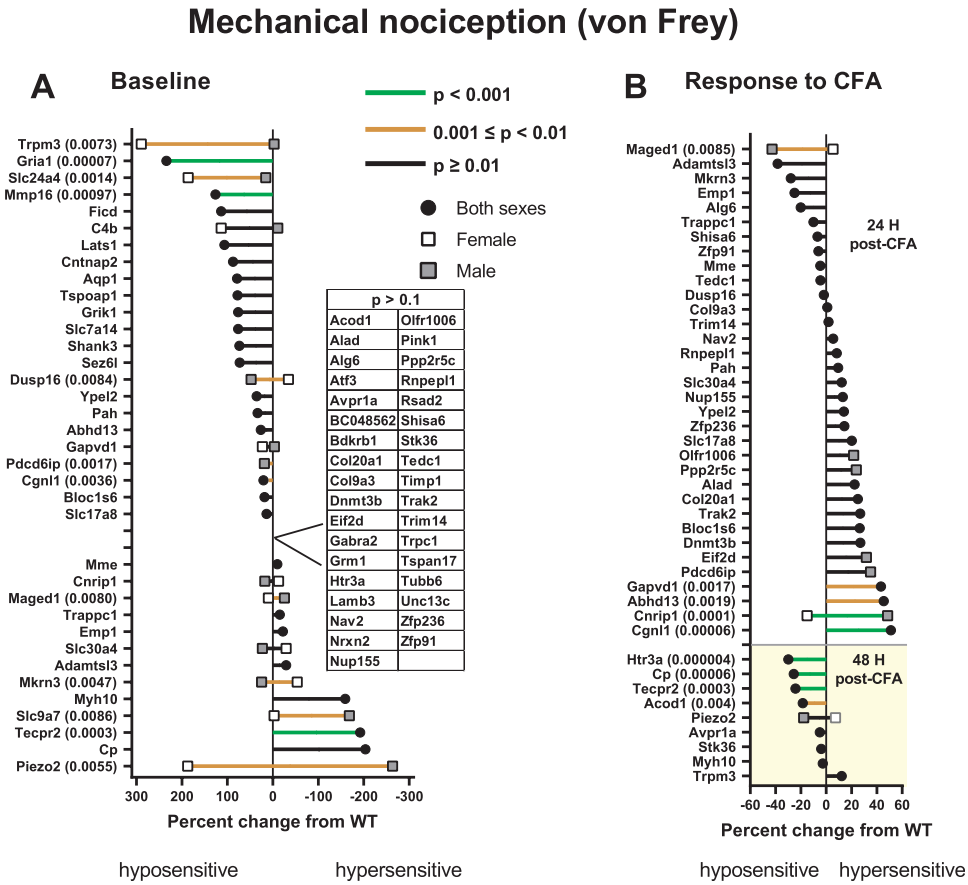

Figure 2. Relative effect size of mutant compared with wild type (WT) mice for mechanical nociception. (A) The percentage effect size of genotype for the baseline von Frey measurement plotted by gene symbol. The mixed model analysis tested log10 baseline scores, and effect sizes are calculated on that basis. The x-axis is reversed to indicate that a negative effect represents a measure of lower force and therefore a hypersensitive response by the mutant, and a positive change indicates higher force (hyposensitive). Only genes with *P* values less than or equal to 0.1 are plotted, and all other genes with *P* value greater than 0.1 are named in the text box. The unadjusted *P* value is inserted after the gene symbol for all significant gene effects and genes of potential interest, reporting the effect of genotype of the null model hypothesis analysis where both sexes are affected or from the sex×genotype interaction where sexual dimorphism is present. A brown bar indicates that the *P* value was less than 0.01 but not considered significant, and a green bar indicates that the *P* value for this test was considered significant with a *P* value below 0.001. Bars with a single symbol represent both sexes. Where sexual dimorphism was detected, the male value is indicated by a gray square and female by a white square. The colors and symbols are maintained for part B. (B) The percentage effect size of genotype for the measured peak change from baseline in response to CFA administration of the von Frey assay is plotted by gene symbol. The mixed model analysis tested the difference of log10 scores, and effect sizes are calculated on that basis. A positive effect indicates a greater change from baseline and represents a hypersensitive response to CFA administration for the mutant, and a negative effect indicates a smaller response (hyposensitive). All genes are shown regardless of *P* value. The unadjusted *P* value is inserted after the gene symbol for all significant gene effects and genes of potential interest, reporting the effect of genotype of the null model hypothesis analysis where both sexes are affected or from the sex×genotype interaction where sexual dimorphism is present. The data above the break in the y-axis are from TCP and UCD and use 24 hours postadministration as the comparison to baseline; data below the break are from JAX and used 48 hours postadministration as the comparison to baseline (yellow shade). CFA, complete Freund's adjuvant.

3.2. Gene selection

The IMPC intends to identify the function of every protein-coding gene in the human–mouse orthologous genome using standardized knockout strain production on a fixed genetic background and systematic high throughput sequential phenotyping tests. All 5 centers contributing data to the current study are members of the IMPC. Each center selected a subset of strains from their active IMPC mouse colonies and assessed them for altered nociception using up to 3 nociception phenotyping assays. Gene selection criteria varied between centers but broadly fell into 3 categories: (1) nominations from domain experts, (2) functional evidence highlighted on GeneWeaver, and (3) unbiased selection.

To reconcile the final list of 110 genes and identify the most up-to-date functional genomics evidence of a role in nociception, a centralized post hoc analysis was run using GeneWeaver.[2] GeneWeaver is a curated repository of functional genomics information compiled from a vast array of data sources. Overlaying GeneWeaver is software tools that allow the interrogation and integration of these data sets to identify convergent evidence of gene function. The GeneWeaver database was queried for nociception or pain-related gene sets that were then manually curated for accuracy. One hundred forty-five gene sets of varying strength were identified. Stronger, more direct evidence included Mendelian disease associations (Online Mendelian Inheritance in Man) and pain-related Mammalian Phenotype Ontology terms. Weaker evidence included positional candidates from QTL and gene expression correlations with pain phenotypes. The number of GeneWeaver associations for the 110 genes ranged from 0 up to a maximum of 9 pain-related gene sets (**Table 1**). This included 98 genes associated with at least 1 piece of functional genomics data (eg, cerebellum gene expression correlates for hot plate measured in BXD recombinant inbred strains or QTL for morphine antinociception on chromosome 9), 67 genes associated with at

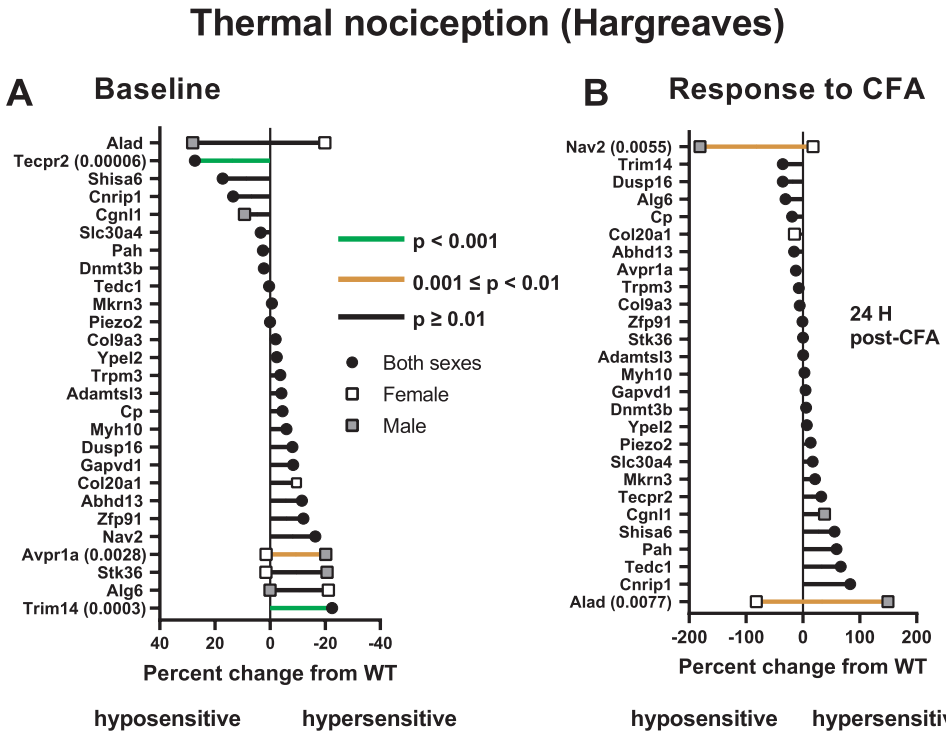

Figure 3. Relative effect size of mutant compared with wild type (WT) mice for thermal nociception. (A) The percentage effect size of genotype for the baseline Hargreaves measurement plotted by gene symbol. The x-axis is reversed to indicate that a negative effect represents a measure of shorter latency to withdraw and therefore a hypersensitive response by the mutant, and a positive change indicates longer latency (hyposensitive). All genes are shown regardless of *P* value. The unadjusted *P* value is inserted after the gene symbol for all significant gene effects and genes of potential interest, reporting the effect of genotype of the null model hypothesis analysis where both sexes are affected or from the sex×genotype interaction where sexual dimorphism is present. A brown bar indicates that the *P* value was less than 0.01 but not considered significant, and a green bar indicates that the *P* value for this test was considered significant with a *P* value below 0.001. Bars with a single symbol represent both sexes. Where sexual dimorphism was detected, the male value is indicated by a gray square and female by a white square. The colors and symbols are maintained for part B. (B) The percentage effect size of genotype for the peak change from baseline in response to CFA administration of the Hargreaves assay is plotted by gene symbol. A positive effect indicates a greater change from baseline and represents a hypersensitive response to CFA administration for the mutant, and a negative effect indicates a smaller response (hyposensitive). All genes are shown regardless of *P* value. The unadjusted *P* value is inserted after the gene symbol for all significant gene effects and genes of potential interest, reporting the effect of genotype of the null model hypothesis analysis where both sexes are affected or from the sex × genotype interaction where sexual dimorphism is present. For all contributing centers, the peak change from baseline was measured 24 hours after CFA administration. CFA, complete Freund's adjuvant.

least 2 pieces, 21 genes with ≥4 pieces, and 12 lacking any functional genomics evidence of a role in pain (full GeneWeaver results are available as a Zenodo repository at https://doi.org/10.5281/zenodo.5178015 [74]). We conclude that our gene set is enriched for genes that play a role in nociception.

3.3. Genes involved in nociception

A total of 110 single-gene knockout mouse strains were tested using at least 1 and up to 3 distinct assays designed to assess nocifensive behavior: formalin administration and Hargreaves and von Frey with or without CFA administration. The results of statistical comparisons for all 110 knockout strains are available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]). Thirteen from the 110 single-gene knockout mouse strains achieved statistical significance in 1 or more assays, defined as $P < 0.001$ (**Table 2**), with a false discovery rate (q-value) of less than 2% (see *2.10 Statistical Analysis* in the Methods section). On inactivation, 6 genes were classified in 1 or more measures as generating hyposensitivity (*Cp*, *Gria1*, *Htr3a*, *Mmp16*, *Oxa1l*, and *Trim2*), 3 genes were classified as generating hypersensitivity (*Cgnl1*, *Cnrip1*, and *Trim14*), 1 gene gave a mixed response depending on the assay (*Tecpr2*), 2 genes were classified as generating an altered recovery response (*Abhd13* and *Alg6*), and the remaining 1 gene was classified as generating an altered phasing of the response (*BC048562*). The breakdown of these results by assay is given below.

Seventy-five knockout mouse strains were tested for their subacute or late phase response to formalin (**Fig. 1**) measured using the sum of the time spent licking or biting between 10 and 60 minutes after formalin was administered. Four of those 75 genes were abnormal based on the primary effect of genotype (**Table 2** and **Fig. 1**). Decreased licking or biting was observed for both sexes of *Trim2*, *Gria1*, and *Oxa1l* knockout mice, indicative of hyposensitivity to formalin. The fourth, *Mmp16*, gave a sexually dimorphic response in which female homozygous knockout mice were strongly hyposensitive while male homozygotes were indistinguishable from control mice.

Baseline von Frey testing was completed on 71 knockout mouse strains, of which 4 yielded an altered response (**Table 2** and **Fig. 2A**). *Tecpr2* presented with a reduced PWT in both sexes, indicative of increased sensitivity to mechanical stimulation. By contrast, both sexes of *Gria1* and *Mmp16* mutant mice presented with increased PWT, consistent with hyposensitivity to mechanical stimulation. The nature of the fourth hit was unusual because of the testing protocol used in the absence of CFA. Specifically, von Frey testing was performed on 3 consecutive

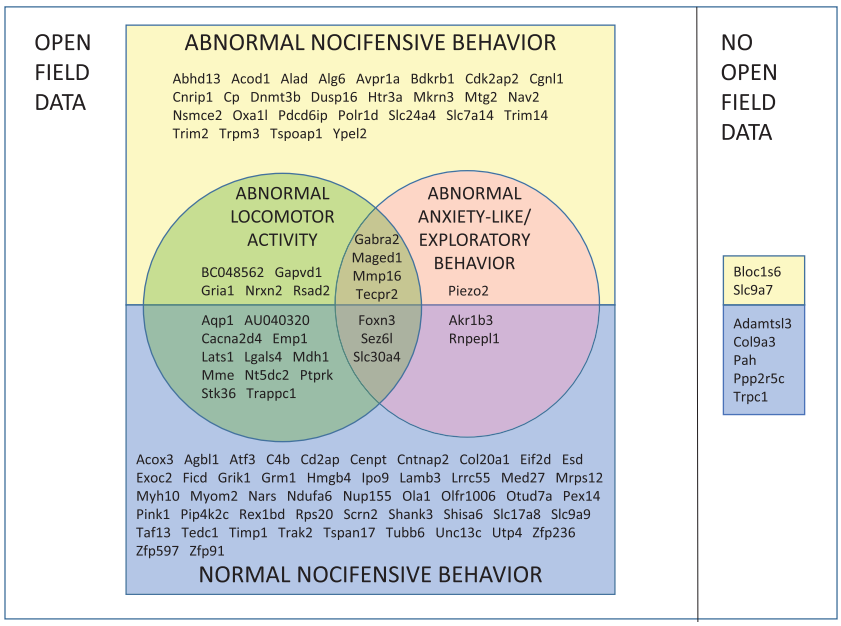

Figure 4. Single-gene knockout strains grouped by nocifensive, locomotor, and anxiety-like or exploratory behavior. One hundred ten single-gene knockout strains were screened for nociception or hypersensitivity. A separate cohort of mice for 103 of these 110 strains was phenotyped using open field. Gene symbols for these knockout strains are grouped by outcome of nociception testing [abnormal (yellow) or normal (blue) nocifensive behavior], the presence or absence of open field data (open field data or no open field data), and the outcome of open field data statistical analyses where abnormalities were detected [abnormal locomotor activity (defined using the MP terms "hyperactive," and "hypoactive," green) or anxiety-like or abnormal exploratory behavior (defined using the MP terms "increased thigmotaxis," "decreased thigmotaxis," "increased vertical activity," "decreased vertical activity," "increased anxiety-related response," "increased exploration in new environment," and "abnormal behavior", pink)].

days, and on the third day of testing, significantly increased PWT was observed in female *BC048562* knockout mice that may indicate a carryover effect resulting from the previous 2 days of testing.

Neither *Gria1*, *Mmp16*, nor *BC048562* mice were further assessed with CFA; however, 43 of the 71 strains assessed for baseline von Frey were subsequently administered CFA, and the change from baseline was measured (**Table 2** and **Fig. 2B**). Intriguingly, *Tecpr2* presented with a reduced sensitivity after CFA; the strain is presented below in full. Other change from baseline hits included 2 hypersensitive lines [*Cgnl1* (both sexes) and *Cnrip1* (sexually dimorphic, presenting in males only)], 2 hyposensitive lines seen in both sexes [*Cp* (presented below in full) and *Htr3a*], and *Abhd13* which presented with a significantly different recovery response (change between baseline and 144 hours (6 days) after CFA administration); however, a floor effect in the data prevented further examination.

Thermal nociception was assessed using the Hargreaves assay in 27 knockout mouse strains at baseline and after CFA administration. Two strains displayed altered sensitivity before CFA administration (**Table 2** and **Fig. 3A**). *Trim14* presented as a hypersensitive strain exhibiting reduced latency to paw withdrawal in both sexes after exposure to the high-intensity heat stimulus administered in the UCD protocol. Prolonged latency to respond was observed in both sexes of *Tecpr2* knockout mice indicating baseline hyposensitivity to thermal stimulation. Two further strains responded abnormally after CFA administration (**Table 2** and **Fig. 3B**). *Alg6* mutants gave a strong, sexually dimorphic response to recovery from CFA administration. Specifically, males displaying thermal hyposensitivity 144 hours (6 days) after CFA administration. By contrast, 48 hours after CFA administration, *Cp* mutant mice (presented below in full) exhibited increased latency to respond indicating that their thermal hyperalgesia resolved more rapidly.

Abnormal locomotor or anxiety-like behavior could influence measurement of nocifensive behavior. For example, motor hyperactivity may skew nocifensive response towards hypersensitivity. To investigate this potential confound, IMPC-generated open-field data were interrogated for 103 of the 110 single-gene knockout mouse strains included in this study, focusing on the IMPC-ascribed MP terms for abnormal locomotor or anxiety-like or exploratory behavior. A χ^2 test of independence was performed to examine the relation between nocifensive phenotypes [including hits ($P < 0.001$) and genes of potential interest ($0.001 \leq P < 0.01$)] and abnormalities in open-field. The relation between these variables was not significant, X^2 (1, N = 103) = 0.0198, $P = 0.89$. To visualize patterns in behavioral outcomes and highlight genes with open-field abnormalities that could potentially confound the outcome of nociception phenotyping, we grouped genes by abnormalities in nocifensive (yellow), locomotor (green), and anxiety-like or exploratory (pink) behavior (**Fig. 4**). Seventy-six from 103 genes had no significant effect in open field, this included 9 nociception hits and 18 genes of potential interest. The remaining 27 genes had a significant open-field effect, of which 4 were nociception hits and 6 were genes of potential interest. A complete list of all abnormal open-field parameters and the associated MP terms for the 27 abnormal strains is available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015,[74]). Open-field phenotyping identified knockout strains for *AU040320*, *Cacna2d4*, *Emp1*, *Gapvd1*, *Gria1*, *Lgals4*, *Mdh1*, *Mme*, *Nt5dc2*, *Ptprk*, and *Trappc1* specifically as hyperactive. Of these, *Gria1* mutants showed reduced nocifensive behaviors, and *Gapvd1* (nociception gene of potential interest) mutants trended towards hypersensitivity. Knockout strains for *Aqp1*, *BC048562*, *Lats1*, *Nrxn2*, *Rsad2*, and *Stk36* were classified specifically as hypoactive from open-field testing. Of these, *BC048562* presented

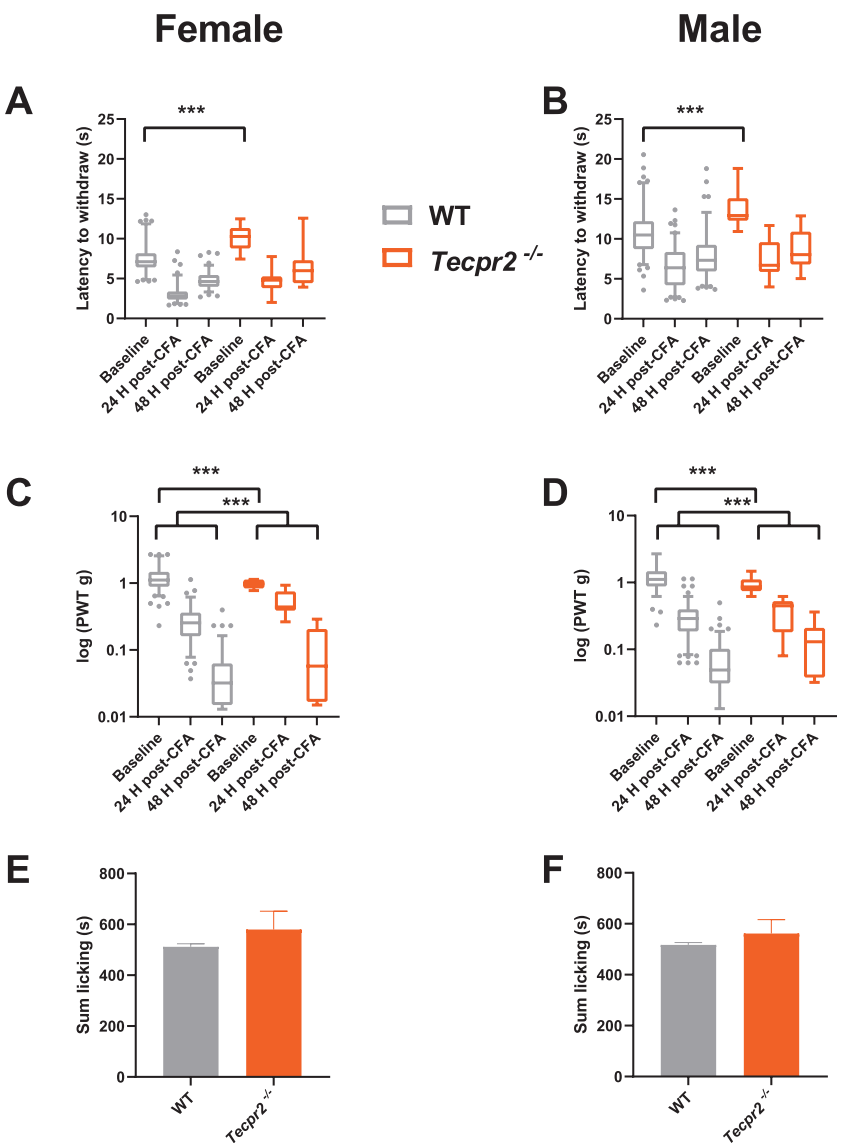

Figure 5. Abnormalities in nocifensive behavior after *Tecpr2* inactivation. (A and B) Latency to withdraw the paw from a thermal stimulus is shown (quartile boxplots with error bars for 5%-95% percentile) in seconds for each test day in the Hargreaves assay for wild type C57BL/6NJ (WT) mice [8 and 17-18 weeks (n = 96F, n = 100M), gray] and *Tecpr2* [17 weeks (n = 12F, n = 10M), orange] homozygous null female (A) and male (B) mice. Baseline withdrawal latency was significantly longer for *Tecpr2* mutant mice (mixed model genotype $F_{(1,196)} = 15.1$, $P < 0.001$,***) than their WT controls. (C and D) Paw withdrawal threshold is plotted in log10 scale as quartile boxplots (with error bars for 5%-95% percentile) for each test day for wild type C57BL/6NJ (WT) [8 and 17-18 weeks (n = 119F, n = 122M), gray] and *Tecpr2* [17 weeks (n = 12F, n = 10M), orange] homozygous null females (C) and males (D) for the von Frey assay. Baseline threshold was significantly lower for *Tecpr2* mutant mice ($F_{(1,256)} = 15.2$, $P < 0.001$, ***), and the CFA response was significantly smaller for *Tecpr2* mutant mice 48 hours postadministration ($F_{(1,255)} = 12.7$, $P < 0.001$, ***) compared with WT controls. (E and F) The mean (with SEM) of licking or biting behavior duration summed over 10 to 60 minutes is shown for wild type C57BL/6NJ (WT) [aged 12-21 weeks (n = 346F, n = 398M), gray] and *Tecpr2* [16-17 weeks (n = 12F, n = 11M), orange] homozygous null mice for females (E) and males (F). No statistically significant difference was detected.

with a putative carryover effect, and *Nrxn2* (trend towards hypersensitivity) and *Rsad2* (trend towards hyposensitivity) were both genes of potential interest. Three strains were reported solely with abnormal anxiety-like or exploratory behavior from open-field data as follows: decreased anxiety-like behavior was measured for *Akr1b3* and *Piezo2* mutant strains and increased vertical activity was reported for *Rnpepl1* mutants. Of these, only *Piezo2* [sexually dimorphic trend towards hyposensitivity (female) or hypersensitivity (male)] was reported as a nociception gene of potential interest. Abnormalities in both locomotor and anxiety-like or exploratory behaviors were ascribed to *Foxn3, Gabra2*, *Maged1*, *Mmp16*, *Sez6l*, *Slc30a4*, and *Tecpr2* knockout strains. Of these *Mmp16* (predominantly hyperactive) showed reduced nocifensive behaviors (females only), *Tecpr2* (predominantly hyperactive) gave a mixed response depending on the nociception assay, whereas *Gabra2* (predominantly increased anxiety-like behavior) was a nociception gene of interest due to a carryover effect and *Maged1* (predominantly hypoactive) was a gene of interest that gave a mixed response (males only) depending on the nociception assay.

These open-field data can aid interpretation of nociception results for individual knockout strains but considered collectively there was no prevalent pattern in open-field behavior that would indicate a confound consistently skewing the outcome of nociception assays.

Turning to human studies, of 800 genes featured as playing a role in inflammatory and neuropathic pain,[56] 16 mouse orthologues were knocked out and characterized in the current study. Of these, 2 achieved significance at $P < 0.001$ [*Gria1* and *Htr3a*]

Table 3

SFARI identified genes ranked by *P* value.

Gene	*P*	Rank of p	Percentile
Htr3a	4.10E-06	1	0.2
Gria1	4.80E-06	2	0.5
Cgnl1	6.08E-05	7	1.7
Alg6	5.67E-04	20	4.9
Avpr1a	2.80E-03	34	8.3
Nrxn2	2.81E-03	35	8.5
Tspoap1	4.90E-03	44	10.7
Nav2	5.50E-03	49	11.9
Tspan17	2.30E-02	96	23.3
Pah	2.34E-02	98	23.8
C4b	2.73E-02	102	24.8
Otud7a	2.95E-02	103	25.0
Cntnap2	3.14E-02	108	26.2
Myh10	4.25E-02	126	30.6
Shank3	4.29E-02	127	30.8
Nup155	9.09E-02	160	38.8
Slc9a9	9.35E-01	396	96.1

The *P* values for all 412 statistical tests performed in the current analyses were ranked from smallest to largest, and percentile scores were calculated for each value. The full table of results for all 110 genes tested is available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]). The smallest *P* value was selected for each gene and used to rank each of the 110 genes. The 17 SFARI genes overlapping with the 110 genes in the current study are shown along with the corresponding *P* value, rank position, and percentile.

and an additional 4 (*Avpr1a*, *Bloc1s6*, *Nav2*, and *Trmp3*) fell below the stringent significance threshold used herein but still achieved $P < 0.008$.

Gria1 and *Htr3a* knockout strains both displayed reduced hyperalgesia (**Table 2** and **Figs. 1, 2A, and 2B**). *Avpr1a* trended towards baseline thermal hyperalgesia in males only (sex × genotype $P = 0.0028$), *Bloc1s6* trended towards delayed or diminished recovery after mechanical sensitization to CFA ($P = 0.003$), *Nav2* males trended towards reduced thermal hypersensitivity 24 hours after CFA administration (sex × genotype $P = 0.0055$), and *Trpm3* trended towards baseline mechanical hyposensitivity in females (sex × genotype $P = 0.0073$). Taken together these associations validate the current study and support the candidacy of the novel pain genes identified herein. The lack of response in the 10 other genes identified in the study by Parisien et al. could be due to the following: incomplete knockdown of target gene expression in the targeted mutation (tm1b and tm2b)[27] and endonuclease-mediated (em1 and em2) alleles used in the current study; the zygosity of the mice that were screened (heterozygotes were screened for one of the 10 strains because of reduced homozygote viability or availability; across the full gene set, 34 of the 110 strains studied were screened as heterozygotes); the genetic background may have influenced phenotypic expression; the nociception screening performed herein was not all encompassing, as for example, it did not include an assay to interrogate neuropathic pain; or the phenotype leading to the association in humans may have resulted from a change other than the loss-of-function mutations characterized herein.

All mutant mouse strains reported here are available from public mouse repositories for further investigation. All data sets, scripts, and output are available as a Zenodo repository (https://doi.org/10.5281/zenodo.5178015 [74]).

3.4. Abnormalities in nociception after Tecpr2 inactivation

Tecpr2 encodes tectonin beta-propeller repeat-containing 2, a cytoplasmic protein broadly expressed in adult mice (Gene Expression Database, queried March 11, 2021) and predicted to play a role in protein exit from the endoplasmic reticulum. Disease-associated loss-of-function mutations of human *TECPR2* have been reported.[32,55,59] The clinical features of this rare peripheral neuropathy include decreased pain and temperature sensitivity, intellectual disability, facial dysmorphia, spastic paraparesis, areflexia, autonomic neuropathy, gastroesophageal reflux disease, and respiratory dysregulation.

Thermal baseline sensitivity of *Tecpr2* mutants was altered compared with that of their age and sex matched wild type controls (**Table 2** and **Figs. 3A, 5A, and 5B**). Specifically, the latency to paw withdrawal from the thermal stimulus was longer in *Tecpr2* mutants in both sexes ($F_{(1,196)} = 15.1$, $P < 0.001$; effect size −27% genotype difference), indicative of reduced thermal hyperalgesia. No significant difference in change from baseline response was detected at 24 or 48 hours after CFA administration.

Mechanical baseline sensitivity and CFA-induced hypersensitivity of *Tecpr2* mutants were both significantly different compared with that of their age-matched and sex-matched wild type controls (**Table 2** and **Figs. 2A and 2B**). Baseline sensitivity was significantly increased ($F_{(1,256)} = 15.2$, $P < 0.001$) as reflected in the lower paw withdrawal threshold with a large effect size (192% logarithm based) corresponding to a substantial difference in grams of force applied (WTmean = 1.23 g, SD = 0.52; *Tecpr2*mean = 0.94 g, SD = 0.23) (**Table 2** and **Figs. 5C and 5D**). There was no significant difference in response to CFA administration at 24 hours, but at 48 hours, both *Tecpr2* sexes showed significantly diminished response ($F_{(1,255)} = 12.7$, $P < 0.001$, effect size relative to baseline of −24%), indicative of reduced mechanical hyperalgesia after CFA-induced inflammation (**Table 2** and **Figs. 5C and 5D**).

No difference was detected in the sum of the time spent licking or biting between 10 and 60 minutes after formalin injection (**Figs. 1, 5E, and 5F**). An independent cohort of *Tecpr2* mutant mice was tested by JAX through the IMPC phenotyping pipeline. The results from open-field testing were indicative of a strong, hyperactive phenotype including increased whole arena average speed, increased total distance travelled, and decreased whole arena resting time (https://www.mousephenotype.org/data/genes/MGI:2144865#phenotypesTab). A similar trend towards

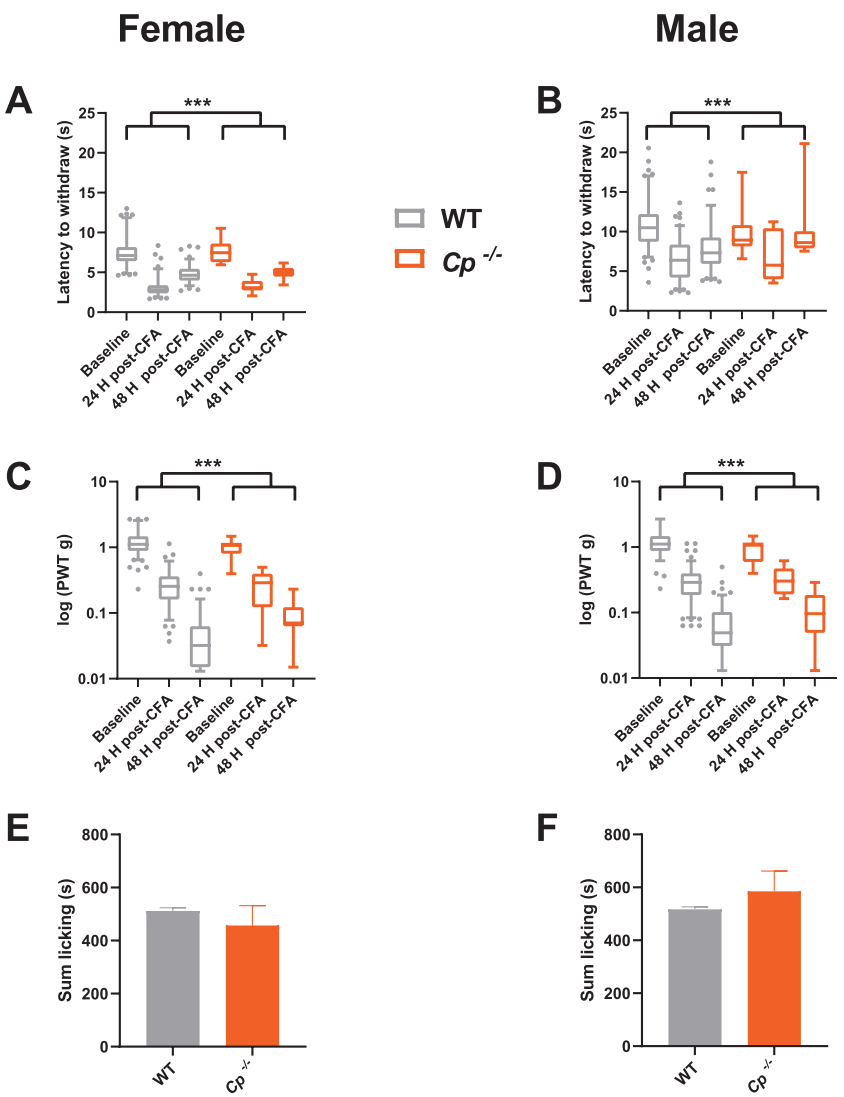

Figure 6. Abnormalities in nocifensive behavior after *Cp* inactivation. (A and B) Latency to withdraw the paw from a thermal stimulus is shown (quartile boxplots with error bars for 5%-95% percentile) in seconds for each test day in the Hargreaves assay for wild type C57BL/6NJ (WT) mice [8 and 17-18 weeks (n = 96F, n = 100M), gray] and *Cp* [16-17 weeks (n = 12F, n = 12M), orange] homozygous null female (A) and male (B) mice. Thermal hyperalgesia resolved more rapidly in *Cp* mutant mice (null genotype $P < 0.001$; sex × genotype $F_{(2,216)} = 7.41$, $P < 0.001$,***) than their WT controls. (C and D) Paw withdrawal threshold is plotted in log10 scale as quartile boxplots (with error bars for 5%-95% percentile) for each test day for wild type C57BL/6NJ (WT) [8 and 17-18 weeks (n = 119F, n = 122M), gray] and *Cp* [16-17 weeks (n = 12F, n = 12M), orange] homozygous null females (C) and males (D) for the von Frey assay. Peak mechanical hyperalgesia 48 hours after CFA administration was significantly reduced for *Cp* mutant mice ($F_{(1,257)} = 16.3$, $P < 0.001$, ***) compared with WT controls. (E and F) The mean (with SEM) of licking or biting behavior duration summed over 10 to 60 minutes is shown for wild type C57BL/6NJ (WT) [aged 12-21 weeks (n = 346F, n = 398M), gray] and *Cp* [16-17 weeks (n = 6F, n = 9M), orange] homozygous null mice for females (E) and males (F). No statistically significant difference was detected.

increased activity was detected in the light–dark transition assay conducted as part of the IMPC pipeline.

3.5. Pain as a comorbidity

The leading role of pain in global morbidity[70] led us to consider the results of our study in the context of pain as a common comorbidity. For example, complex and contradicting literature exists in the field of objective and subjective pain sensitivity in patients with autism spectrum disorders (ASD).[71,75] Cross referencing the list of 110 genes reported in the current study with the Simons Foundation Autism Research Initiative's (SFARI Database, queried March 3, 2021; https://www.sfari.org/) list of 1003 genes associated with ASD identified 17 overlapping genes (**Table 3**), 4 of which were highlighted in the current study as playing a role in pain sensitivity. Although the remaining 13 overlapping genes did not meet the stringent significance threshold used herein, their distribution across the whole 110 genes is noteworthy. That distribution was estimated by ranking the 412 *P* values that resulted from statistical analyses of the current nociception phenotyping data set and selecting the smallest *P* value reported for each gene, irrespective of the biological parameter it represented. When converted into a percentile presentation (**Table 3**), the 4 hits ranked within the top 5% of all tests, 2 additional genes were represented in the top 10 percentile, and a total of 12 from 17 overlapping genes were represented in the top quartile of all tests. Although we did not call these overlapping genes hits in the current study, the nonrandom distribution across the genes list is of interest and indicates that more subtle effects may exist and could potentially be detected with larger group sizes.

Cp was not included in the 1003 genes reported on the SFARI database; however, significant literature supports a role for *Cp* in ASD.[15,22,23,77] *Cp* encodes ceruloplasmin, a neuroprotective antioxidant protein involved in iron homeostasis. The change from baseline thermal sensitivity after CFA administration was altered in *Cp* mutants compared with that of their age-matched and sex-matched wild type controls (**Table 2**, **Figs. 6A and 6B**). Specifically, 48 hours after CFA administration when strains typically have partially resolved sensitivity to CFA, the response of *Cp* mutant mice, particularly males, closely resembled that of the baseline response (sex × genotype $F_{(2,216)} = 7.41$, $P < 0.001$; male genotype effect size −123% and female genotype effect size −5%), indicating that their thermal hyperalgesia resolved more rapidly. Furthermore, peak change from baseline mechanical nociception of *Cp* mutants, measured at 48 hours after CFA administration, was reduced compared with that of their age-matched and sex-matched wild type controls (**Table 2** and **Figs. 2B, 6C, and 6D**), indicating reduced mechanical hyperalgesia ($F_{(1,257)} = 16.3$, $P < 0.001$, percent effect size −26% genotype difference). There was no significant difference in formalin response for this mutant strain (**Figs. 6E and 6F**), and no open-field abnormalities were reported by the IMPC for an independent cohort of *Cp* knockout mice.

4. Discussion

Our study aimed to identify novel genetic determinants of nociception. Our approach was to use the mouse as a genetic model from which we could glean insights into human pain.

Although the direction of response for each assay-center combination was as expected, absolute values varied between centers, even when the same C57BL/6N substrain was used. Such cross-center differences were observed for other phenotypic domains the IMPC investigated[66] and indicate that strain × center interactions influenced the outcome. Genotype × environment interactions are well documented[38] and in response we implemented a local control strategy whereby mutant–wild type comparisons were performed only on data generated within the same center.

One hundred ten single-gene knockout mouse strains were tested using up to 3 nociception assays. A stringent statistical threshold was implemented intentionally to reduce the risk of false discovery to below 2%. Thirteen strains achieved statistical significance in 1 or more assays. Measures of locomotor activity and anxiety-like behavior were available from the IMPC for 103 of the lines reported herein, and a review of these data indicated there were no prevalent confounds skewing the outcome of the nociception assays. Our review of published phenotypes reported in the Mouse Genome Informatics database[9] (queried March 11, 2021) revealed that 7 of the 13 genes (*Abhd13*, *Alg6*, *BC048562*, *Cgnl1*, *Cnrip1*, *Oxa1l*, and *Tecpr2*) had no non-IMPC mouse alleles registered; therefore, these IMPC alleles represent novel strains. Of interest is *Cnrip1,* which encodes cannabinoid receptor-interacting protein 1. In humans, CNRIP1 has been shown to interact with cannabinoid receptor 1 (CB1) to suppress CB1-mediated tonic inhibition of voltage-gated calcium channels[54] that are highly expressed on peripheral afferent nerve fibers and play a key role in pain modulation. Previous mouse strains exist for the remaining 6 genes, including 2 (*Gria1* and *Htr3a*) with a reported role in nociception, 3 (*Cp*, *Mmp16*, and *Trim2*) with a role in neurobehavior but not reported specifically in nociception, and 1 (*Trim14*) with no reported phenotypes related to nociception or neurobehavior. The 2 genes with previously reported nociception phenotyping were used for benchmarking. Consistent with our findings, both *Gria1*[29] and *Htr3a*[80] knockouts were reported to display reduced pain sensitivity.

The genetic dissociability of nociception and hypersensitivity[42,49,51] was referenced to select phenotyping assays belonging to distinct genetic categories. Two of the 13 strains achieving statistical significance were tested with only 1 nociception assay, whereas the remaining 11 strains were tested with multiple assays. Interestingly, 7 of those 11 strains achieved statistical significance in only 1 assay, variously affecting baseline sensitivity, response after inflammation or recovery. The remaining 4 strains achieved significance across 2 assays [$Cp^{-/-}$, hyposensitive in both von Frey and Hargreaves at 48 hrs after CFA administration (**Fig. 6**); $Gria1^{-/-}$ and $Mmp16^{-/-}$, both hyposensitive in formalin and baseline von Frey (response to CFA was not assessed); and $Tecpr2^{-/-}$, baseline thermal hyposensitivity, baseline mechanical hypersensitivity but reduced mechanical hypersensitivity after CFA administration (**Fig. 5**)], indicating a more general or centralized role in nociception for these genes.

In humans, multiple cases of *TECPR2* mutation have been reported[59] as causing a multisystem disorder classified within a broader group of neurodegenerative diseases called hereditary spastic paraparesis.[55] Hereditary spastic paraparesis is characterized by axonal degeneration in the corticospinal tract which controls limb motor function. The age of clinical presentation ranges from early infancy to age 70 years,[55] although reported mutations affecting TECPR2 present in early infancy.[59] TECPR2 is a positive regulator of autophagosome accumulation.[4] Autophagosomes sequester bulky cellular constituents such as protein aggregates and organelles and deliver them into the lysosomal degradation pathway. This process is a key cellular mechanism for the turnover of proteins, the disruption of which is linked to neurodegenerative and neuromuscular disorders, such as amyotrophic lateral sclerosis, Huntington disease, and lysosomal storage disorders.[43] TECPR2 inactivation will result in defective autophagy leading to neuronal dysfunction[53,72] that would explain the $Tecpr2^{-/-}$ hyposensitive phenotypes reported herein. Note that $Tecpr2^{-/-}$ mice were hyperactive when tested by the IMPC in open field and light–dark transition at age 8-9 weeks. Nociception testing was performed at age 17 weeks and although outside the scope of this study, it would be valuable to conduct a longitudinal study of locomotor activity and nocifensive behavior to determine changes in response with time.

Of the 13 genes with statistically significant pain phenotypes, human orthologues for 5 have been linked to ASD. Referencing the SFARI database, 1 gene (*ALG6*) is classified as syndromic based on the strength of evidence to support a role in ASD, 2 genes (*CGNL1* and *GRIA1*) are considered strong candidates (SFARI confidence score = 2) and 1 gene (*HTR3A*) has suggestive evidence (SFARI confidence score = 3). Independently of SFARI, *CP* has been linked to ASD in children[15,23,77] and is featured herein. *CP* encodes ceruloplasmin, a ferroxidase involved in iron homeostasis. Ceruloplasmin deficiency in humans results in aceruloplasminemia, a rare neurodegenerative disorder with brain iron accumulation.[79] Neurological symptoms are detected in the third to seventh decade of life and include movement disorders, ataxia, dysarthria, and progressive dementia.[79] Reduced CP levels have also been associated with ASD through biochemical measurement of circulating ceruloplasmin[15,23,77] and integrated transcriptomic analyses.[22] The reduction or absence of ceruloplasmin activity results in cell death through at least 2 mechanisms.[16,36] Ceruloplasmin deficiency in cerebellar astrocytes results in an intracellular build-up of ferrous (Fe^{2+}) iron that is believed to cause cell death through the generation of toxic reactive oxygen

intermediates. By contrast, Purkinje neurons are believed to be lost in patients with aceruloplasminemia because of iron deficiency.[16,36] Multiple *Cp* loss-of-function mutations have been reported to phenocopy aspects of human aceruloplasminemia,[79] including motor coordination deficits observed at age 16 months.[58] However, to the best of our knowledge, altered nocifensive behaviors have not been reported. We detected altered nociception in 17-week-old mice, which is significantly earlier than previously reported abnormal phenotypes. A similar pattern of response that included normal formalin-induced nocifensive behavior, attenuated thermal allodynia and mechanical hyperalgesia, and also coincided with reduced astrocyte function was reported previously in hemokinin-1 knockout mice[34] and may merit deeper investigation.

Pain has been implicated as a comorbidity in several neuropsychiatric conditions including ASD.[8] An estimated 90% of individuals with ASD experience sensory perception abnormalities in every sensory modality. These abnormalities contribute to ASD core symptoms such as social and communication deficits and are considered primary characteristics of ASD neurobiology.[64] Abnormalities in processing tactile and pain sensitivity, ranging from exaggerated response to touch, stimuli hyposensitivity, and self-harming, have been reported in individuals with ASD [10,17,44,46,52,62,63,69] and in rodent models[65] and are consistent with data reported herein. For example, *Htr3a* knockout mice were reported previously to display impaired pain response to formalin,[80] but no other behavioral data related to ASD-like phenotypes were reported. Interestingly, the remaining 4 of the top 5 ASD-related hits (*Cgnl1*, *Gria1*, *Alg6,* and *Cp*) are not on the SFARI list of 209 ASD genes with mouse models although genetically altered models of *Gria1*[29,78] and *Cp*[58,79] have been reported. In fact, of the 17 genes which overlap between the 110 genes in the current study and the 1003 ASD-associated gene set listed on SFARI, only 7 are reported in the SFARI list of relevant mouse models, resulting in 10 novel strains available through the IMPC to the scientific community.

One hundred ten single-gene knockout mouse strains were assessed for their role in nociception and hypersensitivity using up to 3 commonly used phenotyping assays. Five independent mouse phenotyping facilities spanning 2 continents contributed data. Owing to local restrictions pertaining to capacity and ethical approval, not every strain was tested for every assay. However, we present herein 173 unique gene×assay combinations which represents a significant contribution to the pain field. Unsurprisingly for a complex behavioral phenotype, genotype × environment interactions[38] were found to influence nociception screening results. In response, a local control strategy was implemented whereby mutant–wild type comparisons were performed only on data generated within the same phenotyping facility. This is an established control strategy used for all IMPC phenotyping data. We also implemented a stringent significance threshold, thereby highlighting only strains presenting with large and highly significant effects. The results expand our understanding of the genetic mechanisms of inflammatory pain and provide new and freely available mouse models to pursue further studies to better understand pain sensitivity and its interactions with potential ASD-related phenotypes.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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