Archival Report

Biological Psychiatry

# Sex Differences in Nociceptor Translatomes Contribute to Divergent Prostaglandin Signaling in Male and Female Mice

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## ABSTRACT

**BACKGROUND:** There are clinically relevant sex differences in acute and chronic pain mechanisms, but we are only beginning to understand their mechanistic basis. Transcriptome analyses of rodent whole dorsal root ganglion (DRG) have revealed sex differences, mostly in immune cells. We examined the transcriptome and translatome of the mouse DRG with the goal of identifying sex differences.

**METHODS:** We used translating ribosome affinity purification sequencing and behavioral pharmacology to test the hypothesis that in Nav1.8-positive neurons, most of which are nociceptors, translatomes would differ by sex.

**RESULTS:** We found 80 genes with sex differential expression in the whole DRG transcriptome and 66 genes whose messenger RNAs were sex differentially actively translated (translatome). We also identified different motifs in the 3′ untranslated region of messenger RNAs that were sex differentially translated. In further validation studies, we focused on *Ptgds*, which was increased in the translatome of female mice. The messenger RNA encodes the prostaglandin $PGD_2$ synthesizing enzyme. We observed increased PTGDS protein and $PGD_2$ in female mouse DRG. The PTGDS inhibitor AT-56 caused intense pain behaviors in male mice but was only effective at high doses in female mice. Conversely, female mice responded more robustly to another major prostaglandin, $PGE_2$, than did male mice. PTGDS protein expression was also higher in female cortical neurons, suggesting that DRG findings may be generalizable to other nervous system structures.

**CONCLUSIONS:** Our results demonstrate sex differences in nociceptor-enriched translatomes and reveal unexpected sex differences in one of the oldest known nociceptive signaling molecule families, the prostaglandins.

https://doi.org/10.1016/j.biopsych.2020.09.022

For decades, studies in the neuroscience field have been done almost entirely on male animals (1). However, there are physiological and molecular differences in the peripheral and central nervous systems between males and females. Many neurological disorders have been shown to have different incidence proportion, age of onset, symptoms, and response to treatment between males and females. On the one hand, schizophrenia tends to develop at an earlier age in men (2,3); Parkinson's disease is twice as common in men than in women (4), and it has sex differences in symptoms and response to treatment (5). On the other hand, major depressive disorder (6), anxiety disorders (7), and Alzheimer's disease (8) affect more women than men. There are also sex differences in pain syndromes. Neuropathic pain, osteoarthritis, migraine, and fibromyalgia are more frequently reported in women than in men (9–13). A common thread in all of these neurological disorders is that they are poorly treated by current therapies. The exclusion of one of the sexes in preclinical studies has likely been detrimental to the success of translational research.

As noted above, there are clear sex differences in pain mechanisms, yet we are only beginning to understand how these differences emerge (14). Nociceptors of the dorsal root ganglion (DRG) and trigeminal ganglion are the neurons that send nociceptive information to the brain and are a possible source of mechanistic diversity that causes sex differences in pain. A previous study suggested that there are some sex differences in sensory neuron transcriptomes (15). However, transcription and translation are not directly coupled in eukaryotes, so there can be important divergences between transcriptomes (the cellular RNA profile) and translatomes (the subset of the transcriptome that is bound to ribosomes for translation) in cells (16). An example in nociceptors is the similar transcription of the prolactin receptor (*Prlr*) in male and female nociceptors, but the female-selective localized translation of the *Prlr* messenger RNA (mRNA) in nociceptor terminals (17). This sex difference in localized *Prlr* mRNA translation causes an important sex difference in prolactin-evoked pain responses (17–19).

 

ISSN: 0006-3223

The primary goal of our study was to identify differences in translatome between male and female DRG neurons using translating ribosome affinity purification (TRAP) methodology (20–22). We demonstrate that specific mRNAs are differentially bound by ribosomes (hence likely differentially translated) in Nav1.8-positive neurons [most, but not all, of which are nociceptors (23)] of the DRG between males and females. One of the differentially translated mRNAs identified using our TRAP approach was *Ptgds* (prostaglandin D-synthase). We chose this target for further validation because PTGDS is an abundant enzyme in neuronal cells that converts the prostaglandin $PGH_2$ to $PGD_2$ that can be targeted with pharmacological tools. We find that it is upregulated in female neurons. Consistent with this, we observed significant differences in behavioral responses between males and females when inhibiting this enzyme. We also noted sex differences in the response to $PGE_2$. Our use of TRAP technology to delve into sex differences in nociceptor translatomes reveals a fundamental difference in how male and female mice respond to one of the oldest known families of nociceptive signaling molecules, the prostaglandins (24).

## METHODS AND MATERIALS

Detailed methods are provided in Supplement 1. All animal procedures were approved by the Institutional Animal Care and Use Committee at University of Texas at Dallas. DRGs from Nav1.8-TRAP male and female mice were quickly dissected and homogenized using Precellys Minilys Tissue Homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). An aliquot of the lysate was saved for use as input (IN) (bulk RNA sequencing [RNA-seq]), and the remaining was used for immunoprecipitation (IP) (TRAP sequencing [TRAP-seq]) by incubating the lysate with protein G–coated Dynabeads (Invitrogen, Carlsbad, CA) bound to anti-GFP (green fluorescent protein) antibodies for 3 hours at 4°C. RNA was eluted from all samples using the Direct-zol kit (Zymo Research, Irvine, CA) and complementary DNA libraries were prepared with total RNA Gold library preparation (Illumina, San Diego, CA). After standardizing the amount of complementary DNA, the libraries were sequenced on Illumina NextSeq500 sequencing machine with 75-bp single-end reads. Reads were then mapped against the reference genome and transcriptome (Gencode vM16 and GRCm38.p5) using STAR v2.2.1 (25). Relative abundances in transcripts per million (TPM) for each gene of each sample were quantified by Stringtie v1.3.5 (26). Downstream analyses were restricted to protein-coding genes and excluded mitochondrial chromosome genes. For each expressed coding gene, we report $\log_2$ fold change, Bhattacharyya coefficient (27), and strictly standardized mean difference (SSMD) (28,29). We used immunohistochemistry, enzyme-linked immunosorbent assay, and behavioral tests to understand the consequences of sex differences in *Ptgds* expression.

## RESULTS

Nav1.8$^{cre}$ mice were crossed with Rosa26$^{fs\text{-}TRAP}$ (30) in order to create mice expressing eGFP (enhanced GFP) fused to the ribosomal L10a protein in Nav1.8-positive neurons (Nav1.8-TRAP mice) (Figure 1A). The specificity of the TRAP

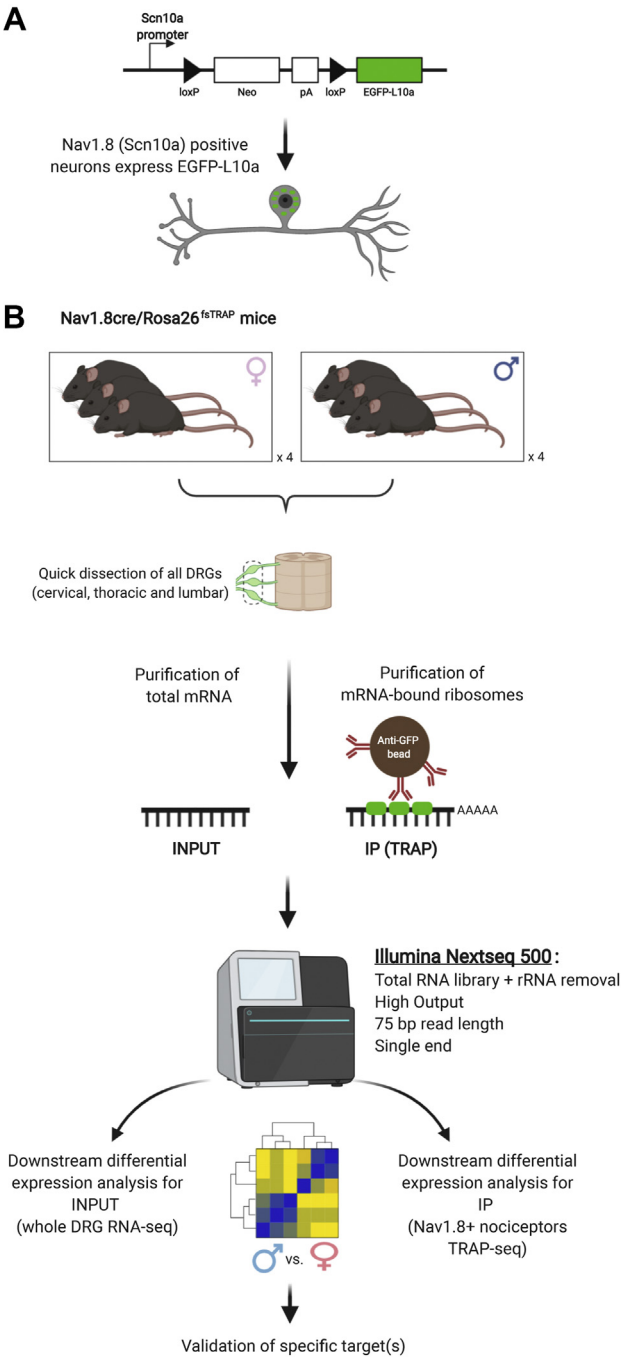


**Figure 1.** Outline of workflow for TRAP-seq to reveal sex differences in nociceptor translatomes. **(A)** eGFP-L10a protein is expressed in Nav1.8-positive nociceptors. **(B)** Schematic representation of the methodology shows dissection of all DRGs (cervical, thoracic, and lumbar) from Nav1.8-cre/Rosa26$^{fsTRAP}$ mice followed by isolation of total RNA (INPUT), and mRNA-bound to the ribosome (IP) using anti-eGFP–coated beads. Samples were sequenced and processed for downstream analysis of differentially expressed genes as shown. DRG, dorsal root ganglion; eGFP, enhanced green fluorescent protein; IP, immunoprecipitation; mRNA, messenger RNA; RNA-seq, RNA sequencing; rRNA, ribosomal RNA; TRAP-seq, translating ribosome affinity purification sequencing.

approach has been previously characterized, in which it was shown that eGFP-RPL10a is expressed in sensory neurons in the DRG including most nociceptors (21,22). We used Nav1.8-TRAP mice to characterize the translatome of male and female DRG neurons by immunoprecipitating actively translating ribosomes and purifying the associated mRNA (Figure 1B). We sequenced bulk RNA (designated INPUT or IN in later plots), corresponding to total RNA from whole DRGs, and IP mRNA, correspondent to mRNA bound to GFP-tagged ribosomes in Nav1.8-positive neurons. This approach allowed us to characterize differences at the steady-state transcriptional and active translational levels between female and male DRG.

A heatmap of the correlation coefficients for coding gene TPM of each biological replicate showed a clear separation between TRAP-seq and bulk RNA-seq, as expected given the different cell populations from which the complementary DNA library is constructed (Figure 2A). Consequently, in our hierarchical clustering analysis using these correlation coefficients, we found two distinct clusters of the bulk RNA-seq and TRAP-seq molecular profiles (Figure 2B). Subclusters within each cluster were very similar to each other and did not segregate by sex, showing that whole-transcriptome molecular profiles in each assay type (RNA-seq and TRAP-seq) were consistent across sexes for the DRG. All biological replicates for each sex in the input and IP samples showed high correlation coefficients across gene TPM, suggesting high reproducibility across experiments (Figure 2C).

Because TRAP-seq purifies translating mRNAs in a cell type–specific manner, we tested the specificity of our approach using a group of control genes. We analyzed a subset of genes known to be enriched in specific cell populations in the DRG and verified that neuronal mRNAs, such as *Calca*, *Trpv1*, *Scn10a*, and *Prph*, had enriched relative abundance in IP fraction. In contrast, non-neuronal genes such as glial markers (*Mpz*, *Mbp*, *Gfap*) were depleted in IP samples (Figure 2D).

Percentile ranks were calculated (Tables S1A and S2A in Supplement 2) for gene expression levels (in TPM) for each RNA-seq and TRAP-seq sample. Based on these order statistics, we conservatively determined a set of 15,072 genes ($\geq$30th percentile) that were consistently detected in at least one sex in the RNA-seq samples, and of those, 12,542 genes ($\geq$15th percentile) that were consistently detected in at least one sex in the TRAP-seq samples. These numbers are consistent with previous mouse DRG RNA-seq and TRAP-seq studies (21,31).

For each biological replicate in IN and IP, we plotted the empirical probability densities of coding gene TPM and noted a distinctly bimodal distribution for genes that are consistently detected versus those that are lowly expressed or undetected in each assay (Figure 2E, F). The TPM expression levels were finally quantile normalized (represented as quantile-normalized TPM) in order to correct for sequencing depth and thus ensure comparability between samples.

To determine differentially expressed (DE) genes, we calculated the $\log_2$ fold change of TPM across sexes, and two related statistics—SSMD of TPM percentile ranks (28,29) and Bhattacharyya coefficient of quantile-normalized TPM (27) between sexes for quantifying the effect size and controlling for within-group variability (Tables S1A, B in Supplement 2 for IN; Tables S2A, B in Supplement 2 for IP). For stringency, we required DE genes to have $|\log_2\text{-fold change}| > 0.41$ (corresponding to fold change $>1.33$), $|\text{SSMD}| > 0.9$, and Bhattacharyya coefficient $>0.5$. We plotted the SSMD values against the fold change ($\log_2$ scale) for the autosomal genes for IN and IP (Figure 3A, D).

We found a total of 80 genes for IN (transcriptome) (Figure 3A, B; Table S3 in Supplement 2) and 66 genes for IP (translatome) (Figure 3C, D; Table S4 in Supplement 2) that were DE between sexes. Given that we used mice that had not been experimentally manipulated, we anticipated finding a relatively small number of genes using both approaches. Interestingly, we did not observe a substantial overlap between genes DE in IN and IP, except for sex-chromosomal genes such as *Eif2s3x*, *Eif2s3y*, *Uty*, and *Kdm5d* and a few autosomal genes (Figure 3E). We found that *Lepr* (leptin receptor) and *Lbp* (lipopolysaccharide binding protein) were upregulated in both IN and IP, in females. *Ep400* (E1A binding protein P400) was upregulated in male IN and female IP, highlighting discrepancies between transcriptome and translatome. *Atf3*, a known nerve injury marker (32), was upregulated in both IN and IP in males. Because *Atf3* can be induced by minor injuries, like scratches (33), this may be explained by more frequent fighting in male cages.

Next, we conducted gene set enrichment analysis for DE genes using Gene Ontology Enrichment Analysis resource PANTHER (34). We did not find any statistically significant Gene Ontology terms for DE genes in IN. In contrast, we identified 6 Gene Ontology terms (Biological Process) statistically significant (false discovery rate $< .05$) for genes DE in IP (Figure 3F). The majority of the genes DE in IP were involved in the regulation of cell communication and signaling (Figure 3G). We identified several genes (e.g., *Sfrp4*, *Sema6a*) that encode for membrane proteins involved in cell signaling. Genes such as *Fbln1*, an extracellular matrix structural protein, and *Fgf9*, a growth factor, are involved in cell to cell communication. Genes such as *Map3k1* and *Mapk1ip1* are involved in the regulation of MAPK (mitogen-activated protein kinase) cascade. These findings suggest that there may be sex differences in proteins that regulate fundamental signaling pathways in male and female nociceptors. We expanded our functional analysis by manually curating relevant information regarding DE genes for IN and IP (Tables S5 and S6 in Supplement 1 contain detailed information).

Within the DE genes in the IN, we identified several transcription factors such as *Hoxd4* in females and *Foxd3* (known to be glially expressed) in males. We also noted different immune-related genes identified in male and female DRG IN, consistent with previous work in mice (35,36) and humans (37). These included *Cxcl16*, a T cell signaling molecule, and *Jchain* (immunoglobulin joining chain), which were upregulated in female IN. *Cd276* was upregulated in male IN and plays a role in inflammatory responses by regulating cytokine production and T cell receptor signaling.

In the IP fraction, as expected, the identified DE genes were neuronally enriched in expression when compared with the IN fraction (Tables S5 and S6 in Supplement 1). In the female translatome (IP), we found several upregulated DE genes that are involved in neuronal functions, such as *Pcdha8*, *Zmynd8*, and *Slc6a13*. In the male translatome, genes with known

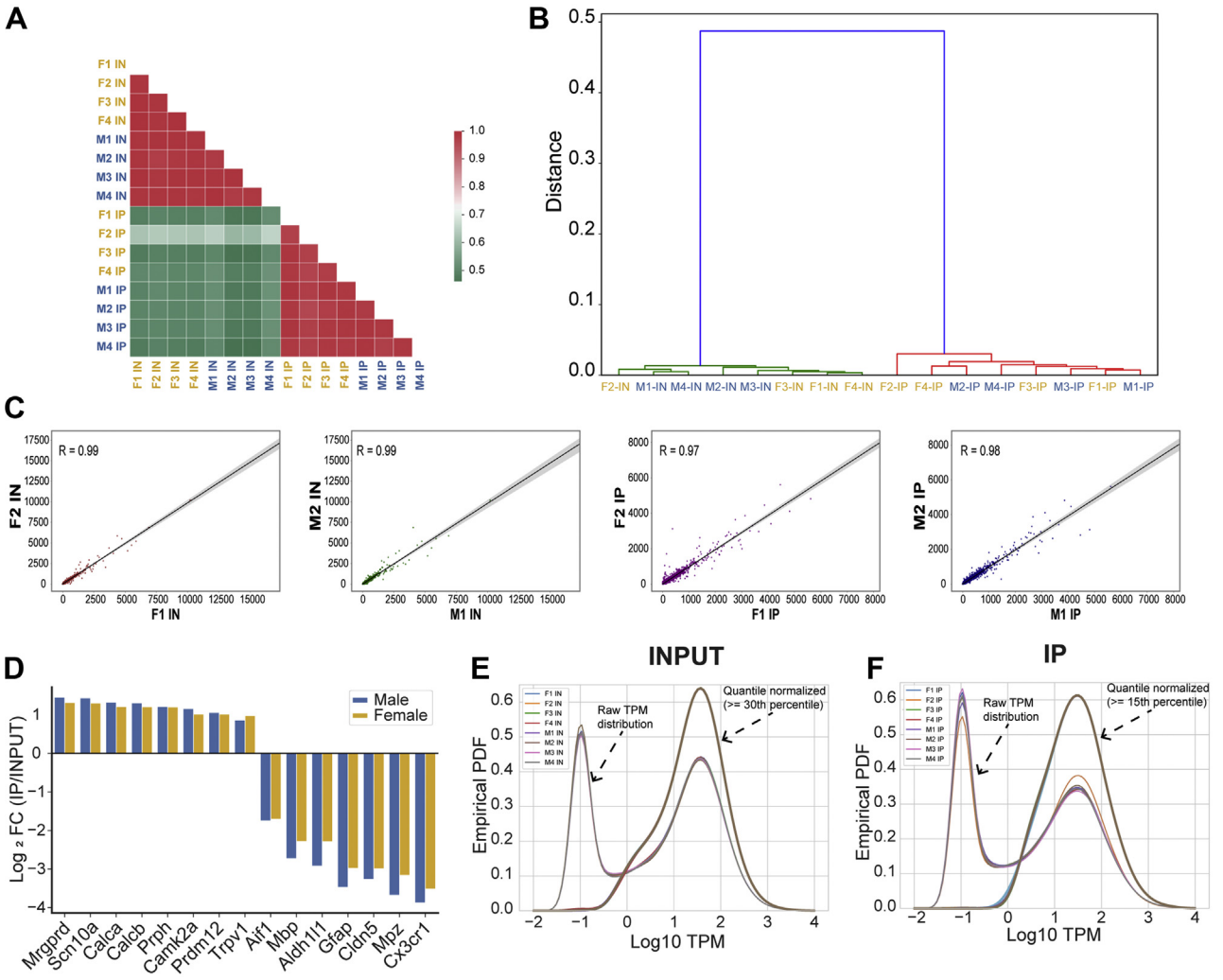


**Figure 2.** Nociceptor TRAP-seq quality control. **(A)** Hierarchical clustering analysis and **(B)** heatmap of the correlation coefficient show clear separation between TRAP-seq and bulk RNA-seq. However, we did not observe a clear distinction between male and female samples. **(C)** Linear correlation plots shows high correlation coefficients of gene TPM within biological replicates for the IN and IP fractions (shown for 2 replicates in each sex and assay), suggesting high reproducibility between replicates. **(D)** Neuronal markers were enriched in IP fractions, such as *Calca* (encoding CGRP) or *Prph*, while glial markers such as *Mbp*, *Mpz*, and *Gfap* were depleted (based on FCs of median TPM in each assay). **(E, F)** The empirically estimated probability density of the raw TPM and qTPM distributions for the IN and IP fractions of all samples are shown. For IN samples, TPM distributions are shown for all coding genes and qTPM shown for systematically transcriptome-expressed genes. For IP samples, TPM distributions were plotted for all coding genes, and qTPM are shown for systematically translatome-expressed genes. CGRP, calcitonin gene-related peptide; FC, fold change; IN, input; IP, immunoprecipitation; PDF, probability density function; qTPM, quantile-normalized TPM; RNA-seq, RNA sequencing; TPM, transcripts per million; TRAP-seq, translating ribosome affinity purification sequencing.

neuronal functions such as *Chka* and *Sema6a* were found to be upregulated. Several enriched gene set categories were similar between males and females, but these were driven by different genes, suggesting that unique genes may control similar functions in male and female nociceptors.

Because translation efficiency can be controlled by sequence elements within the mRNA (38,39), we examined whether there were motifs in the 5′ untranslated regions (UTRs) or 3′ UTRs of DE mRNAs in IP. We found 3 enriched motifs in the 3′ UTRs of the genes upregulated in males (Figure 4A) and 1 enriched motif in the 3′ UTRs of the genes upregulated in females (Figure 4B). The 3′ UTR of a gene is known to influence the localization, degradation, and translation efficiency of an mRNA (6,7). The identified motifs are involved in neuron differentiation and migration, cell communication, and signal transduction, in agreement with the biological functions of DE genes identified in our IP fractions. We did not find any enriched motifs in the 5′ UTRs of male or female DE mRNAs.

Next, we proceeded to validate our TRAP-seq approach by linking sex differences in the nociceptor active translatome to functional differences in expression and/or behavior. We decided to focus on a gene that was upregulated in the female IP: *Ptgds*. PTGDS catalyzes the conversion of $PGH_2$ to $PGD_2$ (Figure 5A), which is known to be the most abundant prostaglandin in the brain (40,41) and regulates nociception, sleep, and temperature homeostasis (42–52). Prostaglandins are

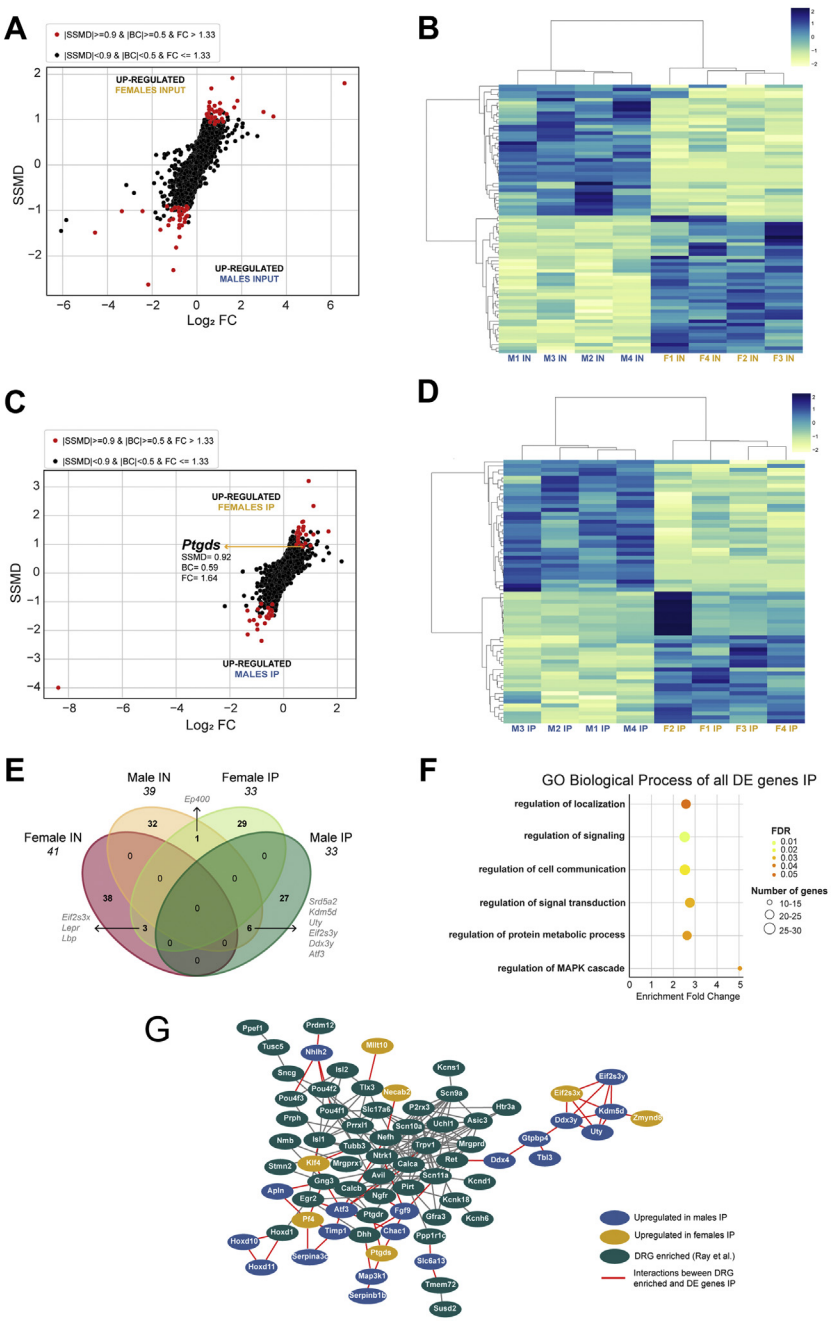


**Figure 3.** Differentially translated mRNAs in male and female DRG nociceptors. **(A)** Dual-flashlight plot of IN samples showing SSMD and $\log_2$ FC values for all autosomal genes on or above the 30th percentile. **(B)** Heatmap shows the *z* scores of the DE genes in IN samples. Labels represent sex and biological replicate number. **(C)** Dual-flashlight plot of IP samples showing SSMD and $\log_2$ FC values for all autosomal genes on or above the 15th percentile. **(D)** Heatmap shows the *z* scores of the differentially translated mRNAs in IP samples. **(E)** Venn diagram comparing the genes identified as DE. There were few overlaps between IN and IP autosomal genes. **(F)** GO terms enriched for all genes DE in IP. **(G)** Network of interactions between genes differentially translated between males and females in IP and genes enriched in DRG neurons (network generated using STRING database and Cytoscape). BC, Bhattacharyya coefficient; DE, differentially expressed; DRG, dorsal root ganglion; FC, fold change; FDR, false discovery rate; GO, Gene Ontology; IN, input; IP, immunoprecipitation; MAPK, mitogen-activated protein kinase; mRNA, messenger RNA; SSMD, strictly standardized mean difference.

among the most widely studied of pain-inducing molecules, and many drugs are currently marketed as analgesics that target this class of molecules (53–55). We reasoned that previously unknown sex differences in prostaglandin signaling could have a dramatic impact on further our understanding of sex differences in pain.

First, we reanalyzed single-neuron DRG RNA-sequencing (56) and observed *Ptgds* mRNA expression in several subpopulations of neurons, including ones expressing *Calca* (a marker for peptidergic neurons) and *P2rx3* (a marker for non-peptidergic neurons) (Figure 5B). We noted that a receptor for $PGD_2$, *Ptgdr1* ($DP_1$), was also expressed, especially in non-peptidergic neurons, but *Ptgdr2* ($DP_2$) was not expressed in DRG neurons. We confirmed, using immunohistochemistry, that PTGDS is expressed in almost all neurons in the mouse DRG (Figure 5C). Next, we sought to verify whether there were any sex differences in PTGDS expression at the protein level in the mouse DRG. PTGDS expression was markedly higher in

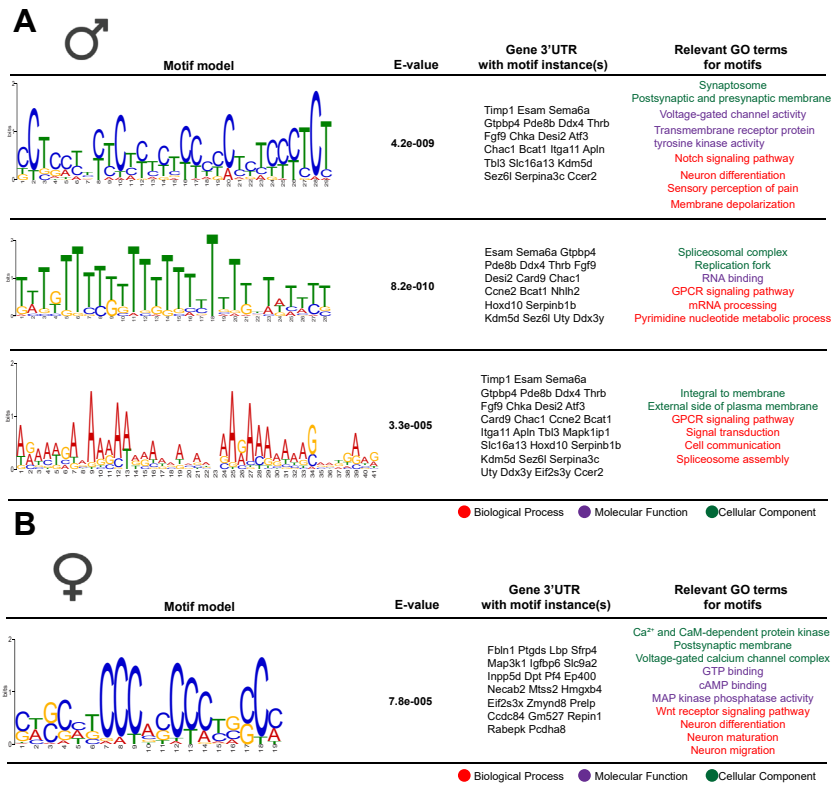


**Figure 4.** Enriched motifs identified in the 3′ UTRs of mRNAs differentially translated in males or females. The motif analysis was conducted on the list of upregulated mRNAs in both male and female IP fractions. **(A)** We found 3 motifs significantly enriched in the 3′ UTR of upregulated male mRNAs compared with the female mRNAs. **(B)** We identified one motif significantly enriched in the 3′ UTR of upregulated female mRNAs compared with the male mRNAs. This motif is involved in several neuronal functions and it is present in multiple mRNAs, such as *Ptgds*. cAMP, cyclic adenosine monophosphate; GO, Gene Ontology; GPCR, G protein–coupled receptor; GTP, guanosine triphosphate; IP, immunoprecipitation; MAP, mitogen-activated protein; mRNA, messenger RNA; UTR, untranslated region.

female DRG (Figure 5D, E). To verify our previous TRAP experiment, we conducted an independent experiment in which we tracked the estrous cycle stage in the female mice. We found that *Ptgds* mRNA associated with ribosomes was substantially higher in this group of female mice with some variation within the estrous cycle, with the highest at the estrous phase (Figure 5F). In addition, we also investigated whether PTGDS showed sex differences in expression in brain neurons (Figure S1A in Supplement 1). Similar to observations in the DRG, PTGDS expression was higher in female cortical neurons (Figure S1B in Supplement 1).

Having confirmed that PTGDS is more highly expressed in female DRG neurons, we investigated whether this would lead to functional sex differences. First, we measured $PGD_2$ levels in female and male DRGs. Because $PGD_2$ is highly unstable and can degrade very rapidly, we opted for converting $PGD_2$ to a more stable derivative $PGD_2$-MOX (by treating our samples with MOX [methoxylamine] hydrochloride) and performed an enzyme-linked immunosorbent assay. Our results demonstrated that $PGD_2$ levels are higher in female DRGs (Figure 5G) in concordance with the higher levels of PTGDS. Next, we tested whether inhibiting PTGDS would produce differential behavioral effects in male and female mice. Unexpectedly, in pilot experiments, we noted intense grimacing behavior in male mice, so we focused on this behavioral output because it is driven by nociceptor input to the central nervous system (57). We used three different doses (1 mg/kg, 3 mg/kg, and 10 mg/kg) of AT-56, a selective and competitive inhibitor of PTGDS (58), observing a robust grimacing effect after intraperitoneal injections of AT-56 (10 mg/kg), particularly in male mice (Figure 6A). At each dose, female mice exhibited less grimacing that lasted for a shorter time (Figure 6B). This finding suggests that females are protected against PTGDS inhibition-evoked pain because they have higher basal $PGD_2$ levels and more enzyme.

A previous study demonstrated that *Ptgds* gene knockout led to a loss of $PGE_2$-evoked mechanical pain hypersensitivity (42), suggesting an interplay between these closely related molecules (Figure 5A) in pain signaling. Moreover, a previous clinical study suggested a sex difference in ibuprofen-induced analgesia in an experimental pain model wherein only males showed analgesia in response to this drug, which lowers $PGE_2$ levels (59). We investigated whether there were any differences in mechanical behavior or grimace between males and females in response to $PGE_2$. While intraplantar injection of $PGE_2$ did not lead to any response to von Frey filaments in male mice at doses of 300 ng or 1 μg (Figure 7A), it produced mechanical hypersensitivity in female mice (Figure 7B). We did not observe any significant grimacing behavior in male mice (Figure 7C) after $PGE_2$ injection. Female mice, however, displayed grimacing up to 60 minutes after $PGE_2$ injection (Figure 7D).

## DISCUSSION

We used TRAP-seq to reveal sex differences in the translatomes of these neurons that are crucial for nociception. We reached several conclusions based on our work. Consistent

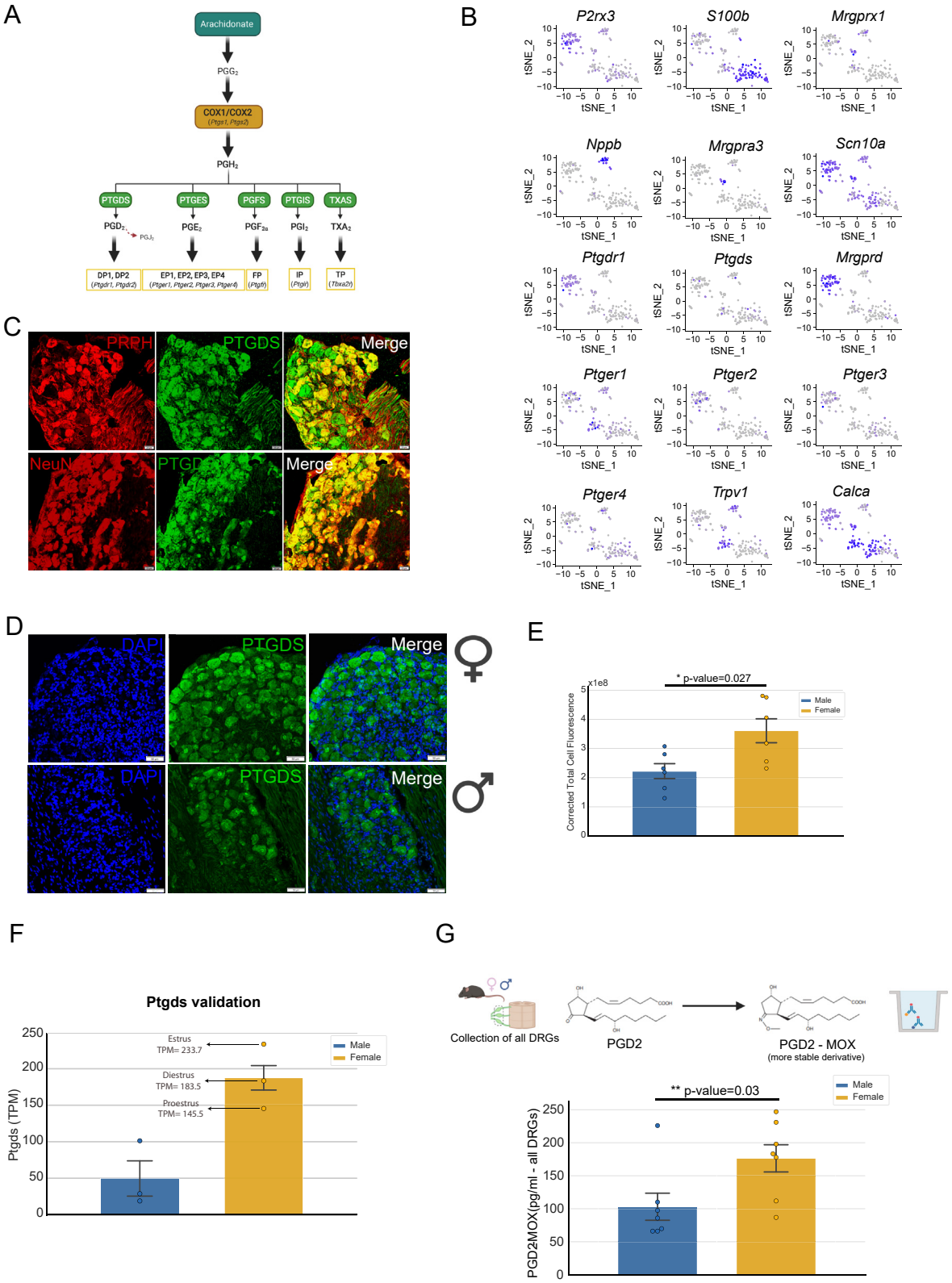


**Figure 5.** *Ptgds* expression is higher in female DRGs and leads to higher production of $PGD_2$. **(A)** PTGDS converts $PGH_2$ to $PGD_2$, which has a very short half-life and is rapidly metabolized to $PGJ_2$. **(B)** Single DRG neuron sequencing shows that *Ptgds* and $PGD_2$ receptor $DP_1$ (*Ptgdr1*) are expressed in neurons in

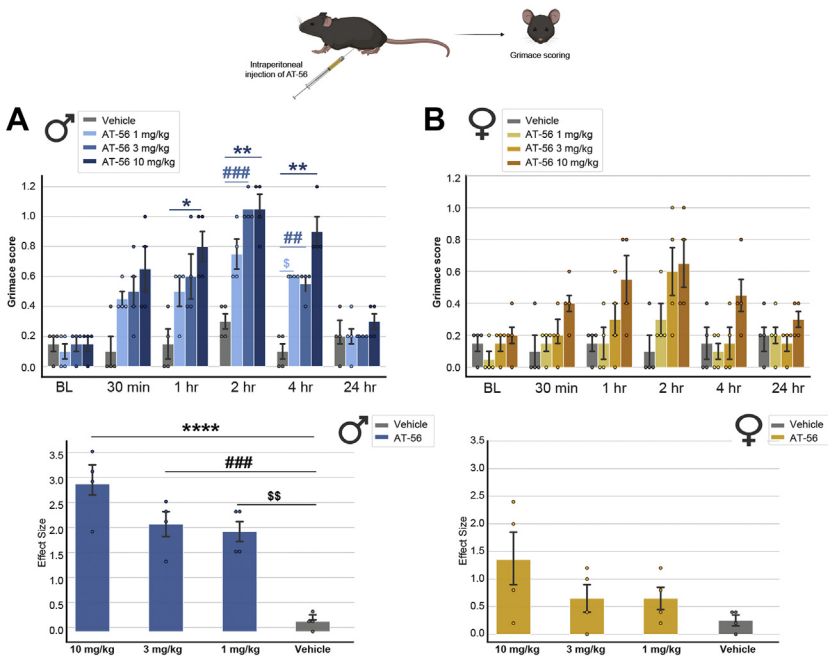


**Figure 6.** Inhibition of PTGDS produces robust grimacing behavior in mice that is greater in males. **(A)** Intraperitoneal injection of AT-56, a selective inhibitor of PTGDS, led to grimacing behavior in male mice (2-way ANOVA [$F_{15,60} = 4.279$, $p < .0001$], post hoc Sidak's test, *Vehicle vs. AT-56 10 mg/kg male at 1 hour, $p = .0314$; **Vehicle vs. AT-56 10 mg/kg male at 2 hours, $p = .0071$; ***Vehicle vs. AT-56 10 mg/kg male at 4 hours, $p = .0068$; ###Vehicle vs. AT-56 3 mg/kg male at 2 hours, $p = .0004$; ##Vehicle vs. AT-56 3 mg/kg male at 4 hours, $p = .0068$; $Vehicle vs. AT-56 1 mg/kg male at 4 hours, $p = .0193$). We also calculated the effect size (difference from the baseline) and observed a significant difference for the 3 doses of AT-56 compared with vehicle in males (1-way ANOVA [$F_{3,12} = 22.00$, $p < .0001$], post hoc Tukey's test; ****10 mg/kg AT-56 vs. vehicle, $p < .0001$; ###3 mg/kg AT-56 vs. vehicle, $p = .0006$; $$1 mg/kg AT-56 vs. vehicle, $p = .0012$). **(B)** Grimacing behavior in female mice following AT-56 injection was not different from vehicle (2-way ANOVA [$F_{15,60} = 1.136$, $p = .3462$]). When calculating the effect size (difference from the baseline), we did not observe any significant differences between groups in female mice (2-way ANOVA [$F_{3,12} = 2.109$, $p = .1524$]). ANOVA, analysis of variance; BL, baseline.

with previous studies done at the whole-transcriptome level for the DRG, most differences can be attributed to immune genes (35,37, 60), a finding that may be important for chronic pain (14). At the translatome level, we observed differences in mRNAs bound by ribosomes in nociceptors between males and females, and many of these likely play an important role in the function of these cells. The Nav1.8-positive neuronal specificity of our TRAP-seq approach did not identify similar sex differences at the transcriptome and translatome levels for autosomal genes, supporting the important role of translation regulation in nociception (61). We validated higher PTGDS expression in female neurons. We found that blocking $PGD_2$ synthesis led to profound behavioral sex differences, with larger behavioral effects in male mice. We also found sex differences in response to $PGE_2$, demonstrating that there is a fundamental divergence in prostaglandin signaling between males and females in relation to nociception.

There is a growing body of evidence of mechanistic sex differences in nociception and pain (13,14,62). Immune cells have been shown to play an important role in the development and resolution of chronic pain, many of them with sex-specific mechanisms [e.g., macrophages in male mice (63)], in which different genes are upregulated following injury in male and female mice (14). We identified nonneuronal genes with potential roles in inflammatory and immune responses that had differential baseline expression between males and females. These included genes that regulate T cell signaling, such as *Cxcl16* (upregulated in females) and *Cd276* (upregulated in males). These findings are consistent with previous literature showing sex differential roles of T cells in opioid analgesia (64) and where distinct proteins produced by T cells are upregulated following spared nerve injury—PPAR$\alpha$ (peroxisome proliferator-activated receptor alpha) in male mice and PPAR$\gamma$ in female mice (65). Interestingly, in the latter study, it was reported that T cells play a key role in driving neuropathic pain in females (65), whereas monocyte-associated genes such as *Tlr4* contributed to neuropathic pain in males (65,66).

While sex differences in immune contributions to pain have garnered extensive attention, differences at the neuronal level have not been found in some transcriptomic studies (35) and have not been examined in others (67). However, some recent studies examining specific neuronal populations have found sex differences (15), and some genes, such as the prolactin receptor, show sex differences in expression within neuronal populations (68). Using the TRAP technique, which we have previously used to characterize nociceptor translatome changes in neuropathic pain (21) and between ganglia (22),

the DRG. *Ptgds* is coexpressed with most neuronal markers, suggesting that it is expressed in all neurons; *Ptgdr1* is mostly expressed in nonpeptidergic neurons (coexpressed with *P2rx3*); $PGE_2$ receptors (*Ptger1*, *Ptger2*, *Ptger3*, *Ptger4*) were expressed by most neuronal subtypes. **(C)** We confirmed using IHC that PTGDS is expressed in neurons. **(D, E)** We found that PTGDS has higher expression in female DRG neurons compared with male DRGs, at the protein level (unpaired *t* test [$t_{10} = 2.584$, $p = .0272$]). **(F)** In a separate TRAP experiment, we monitored the female estrous cycle and validated that *Ptgds* is higher in females compared with females regardless of estrous cycle. **(G)** $PGD_2$-MOX ELISA demonstrated that $PGD_2$ levels are higher in female DRGs (unpaired *t* test [$t_{12} = 2.381$, $p = .0347$). Scale bars = 20 μm **(C)**, 50 μm **(D)**. DRG, dorsal root ganglion; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; MOX, methoxylamine; PG, prostaglandin; TPM, transcripts per million; TRAP, translating ribosome affinity purification.

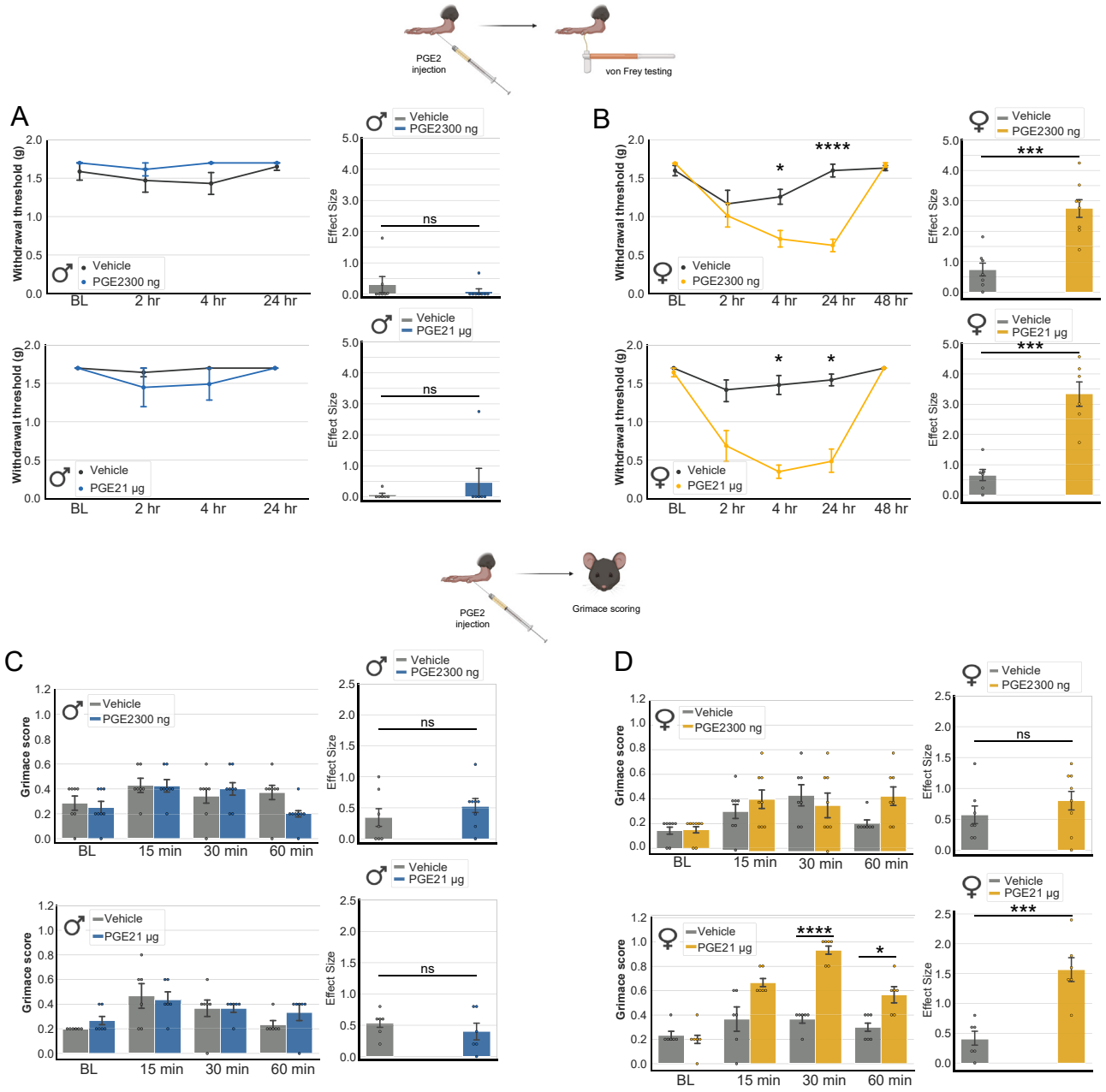


**Figure 7.** Intraplantar administration of $PGE_2$ produces greater mechanical allodynia and grimacing in female mice. **(A)** Male mice did not respond to von Frey filaments after injection of 300 ng (2-way RM ANOVA [$F_{3,39}$ = 1.030, $p$ = .3900]) or 1 µg (2-way RM ANOVA [$F_{3,30}$ = 0.7260, $p$ = .5444]) of $PGE_2$. When calculating the effect size, we did not observe any statistically significant differences between groups in males (effect size 300 ng: unpaired $t$ test [$t_{13}$ = 0.8756, $p$ = .3971]; effect size 1 µg: unpaired $t$ test [$t_{10}$ = 0.8709, $p$ = .4042]). **(B)** Female mice showed mechanical allodynia up to 24 hours after injection of both 300 ng of $PGE_2$ (2-way ANOVA [$F_{4,52}$ = 12.35, $p$ < .0001], post hoc Sidak's test: *Vehicle vs. 300 ng $PGE_2$ at 4 hours, $p$ = .0161; ****Vehicle vs. 300 ng $PGE_2$ at 24 hours, $p$ < .0001) and 1 µg of $PGE_2$ (2-way RM ANOVA [$F_{4,40}$ = 10.78, $p$ < .0001], post hoc Sidak's test: *** Vehicle vs. 1 µg $PGE_2$ at 4 hours, $p$ = .0005; **Vehicle vs. 1 µg $PGE_2$ at 24 hours, $p$ = .0026). We also observed an effect size difference between groups in female mice (***Effect size 300 ng: unpaired $t$ test [$t_{13}$ = 4.980, $p$ = .0003]; ***Effect size 1 µg: unpaired $t$ test [$t_{10}$ = 5.569, $p$ = .0002]). **(C)** Male mice did not show any significant grimacing behaviors following administration of 300 ng of $PGE_2$ (2-way RM ANOVA [$F_{3,39}$ = 1.996, $p$ = .1305]). At 1 µg of $PGE_2$, we also did not observe any significant grimacing in male mice (2-way RM ANOVA [$F_{3,30}$ = 0.5376, $p$ = .6601]). Similarly, we also did not observe any effect size between groups in grimacing of male mice (effect size 300 ng: unpaired $t$ test [$t_{13}$ = 0.9225, $p$ = .3731]; effect size 1 µg: unpaired $t$ test [$t_{10}$ = 0.8305, $p$ = .4257]). **(D)** Female mice exhibit robust grimacing after 1 µg $PGE_2$ injection (2-way RM ANOVA [$F_{3,30}$ = 11.34, $p$ < .0001], post hoc Sidak's test: ****Vehicle vs. 1 µg $PGE_2$ at 30 min, $p$ < .0001; *Vehicle vs. 1 µg $PGE_2$ at 60 min, $p$ = .0257) but not at 300 ng (2-way RM ANOVA [$F_{3,39}$ = 1.197, $p$ = .1303]). We also observed an effect size in the grimacing scores between 1 µg $PGE_2$ and vehicle grimacing in female mice (effect size 300 ng: unpaired $t$ test [$t_{13}$ = 0.9458, $p$ = .3615]; ***Effect size 1 µg: unpaired $t$ test [$t_{10}$ = 4.669, $p$ = .0009]). ANOVA, analysis of variance; BL, baseline; $PGE_2$, prostaglandin $E_2$; RM, repeated measures.

we have identified a number of sex differences in translation of mRNAs in DRG nociceptors. *Inpp5d* was upregulated in female nociceptors. This gene has been associated with dementia and Alzheimer's disease (69,70), both of which are more common in women (8). It would be of interest to investigate whether translation of this gene is also upregulated in the brain of females. In contrast, *Apln*, which is a neuroprotectant neuropeptide and anti-inflammatory protein, is upregulated in male nociceptors, and it has been reported as a promising target to treat Alzheimer's disease (71). We also identified several genes with sex differences in mRNA association with ribosomes at the baseline level that have been previously linked to pain (see Table S6 in Supplement 1). For instance, genes in the MAPK cascade have been linked to inflammatory responses in sensory neurons (72), with *Map3k1* being upregulated in a model of carrageenan-induced hyperalgesia (73). *Map3k1* was upregulated in female nociceptors in our study, while *Mapk1ip1* was upregulated in male nociceptors. These findings suggest that although MAPK signaling likely plays a key role in pain in both males and females (74), there may be underlying nuances in signaling that are sex specific, such as the prominent role of p38 in pain signaling in males (75–77).

We saw little overlap in transcriptome differences in the DRG between males and females and in nociceptor translatome differences between males and females. Some of the reason for this is almost certainly technical; however, this divergence might also be partially explained by sex differential translation regulation. This area of research has not garnered much attention but may be important for neuronal function, given the key role of translation regulation in synaptic plasticity (78). In the context of pain, we recently demonstrated that female-selective translation of the prolactin receptor mRNA in central terminals of nociceptors is a causative factor in the pain-promoting effects of prolactin in female mice (17,79).

The increased abundance of *Ptgds* mRNA in the active translatome of female Nav1.8-positive neurons intrigued us because of the well-known role of prostaglandins in pain signaling. A previous study showed higher PTGDS expression in the neonatal female brain (80), but we are not aware of any other reports of sex differences for this enzyme in neuronal tissue. PTGDS converts $PGH_2$ to $PGD_2$, which is one of the most abundant prostaglandins in the brain (40,41,81). $PGD_2$ is known to have important roles in the regulation of nociception (42,82), temperature (52), and sleep (51). There is also evidence that PTGDS is involved in the transport of retinoids in the brain (83), thus playing essential roles in the nervous system. In behavioral experiments, inhibition of PTGDS caused robust pain behavior in male mice, while female mice showed effects only at high doses of inhibitor. Higher baseline levels of PTGDS and $PGD_2$ in female DRG likely explain these sex differences in response to AT-56. Previous studies have shown both antinociceptive (45,46) and pronociceptive (47) roles of $PGD_2$. We were not able to test the effect of AT-56 in pain models owing to the effect of the drug alone. Interestingly, we also found higher levels of PTGDS protein in female cortex, indicating that our nociceptor findings may be generalizable to other neuronal populations.

Mice lacking the *Ptgds* gene from birth do not develop tactile pain following $PGE_2$ injection (42). This suggests an interaction between the *Ptgds* gene and $PGE_2$ that prompted us to look for sex differences in response to $PGE_2$. We found that female mice responded to lower doses of $PGE_2$ with mechanical hypersensitivity and grimacing than did male mice, reminiscent of similar recent findings with calcitonin gene-related peptide (84). This suggests a complex balance between $PGD_2$ and $PGE_2$ in nociceptive signaling that will take more work to fully understand. Nevertheless, these sex differences in prostaglandin signaling have implications for some of the most commonly used pain relievers—the COX (cyclooxygenase) inhibitors. Studies in rodent arthritis models have described reduced inflammation in COX isoform knockout mice in females but not in males (85). In a study using an experimental pain model in healthy human subjects, ibuprofen produced analgesia in men but not in women, despite equal blood levels of the drug (59). Collectively, these results point to profound sex differences in prostaglandin signaling. Our findings of enhanced translation of *Ptgds* mRNA in the female nervous system can help to understand the molecular underpinnings of these differences better. However, further studies employing genetic tools will be necessary to determine whether these differences are driven primarily by neuronal *Ptgds* expression. Nearly a century after the discovery of prostaglandins (86), and centuries after humans started targeting them for pain, mechanistic sex differences in their actions are only beginning to come into focus. In our view, this is a testament to the dire need for consideration of sex as a biological variable in basic neuroscience research.

## ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by National Institutes of Health Grants Nos. NS065926 (to TJP) and NS102161 (to TJP and ANA).

We thank members of the Price lab for useful discussions throughout the course of the project. We also thank the Genome Center at The University of Texas at Dallas for the services to support our research.

AW is a co-founder of Doloromics. PB-I reports research funding from IBRO. GD reports research funding from the National Institutes of Health, Alder, and Teva; is a co-founder of CerSci Therapeutics, PARMedics, and Ted's Brain Science; and has consulted for Schedule One Therapeutics. ANA reports research funding from the National Institutes of Health. TJP reports research funding from the National Institutes of Health, Merck, Hoba Therapeutics, and Alder; is a co-founder of CerSci Therapeutics, Ted's Brain Science, Doloromics, PARMedics, and 4E Therapeutics; and has consulted for Grunenthal. All other authors report no biomedical financial interests or potential conflicts of interest.

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Received Aug 4, 2020; revised Sep 22, 2020; accepted Sep 23, 2020.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2020.09.022.

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