

Temporal and sex differences in the role of BDNF/TrkB signaling in hyperalgesic priming in mice and rats



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ABSTRACT

Brain-derived neurotrophic factor (BDNF) signaling through its cognate receptor, TrkB, is a well-known promoter of synaptic plasticity at nociceptive synapses in the dorsal horn of the spinal cord. Existing evidence suggests that BDNF/TrkB signaling in neuropathic pain is sex dependent. We tested the hypothesis that the effects of BDNF/TrkB signaling in hyperalgesic priming might also be sexually dimorphic. Using the incision postsurgical pain model in male mice, we show that BDNF sequestration with TrkB-Fc administered at the time of surgery blocks the initiation and maintenance of hyperalgesic priming. However, when BDNF signaling was blocked prior to the precipitation of hyperalgesic priming with prostaglandin E₂ (PGE₂), priming was not reversed. This result is in contrast to our findings in male mice with interleukin-6 (IL6) as the priming stimulus where TrkB-Fc was effective in reversing the maintenance of hyperalgesic priming. Furthermore, in IL6-induced hyperalgesic priming, the BDNF sequestering agent, TrkB-fc, was effective in reversing the maintenance of hyperalgesic priming in male mice; however, when this experiment was conducted in female mice, we did not observe any effect of TrkB-fc. This markedly sexual dimorphic effect in mice is consistent with recent studies showing a similar effect in neuropathic pain models. We tested whether the sexual dimorphic role for BDNF was consistent across species. Importantly, we find that this sexual dimorphism does not occur in rats where TrkB-fc reverses hyperalgesic priming fully in both sexes. Finally, to determine the source of BDNF in hyperalgesic priming in mice, we used transgenic mice (*Cx3cr1^{CreER}* × *Bdnf^{flx/flx}* mice) with BDNF eliminated from microglia. From these experiments we conclude that BDNF from microglia does not contribute to hyperalgesic priming and that the key source of BDNF for hyperalgesic priming is likely nociceptors in the dorsal root ganglion. These experiments demonstrate the importance of testing mechanistic hypotheses in both sexes in multiple species to gain insight into complex biology underlying chronic pain.

1. Introduction

BDNF is an important neurotrophin and neurotransmitter for many nociceptive functions (Nijs et al., 2015). It is involved in the maturation of a subset of sensory neurons and, in the adult, is a critical regulator of plasticity in nociceptive synapses (Kandasamy and Price, 2015; Price and Inyang, 2015). BDNF is expressed by many nociceptors in the dorsal root ganglion (DRG) and its expression is markedly increased by inflammation or injury (Kerr et al., 1999; Mannion et al., 1999; Thompson et al., 1999). BDNF is also expressed in microglial cells in the spinal cord and it can be released downstream of ATP signaling acting

on purinergic receptors (P2XR) expressed by these central nervous system immune cells (Coull et al., 2005; Malcangio, 2017). TrkB is the cognate receptor for BDNF and is expressed in some DRG neurons as well as many dorsal horn neurons. BDNF acts via TrkB to promote synaptic plasticity in the dorsal horn (Kerr et al., 1999). This effect can be mediated by both presynaptic (Chen et al., 2014) and postsynaptic receptors (Kerr et al., 1999). BDNF from microglia is released in neuropathic pain conditions and acts via TrkB expressed in dorsal horn neurons to decrease expression of a potassium/chloride cotransporter called KCC2 that controls intracellular chloride concentrations in these neurons (Coull et al., 2005). The result of this signaling cascade is

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decreased GABAergic and glycinergic inhibitory efficacy and altered sensory gating resulting in mechanical allodynia (Price and Prescott, 2015). In inflammatory and some neuropathic pain conditions, BDNF from nociceptors in the DRG is critical for the full expression of pain hypersensitivity (Zhao et al., 2006; Sikandar et al., 2018). Inflammation or other transient injuries can establish a form of pain plasticity called hyperalgesic priming (Reichling and Levine, 2009; Kandasamy and Price, 2015). The initial insult, e.g. exposure to an inflammagen, “primes” the nociceptive system to respond to a previously subthreshold stimulus with a long-lasting pain hypersensitivity. This form of pain plasticity depends on BDNF for its establishment (Asiedu et al., 2011; Melemedjian et al., 2013) and was recently shown to also depend on BDNF from nociceptors (Sikandar et al., 2018).

It has recently been demonstrated that the actions of microglial purinergic receptor (P2XR) and p38 mitogen activated protein kinases (MAPK) signaling in neuropathic pain are sex specific in mice and rats (Sorge et al., 2015; Taves et al., 2016; Mapplebeck et al., 2018). This suggests that many aspects of microglial signaling may play a critical role in regulating chronic pain in males, but a lesser role in females. In line with demonstrations in neuropathic pain models, we have recently shown that microglial mechanisms like P2XR and p38 also contribute to hyperalgesic priming in male, but not female, mice (Paige et al., 2018). There is less clarity on whether BDNF effects are sexually dimorphic, and if they are, if those mechanisms are conserved in multiple species. This is an important gap in knowledge because BDNF signaling is a potential target for reversal of chronic pain states ranging from inflammatory (Li et al., 2008) to neuropathic (Coull et al., 2005) and even including migraine pain (Burgos-Vega et al., 2016). Our hypothesis in designing the experiments described here was that BDNF would play a sexual dimorphic role in the maintenance of hyperalgesic priming in mice and rats. Our findings support this hypothesis in mice, but not in rats.

2. Methods and materials

2.1. Chemicals

Recombinant human IL6 and TrkB-fc were purchased from R & D systems (Minneapolis, Minnesota). PGE₂ was obtained from Cayman Chemicals (Ann Arbor, Michigan). ANA-12 was purchased from Maybridge (Altrincham, United Kingdom). All other chemicals were purchased from Fisher Scientific (Hampton, New Hampshire).

2.2. Animals

The Institutional Animal Care and Use Committees at The University of Arizona and The University of Texas at Dallas approved all use of animal procedures. Mice and rats were housed in a 12-h light-dark cycle with lights on at 7:00 AM. Food and water were available *ad libitum* in their home cages. Figs. 1–3 show data from male ICR (CD-1) mice that were purchased from Harlan while Fig. 4 shows data from C57BL/6J male and female mice bred at University of Texas at Dallas. Fig. 5 shows data from male and female Sprague-Dawley rats that were purchased from Harlan. Fig. 6 shows data from *Bdnf*^{flx/flx} (Jax mice: ^{Bdnftm3.1ae.1}, stock number 004339) and *Cx3cr1*^{CreER} (Jax mice: B6.129P2(Cg)-*Cx3cr1*-Cre^{tm2.1(cre/ERT)Litt}/WganJ, stock number 21160) crossed with *Bdnf*^{flx/flx} mice gifted to us by Michael Salter lab (Sorge et al., 2015), that were bred and raised at The University of Texas at Dallas to produce experimental animals. For mice bred in house, they were weaned between 3 and 4 weeks old and ear clipped to verify genotypes (supplemental Fig. 1A–C). All mice weighed between 20 and 25 g at the time of experimental use. Two doses of 10 mg tamoxifen in 0.5 ml corn oil was administered through gavage 48 h apart to both male and female *Cx3cr1*^{CreER} and *Cx3cr1*^{CreER}-*Bdnf*^{flx/flx} mice 1 month prior to behavioral testing (Sorge et al., 2015) to generate mice lacking BDNF in microglia: *Cx3cr1*^{CreER}-*Bdnf*^{-/-} and mice treated with tamoxifen but

without a loss of microglial BDNF: *Cx3cr1*^{CreER}-*Bdnf*^{+/-}.

2.3. Behavior

Male and female mice and rats were habituated for approximately 1 hr to acrylic behavior boxes prior to beginning the experimental testing on any given day. Hindpaw mechanical thresholds were determined by using the up-down method as described in (Chaplan et al., 1994) using calibrated von Frey filaments (Stoelting Company, Wood Dale, IL). The experimenters were blinded to the genotype and/or treatment of the animals.

2.4. Plantar incision surgery and priming injections

A mouse model of incisional pain was used for this study (Banik et al., 2006). Surgeries were done as previously described in (Tillu et al., 2012). Animals received intrathecal or intraperitoneal injections of TrkB-fc or ANA-12 (Cazorla et al., 2011), respectively, or vehicle at times indicated after incision or IL6 treatment. Animals were allowed to recover for 24 h and then paw withdrawal thresholds were measured at 24, 48 and 72 h and again at 5, 7, 9, 11, and 13 days post-surgery. For hyperalgesic priming experiments, the animals received an intraplantar injection of IL6 (0.1 ng/25 μL (Melemedjian et al., 2010)) or a plantar incision followed by PGE₂ (100 ng/25 μL (Asiedu et al., 2011)) given at the same site 7–9 days later for IL6 or 14 days following plantar incision. The paw withdrawal thresholds were again measured following the PGE₂ injection.

2.5. Statistics

All data are displayed as mean ± standard error of the mean (SEM), with individual samples represented within graphs to depict the n of each group and distribution. GraphPad Prism 6 v 6.0 for Mac OS X was used for analysis. Statistical tests and *posthoc* analyses are described in each figure legend.

3. Results

Previously, we have shown that BDNF/TrkB signaling is important in IL6-induced mechanical hypersensitivity and hyperalgesic priming (Asiedu et al., 2011; Melemedjian et al., 2013). IL6 plays an important role in postsurgical pain (Buvanendran et al., 2006) and spinal BDNF/TrkB signaling contributes to incisional-evoked pain (Li et al., 2008; Masaki et al., 2016; Ding et al., 2018; Tian et al., 2018) but its role in hyperalgesic priming caused by incision has not been explored. To address this gap in knowledge we gave adult, male mice plantar incision with an intrathecal injection of the BDNF sequestering agent, TrkB-fc, given at the same time as the incision. This single dose of TrkB-fc significantly inhibited mechanical hypersensitivity evoked by the incision (Fig. 1A) but did not influence hyperalgesic priming that was revealed by intraplantar injection of PGE₂ 14 days after the incision, after all mice had returned to baseline mechanical thresholds (Fig. 1B). We then gave intrathecal TrkB-fc injections at the time of incision and also 24 hrs later. This dosing schedule led to a significant inhibition of incision-evoked mechanical hypersensitivity (Fig. 1C) and also blocked the development of hyperalgesic priming (Fig. 1D). We conducted similar experiments with the small molecule TrkB antagonist, ANA-12 but with the drug given at the time of incision and then again 24 and 48 hrs later via the intraperitoneal administration route. We chose this dosing schedule based on our previous work in other priming models (Melemedjian et al., 2013). ANA-12 administration reduced incision-evoked mechanical hypersensitivity (Fig. 2A) and also inhibited the development of hyperalgesic priming (Fig. 2B).

We have previously shown that both atypical PKCs, which are regulated by BDNF at spinal synapses, and BDNF/TrkB signaling are critical for the maintenance of IL6-induced hyperalgesic priming

Mouse

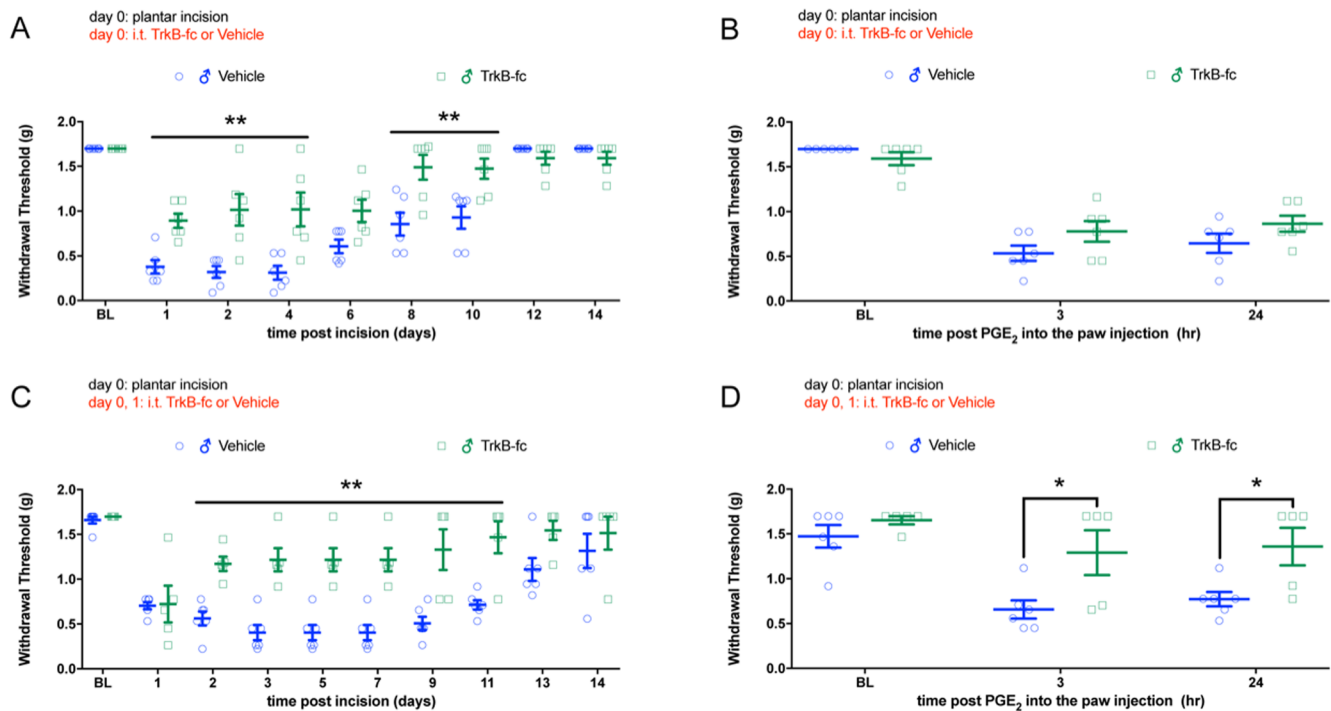


Fig. 1. TrkB-fc blocks mechanical hypersensitivity and hyperalgesic priming induced by plantar incision. Male mice that received an intrathecal (i.t.) injection of TrkB-fc (30 ng) on the day of plantar incision surgery exhibited reduced mechanical hypersensitivity (A; two-way ANOVA; $F(1, 90) = 58.57, P < 0.0001$; Bonferroni's $^{**}p < 0.01$; Dunnett's from BL-Veh-D1-10 $p < 0.0001$; TrkB-Fc D1-6 $p < 0.0001$) but not PGE₂ (100 ng)-induced hyperalgesic priming (B; two-way ANOVA; $F(1, 30) = 2.753, P = 0.1075$) compared to vehicle. Male mice that received two i.t. injections of TrkB-fc (30 ng) on the day of plantar incision surgery and 24 hr after also had decreased mechanical hypersensitivity (C; two-way ANOVA; $F(1, 90) = 95.09, P < 0.0001$; Bonferroni's $^{**}p < 0.01$; Dunnett's from BL-Veh-D1-13 $p < 0.01$; TrkB-Fc D1-2 $p < 0.05$) and PGE₂ (100 ng)-induced hyperalgesic priming (D; two-way ANOVA; $F(1, 27) = 15.27, P = 0.0006$; Bonferroni's $^{*}p < 0.05$) compared to vehicle.

(Asiedu et al., 2011; Melemedjian et al., 2013). To test this in the incision model we gave male mice hindpaw incisions and waited for them to completely recover from mechanical hypersensitivity induced by the incision. We then gave one or two doses of intrathecal TrkB-fc or two intraperitoneal doses of ANA-12. While TrkB-fc had a small, but significant effect on maintenance of hyperalgesic priming only at 24 h after PGE₂ (Fig. 3A), we did not observe a significant effect of ANA-12 (Fig. 3B). From these experiments we conclude that in the incisional pain model in male mice, BDNF/TrkB signaling plays a critical role in acute mechanical hypersensitivity, reproducing similar previous studies

(Li et al., 2008; Masaki et al., 2016; Tian et al., 2018), and establishing hyperalgesic priming but it does not play a key role in the maintenance of hyperalgesic priming. This is different than in the IL6 model where BDNF/TrkB signaling is crucial for establishment and maintenance of hyperalgesic priming (Asiedu et al., 2011; Melemedjian et al., 2013).

Due to some of these differences in the effect of BDNF/TrkB signaling between incisional and IL6 models we returned to the IL6 hyperalgesic priming model to examine potential sex differences in the role of BDNF/TrkB signaling in this system. We were interested in pursuing this question because BDNF from microglia plays a critical

Mouse

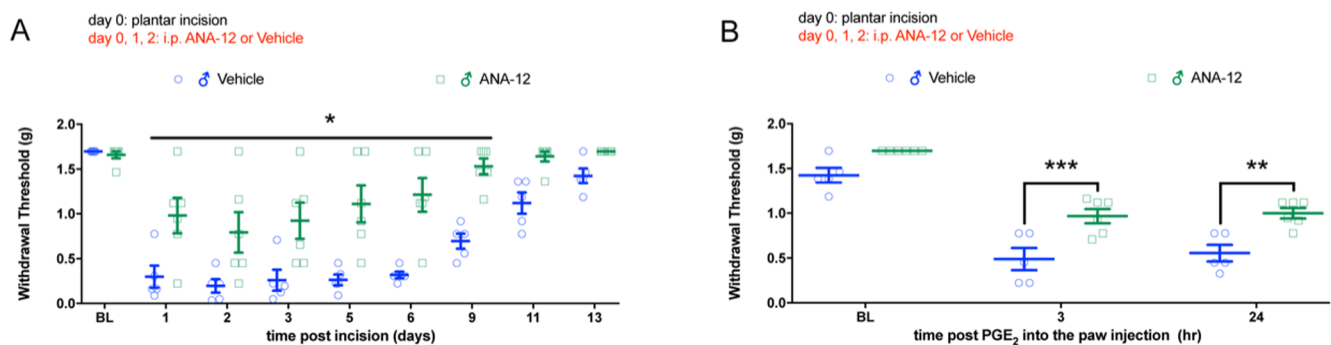


Fig. 2. ANA-12 blocks mechanical hypersensitivity and hyperalgesic priming induced by plantar incision. Male mice that received intraperitoneal (i.p.) injections of ANA-12 (1 mg/kg), a TrkB antagonist, the day of plantar incision surgery, 24, and 48 h after showed reduced mechanical hypersensitivity (A; two-way ANOVA; $F(1, 81) < 0.0001$; Bonferroni's $^{*}p < 0.05$.) and hyperalgesic priming (B; two-way ANOVA; $F(1, 27) = 38.62, P < 0.0001$; Bonferroni's $^{**}p < 0.01$) compared to vehicle.

Mouse

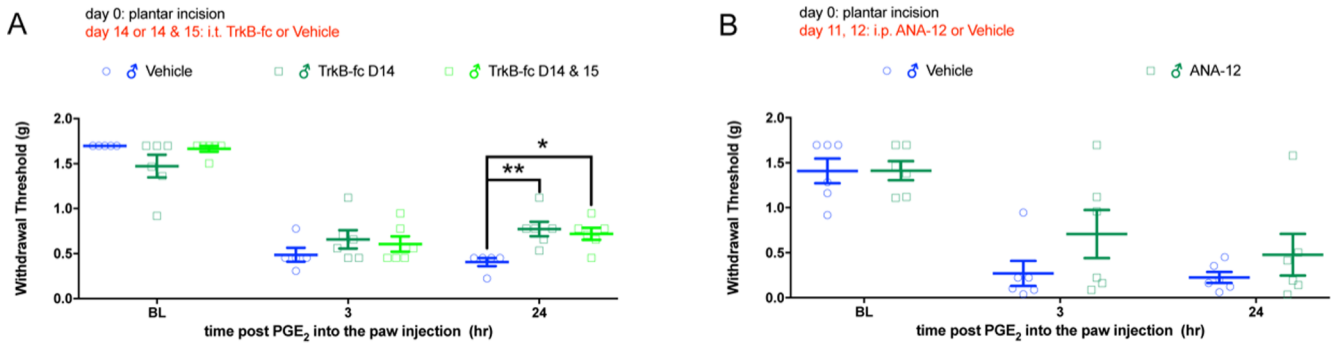


Fig. 3. TrkB-fc, but not ANA-12, reverses plantar incision-induced hyperalgesic priming. Male mice underwent plantar incision surgery and received 1 or 2 i.t. injection (s) of TrkB-fc (30 ng) (A) or 2 i.p. injections of ANA-12 (1 mg/kg) (B) after recovering. Mice that received TrkB-fc reversed the priming effect induced by plantar incision (A; two-way ANOVA; $F(2, 42) = 2.164, P = 0.1276$; Bonferroni's $^*p = 0.0284, ^{**}p = 0.0082$). However, ANA-12 did not reverse PGE₂ (100 ng)-precipitated hyperalgesic priming (B; two-way ANOVA; $F(1, 30) = 2.696, P = 0.1111$).

Mouse

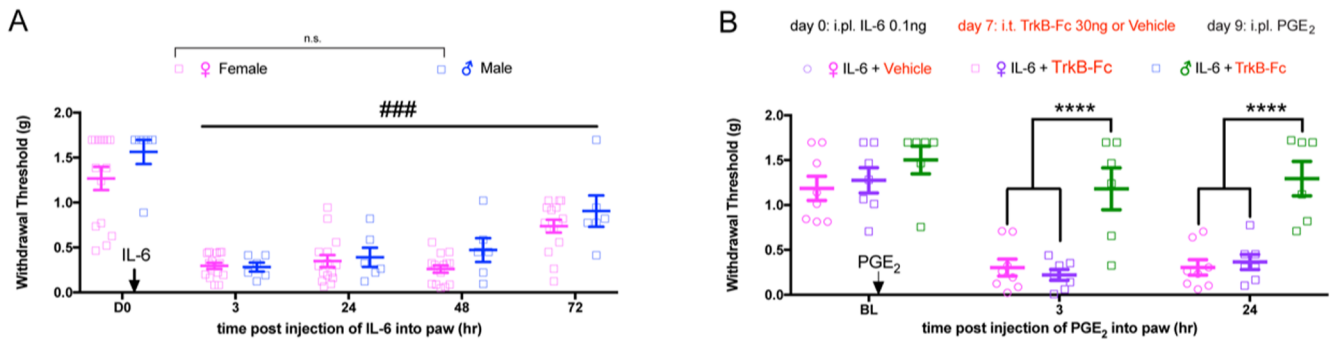


Fig. 4. BDNF/TrkB signaling plays a sexually-dimorphic role in IL6-induced hyperalgesic priming in mice. A) Both male and female mice received an intraplantar (i.pl.) injection of IL6 (0.1 ng) and were tested for mechanical hypersensitivity at 3, 24, 48, and 72 h. IL6 induced equal levels of mechanical hypersensitivity compared to BL in both male and female mice (A; two-way ANOVA; from BL $F(4, 95) = 43.4, P < 0.0001$; between sexes $F(1, 95) = 4.722, P = 0.0323$; Bonferroni's $^{###}p = 0.001$). After recovering, female mice received a single i.t. injection of vehicle or TrkB-fc (30 ng) along with male mice. TrkB-fc significantly reversed IL6-induced hyperalgesic priming in male mice, but not in female mice (B; two-way ANOVA; vs female vehicle, $F(2, 54) = 26.82, P < 0.0001$; vs female TrkB-fc, $F(1, 33) = 33.63, P < 0.0001$; $^{****}p < 0.0001$).

role in neuropathic pain in male but not female mice (Sorge et al., 2015), a sexual dimorphic effect that is apparently conserved in rats (Mapplebeck et al., 2018). We primed mice with IL6 and then administered TrkB-fc after mice had returned to baseline. We then challenged them with PGE₂ into the same paw 48 hrs later. As we have shown previously, TrkB-fc completely reversed hyperalgesic priming maintenance in male mice (Melemedjian et al., 2013). However, the same treatment was completely without effect in female mice (Fig. 4A and B). We then conducted the same experiment in rats. First, male rats recovered from mechanical hypersensitivity more rapidly than females when challenged with IL6 (Fig. 5A) but both sexes showed robust mechanical hypersensitivity in response to treatment. We gave intrathecal injections of TrkB-fc after all rats had recovered from IL6-induced mechanical hypersensitivity and challenged them with PGE₂ 48 hrs later. Male rats treated with TrkB-fc showed a complete reversal of hyperalgesic priming (Fig. 5B). Female rats given TrkB-fc showed a trend to reversal at 3 hrs and a complete reversal at 24 hrs after PGE₂ treatment (Fig. 5B). Therefore, BDNF/TrkB signaling plays a critical role in the maintenance of hyperalgesic priming in male, but not female mice. In rats, the BDNF/TrkB signaling plays a critical role in the maintenance of hyperalgesic priming in both sexes. This latter finding is consistent with strong effects of this signaling pathway in the maintenance of priming in a rat model of migraine in males and females (Burgos-Vega et al., 2016).

Finally, we sought to understand the source of BDNF in hyperalgesic priming. A recently published paper using a nociceptor-specific knockout strategy demonstrated that BDNF from nociceptors is a strong contributor to hyperalgesic priming (Sikandar et al., 2018). On the other hand, many studies have now demonstrated that microglial BDNF is important for neuropathic pain (Coull et al., 2005; Trang et al., 2011), at least in males (Sorge et al., 2015). To clarify the role of microglial BDNF in hyperalgesic priming in male and female mice we obtained *Cx3cr1^{CreER}-Bdnf^{-/-}* and *Cx3cr1^{CreER}-Bdnf^{+/+}* mice and challenged them with IL6 into the paw and then assessed hyperalgesic priming with PGE₂ injections into the same paw. We found no effect of microglial deletion of BDNF either in the acute mechanical hypersensitivity in response to IL6 (Fig. 6A) or in response to PGE₂ (Fig. 6B). Given the previous findings of Sikandar et al. (Sikandar et al., 2018) and our pharmacology data published previously (Melemedjian et al., 2013) and here, we conclude that BDNF from nociceptors is likely the key source of BDNF for the generation of mechanical hypersensitivity in hyperalgesic priming paradigms.

4. Discussion

Hyperalgesic priming models are used to study the transition from acute to chronic pain (Reichling and Levine, 2009; Kandasamy and Price, 2015; Price and Inyang, 2015). We have previously used this

Rat

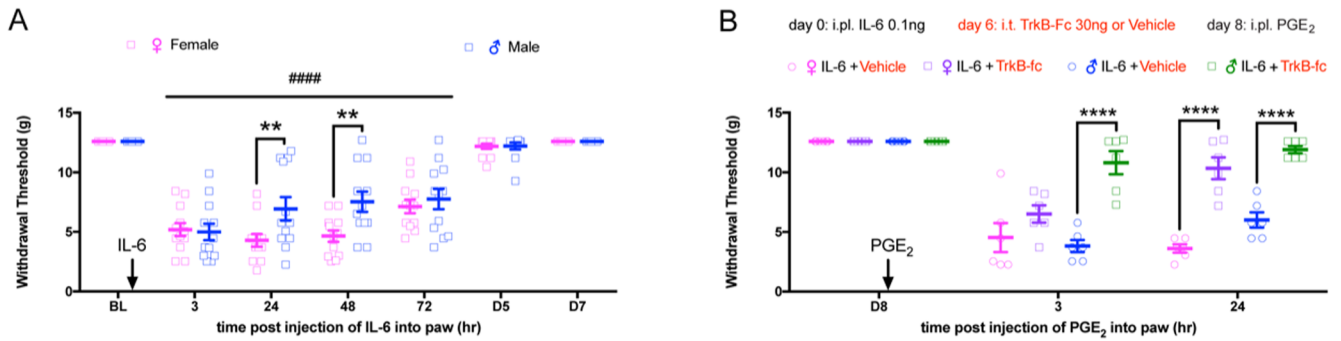


Fig. 5. BDNF/TrkB signaling plays a role in IL6-induced hyperalgesic priming in both male and female rats. Male and female rats received an i.p. injection of IL6 and were observed for mechanical hypersensitivity. A) While IL-6 (0.1 ng) induced equal amounts of mechanical hypersensitivity in both male and female rats compared to BL at 3 hr post injection, male rats recovered faster compared to females (*two-way ANOVA; from BL, F (6, 154) = 81.53, P < 0.0001; ##### p < 0.0001; male vs female rats, F (1, 154) = 8.633, P < 0.0038; ** p < 0.01*). B) After rats recovered, male and female rats received either a i.t. injection of TrkB-fc (30 ng) or Vehicle. Subsequently, PGE₂ was used to precipitate hyperalgesic priming. TrkB-fc was able to reverse IL6-induced priming in both male and female rats (*three-way ANOVA; Time × Sex × Treatment F (2, 60) = 6.429, P = 0.003; Bonferroni's **** p < 0.0001*).

model to identify sex differences in spinal dopamine signaling (Megat et al., 2018) in mice. We used this model system to gain insight into how BDNF/TrkB signaling may control this transition and whether or not there are sexual dimorphisms in these effects in mice or rats. The primary conclusion we reach from our experiments is that BDNF/TrkB signaling plays a critical role in hyperalgesic priming in male mice and rats but that its role is less clear in females, especially in mice. We observed little effect of interfering with BDNF/TrkB signaling on hyperalgesic priming in female mice, but a substantial inhibition of the maintenance of hyperalgesic priming was observed in female rats. This finding is striking similar to previous work from our group in a rat model of migraine pain where BDNF/TrkB is critically required for the maintenance of priming in both male and female rats (Burgos-Vega et al., 2016). We reach different conclusions than the work of Joseph et al., 2003 where sex differences in rats were observed in the hyperalgesic priming model that were dependent on PKCε activation using carrageenan as a priming stimulus. In that work, carrageenan failed to produce priming in female rats. However, Ferrari et al., 2016 demonstrate that if priming stimuli act on mechanisms other than PKCε in female rats (e.g. activation of ryanodine receptors) they induce robust hyperalgesic priming. This suggests that IL6 may act through second messengers downstream of PKCε bypassing the estrogen-dependent effect originally described by Joseph et al., 2003. Whatever the mechanism, our hyperalgesic priming work with male and female mice

and rats clearly shows that IL6 induces priming in both species, in both sexes. While it is not known which species, mice or rats, better resemble humans in terms of spinal BDNF/TrkB signaling, if the rat phenotype is conserved in other mammals, sequestering spinal BDNF may be an appropriate therapeutic approach for treatment of chronic pain in male and female chronic pain patients.

We observed some differences in the magnitude of effect of BDNF/TrkB signaling in hyperalgesic priming depending on the priming stimulus. With IL6 as the priming stimulus there was a clear dependence on BDNF/TrkB signaling for initiation and maintenance of priming in male but not female mice. In rats the maintenance of priming was dependent on BDNF/TrkB signaling in both males and females. We did not test at earlier time points in rats because previous work in the migraine model showed that BDNF/TrkB signaling plays a key role in initiation in males and females (Burgos-Vega et al., 2016). With incision as the priming stimulus we found that initiation of priming was BDNF/TrkB dependent in male mice but that the maintenance effect was only partial with TrkB-fc and there was no effect of ANA-12. Due to this, we decided to focus on IL6 as the priming stimulus for our sex differences experiments. A possible explanation for the differences in the effects of BDNF/TrkB signaling on hyperalgesic priming is that the initiating pain state is substantially longer lasting in the incisional model than the IL6 model. The duration of ongoing afferent discharge from the incisional injury may engage different and/or multiple central mechanisms that

Mouse

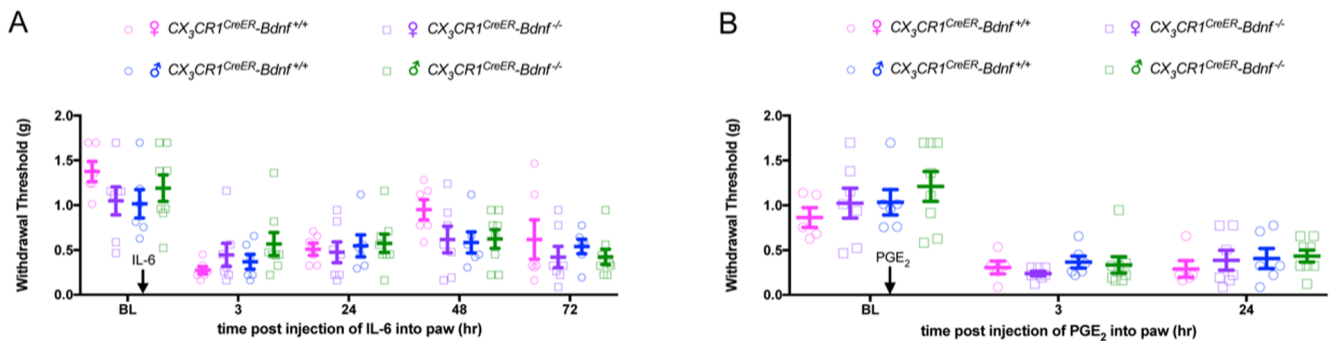


Fig. 6. Male and female mice with BDNF eliminated from microglia show no differences in IL6-induced mechanical hypersensitivity and hyperalgesic priming. A) Tamoxifen-injected male and female *Cx3cr1^{Cre} × BDNF^{-/-}* and *Cx3cr1^{Cre} × BDNF^{+/+}* mice received an i.p. injection of IL6 and showed no differences in mechanical hypersensitivity (*two-way ANOVA; F(3, 115) = 1.339, P = 0.2653*) or PGE₂ (100 ng) hyperalgesic priming (*B; two-way ANOVA; F (3, 66) = 1.209, P = 0.3135*).

are critical for maintaining the primed state after that type of injury and these may be completely independent of BDNF/TrkB signaling. Clearly, this is an important consideration for therapeutic relevance, particularly with respect to chronic post-surgical pain, which can have many different trajectories (Werner and Kongsgaard, 2014; Houle et al., 2017; Booth et al., 2018).

BDNF from microglia is a widely recognized mechanistic driver of neuropathic pain (Coull et al., 2005; Trang et al., 2011). A key downstream target for BDNF is downregulation of the potassium/chloride cotransporter KCC2 that regulates GABAergic inhibition in the dorsal horn (Coull et al., 2005; Trang et al., 2011). Microglial BDNF is controlled by p38 mitogen activated protein kinase signaling downstream of the P2X4 receptor. Somewhat surprisingly, this signaling mechanism is critical for neuropathic pain in male mice and rats but is seemingly not involved in female mice and rats (Sorge et al., 2015; Taves et al., 2016; Mapplebeck et al., 2018). While BDNF from microglia has received much attention in the neuropathic pain literature, BDNF was originally recognized as a pain signaling molecule because it is synthesized by DRG neurons and released at central synapses where it controls forms of synaptic plasticity like long-term potentiation (Kerr et al., 1999; Mannion et al., 1999). BDNF expression in the DRG is induced by peripheral inflammation (Mannion et al., 1999; Moy et al., 2017; Moy et al., 2018a; Moy et al., 2018b) and inflammatory pain is strongly reduced in mice lacking BDNF expression in nociceptors (Zhao et al., 2006). Some forms of neuropathic pain are also reduced in mice lacking BDNF in all sensory neurons and hyperalgesic priming induced by carrageenan is dramatically reduced in these mice (Sikandar et al., 2018). Because BDNF was knocked out in sensory neurons prior to priming in those experiments, the findings of Sikander et al (Sikandar et al., 2018) are consistent with our work wherein BDNF is required to establish hyperalgesic priming in male mice. Unfortunately, the aforementioned paper did not specify the sex of mice used for the hyperalgesic priming model with sensory neuron BDNF knockout (Sikandar et al., 2018). Here, we specifically eliminated BDNF from microglia prior to the priming stimulus in male and female mice using the IL6 priming model. We observed no effect of microglial BDNF knockout in this paradigm. Collectively, our new data and the published work cited above points to nociceptive sensory neuron derived BDNF in the initiation, and likely maintenance, of hyperalgesic priming in male mice and male and female rats.

Sex differences in chronic pain mechanisms is emerging as an important area of research (Rosen et al., 2017) with profound implications for pain therapeutics and clinical trial design (Price et al., 2018). A major area where significant differences have been found is in the spinal cord response to nerve injury. Both astrocytes (Gutierrez et al., 2013) and microglial mechanisms (Sorge et al., 2015; Taves et al., 2016; Mapplebeck et al., 2018) differ between male and female rodents in response to nerve injury. We recently demonstrated that microglial inhibitors that show sexual dimorphisms in neuropathic pain models (p38 inhibitors and P2X4 blockers) also show sexual dimorphisms in male and female mice in hyperalgesic priming (Paige et al., 2018). A recent transcriptomic profiling of male and female mouse microglia found major differences that are determined at birth and independent of adult sex hormone fluctuations. These investigators discovered that male microglia have a more inflammatory phenotype even at baseline in the mouse brain and that this is at least partially responsible for sex differences in response to stroke (Villa et al., 2018). While the state of the science on sex differences in chronic pain mechanisms is far from settled, our work suggests that some of these sex differences observed in mice may not be represented in rats. We think that this highlights the desperate need for greater insight into molecular changes in the nervous system of humans with neuropathic or other forms of chronic pain. For sex differences that are observed in rodent models to ultimately have an impact on clinical translation, we must increase efforts to gain insight into these critical questions in patient populations (Price et al., 2018).

Disclosures

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Author contributions

J.K.M., M.D.B., G.D. and T.J.P. conceived of the project. J.K.M., M.D.B., G.D. and T.J.P. designed experiments. J.K.M. T.S., D.V.T., S.M., G.P., M.K., M.N.A. and M.B.D. performed behavioral experiments. J.K.M. and T.J.P. analyzed data. J.K.M. and T.J.P. wrote the manuscript.

Data and materials availability

Raw data is available for download as a Prism worksheet.

Appendix A. Supplementary data

Cx3cr1^{CreER} and *Bdnf^{flx/flx}* genotyping. Mice used in Fig. 6 were genotyped prior to behavior experiments. Positive bands for *Cx3cr1^{CreER}* are at 300bps (A), whereas WT bands are at ~600bps (B). Positive bands for *Bdnf^{flx/flx}* are at ~500bps (C). Bands in A and C were ran in parallel from the same animals exhibiting the *Cx3cr1^{CreER}-Bdnf^{+/+}* genotype

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ynpai.2018.10.001>.

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