Sex and Gender Differences in Pain and Inflammation: A Rapidly Maturing Field

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In 2001, an Institute of Medicine (IOM) report stated: “Sex matters. Sex, that is, being male or female, is an important basic human variable that should be considered when designing and analyzing studies in all areas and at all levels of biomedical and health-related research... The study of sex differences is evolving into a mature science. There is now sufficient knowledge ... to allow the generation of hypotheses. The next step is to move from the descriptive to the experimental...”(21). The report also concluded that such an effort would require “synergy...between and among basic scientists, epidemiologists, social scientists, and clinical researchers.”

Similarly, a recent review of sex and gender differences in pain concluded that evidence about sex and gender differences in pain and its relief is accumulating rapidly (14). The review noted that epidemiological estimates of the prevalence of painful diseases and psychophysical studies continue to show that the burden of pain is greater, more varied, and more variable for women than for men. Furthermore, some painful disorders evidence themselves differently in women and men, and the efficacy of some therapies—whether drug, somatic or situational—are greater in one sex than the other. Although in some circumstances these differences may seem to be small or the findings conflicting, the mechanisms by which females and males arrive at a similar outcome appear to be different. Underlying these differences are powerfully interactive genetic, physiological, anatomical, neural, hormonal, psychological, lifestyle and sociocultural factors that change across each individual’s lifespan (Figure). Obviously, therefore, a multidisciplinary approach such as that called for by the IOM continues to be necessary to improve understanding.
The fourteen papers accepted so far for publication that were attracted by the present “Call for Papers” show that this obligation is being avidly pursued in the fields of pain and inflammation. Thirteen papers are published in this issue; another one was published last year (13); several more are in the review process. The studies accepted for the “Call” so far run the gamut from research with humans involving brain imaging (1, 17), psychophysics (8), methodology (18), and a study focused on men (19) to research with animals involving actions of analgesics such as morphine (5, 16, 20), non-steroidal anti-inflammatory agents (9) and acid-sensing ion channels (6), actions of hormones (11, 12), cannabinoid molecular biology of the entire brain (3), and psycho-social issues (13).

Studies with humans

Brain imaging (fMRI). Two studies on healthy men and women, assessed brain activations during equally painful thermal stimulation of the foot (17) or during uncomfortable or mild painful rectal distention (1). Both studies observed activation of some common areas in both men and women, but Berman et al. (1) also found additional areas to be activated only in men. Importantly, however, both studies report a new observation: that females show a greater proportion of areas that are deactivated during the experience of pain. The authors offer several explanations for this difference: that the deactivations indicate an enhanced ability by females to “dampen arousal networks during lower levels of discomfort” (1), and/or that the deactivations reflect
physiological differences in “baseline cerebral blood flow” (17), which are higher in women, thereby making it more likely that deactivations would be observed.

**Psychophysics.** Chang et al. (8) examined rectal discomfort thresholds to distention of the rectum and sigmoid colon before and after repeated noxious sigmoid stimulation in healthy men and women compared to those suffering from irritable bowel syndrome (IBS). Several important differences, not previously observed, emerged: Rectal discomfort thresholds were higher in healthy women than in healthy men. Whereas for men the thresholds did not differ between healthy and IBS subjects, for women, they were lower in women with IBS. Interestingly, in women, both healthy and with IBS, but not in any of the men, repetitive sigmoid stimulation induced visceral sensitization (i.e., rectal thresholds were reduced). These findings may reflect that sex differences in mechanisms of central sensitization, are of relevance to the greater vulnerability of women for developing co-existing painful conditions (reviewed in 14), and have implications for colonoscopy.

**Menstrual cycle variations.** One of the biggest issues in pain research concerns potential variations in pain perception associated with the menstrual cycle and how to deal with the issue experimentally. Sherman and LeResche addressed this issue by carrying out a systematic review of 14 papers that fulfilled strict methodological criteria (18). Their clearly-illustrated analysis shows that cyclical effects are inconsistently observed. They state that this situation could reflect the fact that cyclical differences are small, but argue convincingly that any conclusion is unwarranted until methodological problems are addressed: such as inconsistencies in cycle nomenclature, stimulus...
procedures, outcome measures, and hormonal assessment. As noted by these authors, these methodological issues are discussed in a recently published article (2).

**Estradiol in men: effects on systemic inflammatory responses to exercise.** The finding that exercise-induced muscle inflammatory response is smaller in women than men suggests that estradiol may exert an anti-inflammatory effect. Accordingly, Timmons et al. (19) examined the effect of eight days of supplementary estradiol treatment in men (which produced plasma levels of estradiol similar to the luteal phase in women and reduced testosterone levels). Surprisingly, although exercise increased levels of cortisol, IL6 and neutrophil counts, estradiol supplementation had no effect on these increases. These negative findings (as important to report as positive ones) need further investigation into possible sex differences in mechanisms. Indeed, the relationship between inflammatory cytokines and activity are complex and cytokine-specific (22). Future studies should also involve direct assessment of inflammatory mediators within the muscles themselves. Such studies would have considerable basic and clinical implications since inflammatory cytokines influence protein synthesis in muscle precursor cells, and are associated with muscle weakness and wasting (4).

**Studies with experimental non-human animals**

**Actions of analgesics-morphine:** Morphine is considered the “gold standard” treatment for pain. In most animal studies to date, the effects of morphine appear more potent in males than in females, but the situation for women and men is not as clear. Furthermore, the mechanisms underlying these differences are complex. It is therefore
encouraging that three papers in this issue present information that advances our understanding of this topic in rodent models.

Wang et al. (20) extended studies of sex differences in morphine’s effects on acute nociception (e.g., tail flick; hot plate assays) to its effects on persistent nociception (up to 21 days) induced by injection of an inflammatory agent (complete Freund’s adjuvant, CFA) into the hindpaw. As in previous studies, morphine’s anti-nociceptive and anti-hyperalgesic effects were greater in males than in females. Of additional interest was their observation, reported also by others (10) that the inflamed rats ceased cycling. The important issue of the influence of painful inflammatory conditions on reproductive health is poorly understood, and this observation strongly encourages such research.

J Ji et al. (16) extended studies of sex differences in morphine’s effects on nociception induced by somatic stimulation to that induced by visceral stimulation, in this case by colorectal distention, using the visceromotor response as an outcome measure. Again, as in other studies, morphine was more potent in males than females. Importantly, in addition, by using a peripherally-acting morphine agonist and comparing its effects with morphine delivered intrathecally (spinal cord) or intracerebroventricularly (supraspinal), it was observed that the greater male potency was mediated both peripherally and supraspinally, but not spinally.

Bryant et al. (5) extended studies of sex differences in morphine’s effects to sex differences in morphine tolerance. Using mice, this was done by assessing the effects of co-administration of NMDA receptor antagonists and morphine on the hot plate and tail withdrawal assays. They found that despite the fact that NMDA antagonism prolongs
the effects of the acute delivery of morphine equally in males and cycling or ovariectomized females, it produces a male-specific attenuation of morphine tolerance in the hot plate assay and a male-specific facilitation of tolerance in the tail withdrawal assay. These results suggest that the sensitivity to modifications of the NMDA system’s involvement in morphine tolerance is greater in males than females. Such findings warrant further investigation and have implications for development of morphine tolerance in the clinic.

**Actions of analgesics-non-steroidal anti-inflammatory drugs (NSAIDs):** Because the analgesic effects NSAIDs are likely to occur by inhibition of the cyclooxygenase enzymes Cox-1 and Cox-2, Chillingworth et al. (9) used knockout mice lacking these enzymes to investigate their relative contribution to the development of arthritis and inflammatory nociception (induced by intradermal delivery of CFA around the left tibiotarsal joint). To do this, they measured the CFA-induced edema, joint destruction, thermal hyperalgesia and mechanical allodynia. Although Cox-2 disruption eliminated the development of CFA-induced thermal hyperalgesia and mechanical allodynia in both males and females, it reduced edema and joint destruction only in females. Furthermore, while Cox-1 disruption had little influence on ipsilateral thermal hyperalgesia and mechanical hyperalgesia in either sex, it reduced contralateral allodynia as well as edema and joint destruction only in females. These results point to the interacting roles of the two enzymes in both “houskeeping” and “pathological” processes associated with inflammatory events and suggest that the mechanisms by which cyclooxygenase inhibitors influence pain and inflammation differ in males and females in ways that need further exploration.
Actions of analgesics-acid-sensing ion channels (ASICs): Like NSAIDs, ASIC ion channels represent another non-opioid and newly-recognized system involved in nociceptive processing. Using genetically modified mice bearing a dominant-negative mutation of the ASIC3 gene which renders all ASIC-related channel subunits insensitive, Chanda et al. (7) recently reported that only mutant male mice had heightened nociceptive sensitivity relative to wild type mice. Accordingly, Chanda and Mogil assessed the analgesic effect of amiloride, (a non-specific ASIC blocker) on nociception induced by the formalin test in male and female outbred mice, to which females show greater sensitivity (6). Somewhat at odds with the sex differences in the genetic effects, they found that amiloride produced anti-nociception in the formalin test only in females. They also found that gonadectomy switched the sex differences in amiloride efficacy and were switched back by chronic estradiol or testosterone replacement. These genetic and pharmacological sex differences open up an entire new avenue of research on sex differences in non-opioid systems involved in nociception.

Actions of hormones: In addition to the hormonal manipulations done in two of the studies discussed above (5, 6) two other studies in this issue address the topic of hormonal modification of nociception directly. Flake et al. (12) assessed the effects of hormonal manipulations (gonadectomy and estradiol or testosterone replacement) on Evans Blue dye plasma extravasation in the temporomandibular joint (TMJ) of naïve male and female rats as well as in rats whose TMJ had been inflamed by CFA. They found that plasma extravasation in the TMJ of rats was greater in both the naïve and the CFA-inflamed male rats than in the females. Furthermore, whereas the sex difference in
naïve rats reflected actions of testosterone, during TMJ inflammation, testosterone increased extravasation in males, but estradiol decreased in it females. Because plasma extravasation can attenuate joint damage, these results suggest that testosterone mitigates and estradiol exacerbates TMJ damage induced by inflammation. However, Chillingworth et al. (9) reported that there was no sex difference in the joint damage and edema produced by CFA in the tibiotarsal joint of wild type mice. Thus, both of these studies raise interesting new questions about sex differences in and hormonal influences on the protective effects of inflammation after joint injury, but the differences in their findings, one on rats (12) and the other on mice (9) need further exploration.

Evrard (11), discusses data collected mainly in quail, reviews new information indicating synthesis of estradiol in the spinal cord and provides data that support the existence of a rapid paracrine-like mechanism by which estradiol can provide a rapid and segment-specific regulation of nociceptive sensitivity. As discussed in the paper, such a situation may exist in other species and represents an entirely new mechanism in addition to the slower genomic effects of sex steroids that influence nociception differently in males and females.

**Brain function.** An entire new direction of research on mechanisms underlying sex differences in pain has now been opened by Bradshaw et al. (3). Using mass spectrometry, they measured estrous and sex-dependent variations in the levels of four different endocannabinoids (known for their involvement in nociception) in seven different regions of the brain of rats. In all areas except the cerebellum, they observed estrous variations mainly around the time of ovulation and behavioral estrus. Although
small sex differences were observed overall, when data from males was compared with
data from females in different parts of their cycle, clear sex differences were observed in
some brain areas such as the hypothalamus, striatum, midbrain, and hippocampus.
Such findings, like those of Ji et al. (16) and the two brain imaging studies (1, 17)
discussed above underscore the importance of considering the involvement many parts
of the central nervous system in sex and hormonal differences in pain. They also call
attention to the importance of considering the ovarian cycle when comparing males and
females.

**Psycho-social influences-social isolation and inflammation:** Hermes et al. (13),
like Chillingworth et al. (9) and Flake et al. (12) studied factors that mediate the
inflammatory response. In this case, however, the authors used the formation of a
granuloma in response to subcutaneous injection of carrageenin (seaweed) to assess
the influence of long-term social isolation on female and male Sprague-Dawley rats
(housed in groups or isolation). Social isolation delayed the formation of the granuloma
in both sexes. It also reduced the amount of exudate more in the males than the
females, which may relate to the fact that the females in the isolated group had shorter
estrous cycles and therefore more frequent exposure to rising estradiol levels. In
addition, there were sex differences in the response to an additional prior stressor (30
min of restraint) in the isolated rats. The inflammatory response was more robust in the
females than in the males. These results are important for at least two reasons. First,
they demonstrate the possibility of modeling social conditions, specifically long-term
isolation, in experimental animals. Second, they suggest that sex differences in the
effect of social isolation on the mobilization of protective (wound healing) inflammatory
processes may help improve our understanding of the clinical observation that socially-isolated men are more vulnerable to death and disease than women (15).

Conclusions

This series of elegant and informative papers illustrates the enthusiasm, vigor and wide scope of current research on sex and gender differences in pain and inflammation. The studies amply demonstrate that sex differences are fundamental, complex and long lasting. For example, intriguing sex differences in brain activation patterns associated with the experience of pain and in rectal discomfort thresholds are shown in human subjects. Of equal importance are the findings in women that pain perception is inconsistently related to the menstrual cycle, and that estradiol does not affect exercise-induced effects on markers of inflammation in men. Experiments with infrahuman subjects underscore the importance of sex differences in morphine’s effects and reveal multiple levels of action. Studies also reveal sex-specific effects of NSAIDs and ASICs, which play important roles in nociceptive processing, and highlight the importance of hormonal modulation of nociception. Differential and long-term effects of social isolation on inflammation represent another fascinating area of research.

Overall, therefore, the studies presented here support previous research that there are basic sex differences in pain and inflammation with considerable implications for the clinic. Thus, when asked the question, "Does pain and inflammation differ between males and females?" the answer remains a clear yes. The importance of the papers here is how they improve our understanding of what these differences are and
how they come about. The papers provide new hypotheses on fundamental neural, hormonal and developmental factors that mediate these differences, which reinforce the conclusion that no single answer can explain sex differences. Like all complex phenomena, it is important to remain aware that pain and inflammation always occur in a context of individual differences in present circumstances and past history.
REFERENCES  (*PLEASE NOTE: Olivia & Beverly*: The references shown in RED are the ones that will appear in the August issue. We do not know the proper format for referencing them, so please correct.)


3. **Bradshaw HB, Rimmerman N, Krey JF and Walker JM.** Sex and hormonal cycle differences in brain levels of pain-related cannabimimetic lipid mediators.


5. **Bryant CD, Eitan S, Sinchak K, Fanselow MS and Evans CJ.** NMDA receptor antagonism disrupts the development of morphine analgesic tolerance in male, but not female C57BL/6J mice: effect of ovariectomy.

6. **Chanda ML and Mogil JS.** Sex differences in the effects of amiloride on formalin test nociception in mice.


12. Flake NM, Hermanstyne TO and Gold MS. Testosterone and estrogen have opposing actions on inflammation-induced plasma extravasation in the rat temporomandibular joint.


Figure. Conceptualization of some dynamic, interacting factors that can influence pain across the lifespan of men and women differently. This diagram portrays a multifaceted approach towards a better understanding of pain perception in which predominanting factors evolve and change as events occur across an individual’s lifespan (modified and adapted from ref 14).