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Sex differences and hormonal modulation of deep tissue pain

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Abstract

Women disproportionately suffer from many deep tissue pain conditions. Experimental studies show that women have lower pain thresholds, higher pain ratings and less tolerance to a range of painful stimuli. Most clinical and epidemiological reports suggest female gonadal hormones modulate pain for some, but not all, conditions. Similarly, animal studies support greater nociceptive sensitivity in females in many deep tissue pain models. Gonadal hormones modulate responses in primary afferents, dorsal horn neurons and supraspinal sites, but the direction of modulation is variable. This review will examine sex differences in deep tissue pain in humans and animals focusing on the role of gonadal hormones (mainly estradiol) as an underlying component of the modulation of pain sensitivity.

Keywords

Pain; Human; Animal; Viscera; Muscle; Estrogen; Testosterone; Primary afferents; Dorsal horn neuron; Brain imaging

1. Introduction

Chronic pain affects more people than heart disease, diabetes and cancer combined (Institute of Medicine report: Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research (2011)) and visceral pain is the number one reason patients seek medical attention (International Association for the Study of Pain: Global Year Against Visceral Pain, http://www.iasp-pain.org/Content/NavigationMenu/GlobalYearAgainstPain/GlobalYearAgainstVisceralPain/PressRelease/default.htm). Furthermore, sex matters and it is generally accepted there is a sex difference in pain and analgesia (International Association for the Study of Pain: Global Year Against Pain in Women, http://www.iasp-pain.org/Content/NavigationMenu/GlobalYearAgainstPain/RealWomenRealPain/default.htm), although the magnitude of sex differences may be small and the direction can

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differ depending upon many factors including type of test, peripheral organ, and genetics (including species and strain in animals). In the patient population there are significantly more pain conditions/syndromes that are more prevalent in women than men (Berkley, 1997; Greenspan and Traub, 2013.). It is therefore imperative to understand what drives sex differences in deep tissue pain in order to better optimize treatment, perhaps on a sex directed basis. As such, how gonadal hormones modulate deep tissue pain is an important question.

Many excellent and informative reviews have been published on the general topic of sex differences in pain in the last 15 years (Aloisi, 2003; Berkley, 1997; Craft, 2007; Fillingim et al., 2009; Gintzler and Liu, 2012; Greenspan et al., 2007; Greenspan and Traub, 2013.; Heitkemper and Jarrett, 2008; Holdcroft and Berkley, 2006.; Hurley and Adams, 2008; Martin, 2009b; Mogil and Bailey, 2010; Racine et al., 2012a; Racine et al., 2012b; Riley et al., 1998; Unruh, 1996) and this review will not duplicate those efforts. In addition, we will not attempt to review deep tissue pain in general. Rather, the state of understanding of sex differences and the role of gonadal hormones in deep tissue pain conditions (visceral, somatic) will be examined although contrasts to superficial somatic pain will be addressed.

2. Human studies supporting a sex difference and hormonal modulation of deep tissue pain

Many chronic pain conditions involving deep tissue such as irritable bowel syndrome (IBS), fibromyalgia (FM), temporomandibular joint disorder (TMD), painful bladder syndrome/ interstitial cystitis (PBS/IC), and chronic fatigue syndrome (CFS) have greater female prevalence or symptom severity (see (Berkley, 1997; Fillingim et al., 2009; Greenspan and Traub, 2013.; Unruh, 1996) for review). For example, women with IBS had greater abdominal pain vs. males and lower rectal discomfort thresholds than healthy women or men with IBS (Chang et al., 2006; Tang et al., 2012). Likewise, pain in TMD patients was greater in women compared to men (Schmid-Schwap et al., 2013). These conditions involve different peripheral organs but symptoms may coexist in the same patient (Aaron et al., 2000; Chang et al., 2003a; Kurland et al., 2006; Tietjen et al., 2010; Veale et al., 1991). Although genetic predisposition and previous psychological or physical (e.g., inflammation) challenges may contribute to the occurrence of deep tissue pain, in many cases no obvious pathophysiological signs in the peripheral tissue/nerve could be detected at the time of medical evaluation, pointing to dysregulation of central nervous system function as a potential cause of excessive pain.

2.1. Sex differences in deep tissue pain

Temporal summation (the progressive increase in response to a constant, repetitive stimulus) measures increases in central processing of nociceptive stimuli. In a recent review of experimental thermal and mechanical pain studies involving healthy volunteers, more than half of the studies reported increased temporal summation in females compared with males (Racine et al., 2012b). Another review that included patient populations and healthy volunteers reported greater temporal summation in women (Fillingim et al., 2009). Greater temporal summation of pain was observed in female TMD, lower back pain and IBS patients compared with healthy volunteers or male patients (George et al., 2007; Sarlani et al., 2007; Zhou et al., 2011). These studies indicate that central sensitization is more readily evoked in women and is further augmented in women suffering from deep tissue pain.

In contrast to temporal summation measuring pain facilitation, central pain modulation (CPM, formerly called DNIC (diffuse noxious inhibitory controls)) examines the ability of the central nervous system to inhibit pain. CPM measures the ability of a noxious

conditioning stimulus to inhibit the response to a noxious test stimulus. Reduced CPM is associated with greater risk of chronic pain and comorbid pain conditions (see (Lewis et al., 2012; Staud, 2012; Yarnitsky, 2010) for review).

In healthy volunteers repetitive thermal stimulation evoked temporal summation. CPM inhibited the summation in healthy men, but had no effect in healthy women (Staud et al., 2003). In contrast, a different study reported temporal summation was not reduced by a conditioning stimulus in either sex, but CPM did reduce mean pain ratings and peak pain (Tousignant-Laflamme et al., 2008). In another study, distracting and painful conditioning stimuli significantly reduced heat pain intensity and unpleasantness ratings for both sexes, with significantly larger distraction effects on intensity ratings for men than women (Quiton and Greenspan, 2007). In a model of induced muscle pain two bilateral injections of hypertonic saline into the trapezius muscle induced greater pain ratings and lower pressure pain thresholds following the second injection in women compared to men. Correspondingly, the pressure pain threshold increased in men, but not women, in an area of referred pain (Ge et al., 2004; Ge et al., 2006). These data were interpreted to suggest men had greater inhibitory control mechanisms. In a review of 13 studies, approximately half showed less pain inhibition in women compared to men, the remaining studies reporting no sex difference (reviewed in (Fillingim et al., 2009)). These data suggest CPM may be less effective in women although how the experiments were conducted clearly affects the results.

Clinically, several studies have reported significantly less pain inhibition from CPM in women with fibromyalgia compared to healthy controls (Kosek and Hansson, 1997; Normand et al., 2011) although distraction improved the efficacy of CPM in this patient population (Staud et al., 2003). Female patients with IBS or TMD failed to show pain inhibition during CPM compared to healthy subjects and showed a different pattern of brain activation revealed by fMRI (Heymen et al., 2010; King et al., 2009; Wilder-Smith et al., 2004).

2.2. Hormonal regulation of deep tissue pain

A meta-analysis on studies of experimental pain reactivity in healthy women of reproductive age indicated women in the follicular phase had higher pain thresholds than later phases (Riley et al., 1999). A more recent review reported that increased reactivity to pain occurs during the perimenstrual phase or around ovulation (Martin, 2009a), suggesting lower levels of female gonadal hormones or rising /declining of these hormones is correlated to higher pain perception. In addition, greater CPM was observed in ovulatory phase than during the other times of the menstrual cycle (Rezaii et al., 2012; Tousignant-Laflamme and Marchand, 2009), suggesting pain perception and the intrinsic pain inhibitory system are both regulated by female gonadal hormones in healthy premenopausal women.

Several studies in chronic pain patients point to a negative correlation between female gonadal hormone levels and deep tissue pain severity. For example, a population-based questionnaire indicated IBS symptom severity increased after menopause and post-menopausal women had greater abdominal pain/discomfort symptoms compared to pre-menopausal women or men (Olafsdottir et al., 2012). However, another study reported abdominal pain severity decreased in women over age 50 (Palsson et al., 2003). In pre-menopausal women abdominal pain and rectal sensitivity increased during menses in IBS patients, but not healthy women (Houghton et al., 2002). A recent review concluded there is not enough data to make a firm conclusion regarding menopause and IBS symptomatology, although this same review concluded IBS symptoms were heightened around menses (Adeyemo et al., 2010). Estrogen withdrawal from oral contraceptive regimens contributed to pelvic pain and fluctuation in estrogen levels contributed to menstrual migraine (Bitzer, 2013; Mathew et al., 2013). Similarly, TMD pain ratings increased towards the end of the

menstrual cycle and peaked during menstruation when the body had the lowest estrogen and progesterone level. A second peak of pain rating occurred around the time of ovulation when there was a rapid surge in estrogen level. The latter was not seen in women using oral contraceptives (LeResche et al., 2003; Slade et al., 2011).

The modulation of fibromyalgia pain by gonadal hormones is less certain. Several studies report FM did not fluctuate during the menstrual cycle (Alonso et al., 2004; Okifuji and Turk, 2006; Samborski et al., 2005), although another study reported menstrual cycle effects in about 50% of patients (Pamuk and Cakir, 2005). In postmenopausal women with fibromyalgia, hormone replacement did not affect pain ratings (Stening et al., 2011). In general, there was an increase in deep tissue pain symptoms around the time of menses and early menopause, at times of declining or low ovarian hormones, suggesting that estrogen and progesterone withdrawal may contribute either directly or indirectly to pain hypersensitivity.

2.3. Brain imaging of deep tissue pain

Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) measures differences/changes in brain area activation at rest and in response to tasks. It is well acknowledged that the limbic system including cingulate cortex, amygdala, hippocampal formation and other limbic related brain regions such as the insula cortex (IC) and prefrontal cortex are involved in emotion, cognition, anticipation of tasks, attention responses and arousal status of the body. Brain imaging studies indicate the anterior cingulate cortex (ACC), IC, the medial prefrontal cortex (MPFC) and other brain areas are activated by noxious somatic and visceral stimuli (Benson et al., 2012; Craig, 2002; Hayes and Northoff, 2012; Petrovic and Ingvar, 2002; Rainville et al., 1997; Tolle et al., 1999; Vogt et al., 1996).

Sex differences in brain region activation in response to thermal and electrical stimuli have been reported by several groups, with women showing more activation in MPFC (Straube et al., 2009) (Paulson et al., 1998), IC, thalamus (Paulson et al., 1998) and ACC (Derbyshire et al., 2002), but greater deactivation in anterior insula and dorsolateral prefrontal cortex (Moulton et al., 2006). Reduced activity in limbic brain areas during intense pain may reflect transient interruption of regional basal brain activity (Raichle et al., 2001; Vogt et al., 1996). On the other hand, deactivation in the MPFC and ACC have been reported when anticipating painful stimuli (Hsieh et al., 1999; Simpson, Jr. et al., 2001), possibly reflecting a cognitive coping mechanism (Petrovic and Ingvar, 2002).

There is also a sex difference in brain region activation in healthy human subjects in response to deep tissue stimuli. High intensity rectal distention activated the insula and ACC in both men and women, but left thalamus and ventral striatum only in men. Lower intensity distention and expectation of distention activated the insula to a greater extent in men while women had greater deactivation of the amygdala and midcingulate cortex (Berman et al., 2006). Likewise, in women anticipation of esophageal distention produced greater inactivation in the right amygdala and left parahippocampal gyrus and greater activation in the cuneus, precuneus, and supplementary motor area (Kano et al., 2013). However, rectal pain threshold was negatively correlated with distension-induced activation in the ACC, insula, thalamus and somatosensory cortex II in women, but positively correlated with activation of the insula in men, though there was no sex difference in threshold or pain ratings to rectal distention in healthy volunteers. Interestingly, pain ratings were negatively correlated with insula activation in women, but positively correlated with ACC activation in men (Benson et al., 2012). In addition, sex differences in the regional activation of the μ-opioid system in response to persistent pain induced by injecting hypertonic saline into the masseter muscle were detected. Healthy men showed larger magnitude of μ-opioid system

activation in the anterior thalamus, ventral basal ganglia, and amygdala than women during the follicular phase (Zubieta et al., 2002). Taken together, these data suggest there is a sex difference in the brain areas involved in pain perception and modulation.

Patients with deep tissue pain show changes in regional brain activation. In the anterior insular cortex, clinical pain is preferentially processed more rostrally compared with acute experimental pain in healthy subjects (Schweinhardt et al., 2006). The shift in spatial representation under chronic pain conditions may underlie changes in the brain circuit involved in response to painful stimuli, partially explaining the affective/emotional changes in chronic pain patients. In female FM patients, greater activation of the middle ACC subregion has been demonstrated in response to somatic pressure when compared with their age-matched healthy controls (Chang et al., 2003b). In a male predominant study, patients with IBS had expanded activation of right prefrontal cortex, increased activation of rostral ACC and posterior cingulate cortices and reduced activation of brain stem and other brain areas in response to rectal distention (Naliboff et al., 2001). Similarly, female IBS patients showed increased activation of the middle ACC as compared with healthy controls in response to rectal distention (Chang et al., 2003b). Female IBS patients that were hypersensitive to colorectal distention also had greater activation of the insula and reduced deactivation in the pregenual ACC compared to normosensitive IBS patients and healthy controls (Larsson et al., 2012). In vulvodynia patients, noxious stimulation of the thumb evoked greater activation with the insula, dorsal midcingulate, posterior cingulate, and thalamus compared to healthy controls, suggesting central pathology in this disorder (Hampson et al., 2013).

A comparison between male and female IBS patients indicated there is a sex difference in brain areas activated by a visceral stimulus as well as anticipation of a visceral stimulus. Female patients showed greater activation in the ventromedial prefrontal cortex, right ACC and left amygdala in response to the visceral stimulus, whereas male patients showed greater activation of the right dorsolateral prefrontal cortex, insula, and dorsal pons/PAG, suggesting male and female patients with IBS differ in activation of brain areas involved in cognition, memory and emotional aspects of the pain experience, and the descending pain modulatory system (Berman et al., 2000; Labus et al., 2008; Naliboff et al., 2003).

Changes in functional connectivity of the ACC and other brain areas have also been observed under deep tissue pain conditions. Healthy females showed more periaqueductal grey (PAG) - mid-cingulate cortex connectivity while males showed more PAG connectivity with the left medial orbital prefrontal cortex, right insula and prefrontal cortex (Kong et al., 2010). In female FM patients the ACC and thalamus showed reduced connectivity to the amygdala, hippocampus, brainstem and orbitofrontal cortex compared with healthy controls (Jensen et al., 2012). In female TMD patients, increased connectivity between the left anterior insular cortex and pregenual ACC was observed at rest and during pressure pain stimuli. More importantly, the functional connectivity between IC-ACC was negatively correlated with pain rating in patients (Ichesco et al., 2012), suggesting this pain inhibitory circuit is more active under deep tissue pain conditions. Female TMD patients also showed a decrease in gray matter volume in the left ACC, in the right posterior cingulate gyrus, the right anterior insular cortex and other brain areas (Gerstner et al., 2011). Therefore, changes in ACC connectivity might reflect a gain/loss of ACC function, which might be dependent on the subregions of ACC investigated, the origin, time course, severity of deep tissue pain as well as menstrual phases and psychological status at the time of testing. Unfortunately, since many deep tissue pain syndromes are more prevalent in women, most of the above mentioned brain imaging studies only included female subjects and therefore sex difference in brain region activation data are unavailable.

2.4. Summary

Brain imaging studies of experimental and clinical deep tissue pain in humans reveal complex brain networks are involved in sensory, cognitive, emotional aspects of pain. Overall, greater ACC activation in females and insular activation in males have been observed under several chronic pain conditions. Increased ACC-IC connectivity under these conditions may reflect an effort of the body to recruit pain inhibitory mechanisms to suppress pain. Sexually dimorphic brain area activation could contribute to sex difference in pain response, and thereby lay the basis for the development of sexually dimorphic treatment under different conditions. Nevertheless, detailed information about sex differences in neuroanatomical/neurochemical organization in the peripheral and central nervous system is not readily available from these studies, demanding the use of animal models for deep tissue pain research.

3. Animal studies supporting a sex difference and hormonal modulation of deep tissue pain

Clinical and experimental human studies suggest greater pain in women and there is evidence that gonadal hormones contribute to fluctuations in nociceptive processing, especially deep tissue pain in women. A sex difference in processing of nociceptive stimuli and gonadal hormone modulation of deep tissue pain is supported by animal studies with two caveats. First, the direction of the sex difference, are males or females more sensitive, is not straightforward. The direction of sensitivity depends on many factors including, but not limited to test stimuli and measured endpoint, acute or persistent/chronic pain model, hormonal status, species and strain (genetic background). For example, more or less female sensitivity compared to males was reported for different strains of rats and mice in nociceptive and antinociceptive tests (Kest et al., 1999; Mogil et al., 2000). Second, while there is strong evidence that gonadal hormones modulate nociception, the direction and magnitude of effect is controversial.

In several deep tissue pain models, females showed greater sensitivity to noxious stimuli compared to males and gonadal hormones likely contribute to the sex difference. In many of these cases nociceptive sensitivity was greater when estradiol (E2) levels were higher compared to lower (e.g., proestrus vs. met/diestrus, ovariectomized plus E2 replacement (Ovx+E2) vs. ovariectomized). In other cases however, sensitivity was greater when the relative levels of E2 were lower. How then can E2 contribute to sex differences in pain? There are several possible explanations. In some models focusing on lower abdominal/pelvic pain, low plasma E2 levels are associated with increased nociceptive sensitivity which may result from effects of E2 on peripheral tissue. A reduction in the plasma estrogen concentration following ovariectomy or menopause could result in vaginal atrophy increasing pelvic pain and estrogen replacement could alleviate the pain (Pessina et al., 2006; Ting et al., 2004). Alternatively, it may not be the absolute level of E2 that is important, but changes in concentration (i.e., fluctuating levels during the estrous or menstrual cycle). Estrogen or progesterone withdrawal may be more significant in modulating nociceptive sensitivity than maintaining a high level of either hormone (Devall and Lovick, 2010; Heitkemper and Chang, 2009; Ji et al., 2003; Martin et al., 2007; Martin, 2008; Puri et al., 2011; Robbins et al., 2010). Finally, the site where hormones produce their effect, peripheral tissue or the central nervous system (CNS), likely influences any conclusions. E2 has anti-inflammatory effects in the periphery that could lead to anti-nociceptive effect, offsetting pro-nociceptive effects on CNS functioning.

3.1. Sex differences and gonadal hormone modulation of visceral pain (Table 1)

Hollow organ (e.g., colon, bladder, uterus) distention is the prototypical model of visceral pain. A balloon is placed inside the organ (or the organ forms the balloon, e.g., bladder) and distended to different pressures allowing different types of measurements. Hollow organ distention causes pseudoaffective reflex contraction of the abdominal muscles that can be quantified by recording the electromyogram in awake or lightly anesthetized animals (the visceromotor response). Less often distention-evoked elevation of the hindquarters, pressor responses or intraabdominal/intracolonic pressure is measured, or escape responses/learned behaviors quantified. Neuronal responses can be recorded from primary afferents, dorsal horn neurons and supraspinal neurons. The extent of activation can be quantified by immunocytochemical labeling for cellular markers (e.g., c-Fos, pERK, pCREB). Animal imaging techniques have advanced such that PET and fMRI can be used to study brain networks that process nociceptive information. This has facilitated the study of sex differences and the role of gonadal hormones in deep tissue pain.

3.1.1. Sex differences in visceral pain—Our lab studies sex differences in visceral pain using the colorectal distention model. We and others have reported sex differences in the visceromotor response to colorectal distention (Figure 1) with the majority of studies indicating females have greater sensitivity (greater magnitude visceromotor response) than males in both rats and mice (Holdcroft et al., 2000; Ji et al., 2006; Ji et al., 2012b; Kamp et al., 2003). Similar results were reported using alternate responses (hindquarter elevation) to colorectal distention (Bourdu et al., 2005). Different conclusions were reported in studies using different measures. Male mice had greater visceral sensitivity compared to females, but the EMG was only half the duration of the distending stimulus making interpretation difficult (Larsson et al., 2003). Two studies reported no sex difference (Larauche et al., 2012a; Wang et al., 2009), but the Larauche study reported intracolonic pressure, not the visceromotor response. Sex differences in bladder pain were similar in direction to the majority of the colorectal pain studies. The magnitude of the visceromotor response to urinary bladder distention was greater in female rats compared to males (Ball et al., 2010; Ness et al., 2001).

3.1.2. Pronociceptive effects of E2 on visceral pain—Studies in female rats suggest estrogen likely contributes to the sex difference in deep tissue pain. The magnitude of the visceromotor response to colorectal distention fluctuates with the estrous cycle, greatest in proestrus and least in met/diestrus (Gustafsson and Greenwood-Van Meerveld, 2011; Holdcroft et al., 2000; Ji et al., 2008; Sapsed-Byrne et al., 1996). This pattern reflects fluctuations in estradiol (E2) and progesterone plasma concentrations during the estrous cycle, suggesting the level of gonadal hormones or changing hormone concentrations are important in modulating the visceromotor response. Hormone depletion by ovariectomy decreased the magnitude of the visceromotor response to colorectal distention and systemic replacement by bolus injection of E2 four to 48 hours earlier restored the response (Figure 1). The plasma E2 concentration had peaked and was declining 48 hrs following administration supporting the idea that changing levels of E2 are important (Ji et al., 2003). Similarly, the visceromotor response to bladder distention was decreased by ovariectomy and restored by E2 replacement (Robbins et al., 2010). Importantly, Robbins and colleagues demonstrated that E2 replacement in ovariectomized rats by bolus injection with testing 24 hours later increased the visceromotor response to bladder distention, but chronic E2 replacement produced by pellets implanted a week earlier did not increase the visceromotor response. When the pellets were removed, the visceromotor response increased 24 hours later, suggesting E2 withdrawal likely increased visceral sensitivity (Robbins et al., 2010). Further studies showed that E2, acting at the level of the spinal cord could account for the effects of systemic E2. Spinal administration of the estrogen receptor antagonist ICI-182,780

attenuated the visceromotor response to colorectal distention in intact female rats and spinal administration of E2 to ovariectomized rats facilitated the visceromotor response similar to systemic E2 (Ji et al., 2011; Ji et al., 2003).

In a model of mechanical stimulation of the uterine-cervix in rats to emulate labor, ovariectomy decreased the visceromotor response which was reversed by E2 replacement and the response of the innervating hypogastric nerve afferents increased in OVx+E2 rats via activation of TRPV1 (Liu et al., 2005; Yan et al., 2007). Correspondingly, uterine-cervix afferent activity increased as estrogen levels increased during pregnancy (Liu et al., 2008).

NMDA receptor activity could partially account for the sex difference induced by estrogen in deep tissue pain. Spinal administration of the NDMA receptor antagonist APV dose-dependently attenuated the visceromotor response to colorectal distention with greater effect in males. This corresponded with greater cell membrane expression of the GluN1 subunit of the NMDA receptor in the dorsal horn in females, suggesting females had more functional NMDA receptors (Ji et al., 2012b). This was likely due to E2 modulation of NMDA receptor activity. Ovariectomy increased the potency of APV in modulating the visceromotor response to colorectal distention and E2 increased GluN1 and GluN2B expression and PKA-mediated GluN1 phosphorylation in the spinal cord (Ji et al., 2012a; Tang et al., 2008). These data suggest a role for E2 modulation of NMDA receptors in sex differences in visceral pain. This is supported by studies of cross organ sensitization using the pelvic nerve to urethra reflex. Stimulation of the pelvic nerve (either electrically or via colonic inflammation) increased urethra activity. The reflex was more robust in rats in proestrus compared to metestrus and was decreased by ovariectomy (Peng et al., 2010; Peng et al., 2008). The reflex was blocked by APV with greater effect in ovariectomized rats (Lin et al., 2006).

In contrast to the pronociceptive effect of estrogen in these distention models progesterone replacement had no effect on increasing the visceromotor response following ovariectomy, but dampened the effect of E2 when coadministered (Ji et al., 2005). However, the progesterone metabolite 3 alpha-hydroxy- 5 alpha- pregnan-20-one was antinociceptive to duodenal distention (Winfree et al., 1992).

3.1.2.1. Visceral inflammation: Hollow organ distention (e.g., colorectal distention) mimics natural stimuli, such as obstruction without inflammation or a tumor that stretches the organ wall evoking pain. A sensitizing agent increases visceral pain, generally by inducing inflammation, but how does this affect sex differences and hormonal modulation? There are several models that induce colonic or bladder hypersensitivity lasting from hours to weeks in the absence or presence of inflammation. Female hypersensitivity to colorectal distention was reported following mustard oil irritation of the colon and the magnitude of the increase was greater in females compared to males (Ji et al., 2012b; Lu et al., 2007). Similarly, hypersensitivity that persisted several weeks in the absence of colonic inflammation was greater in female rats (Bourdu et al., 2005). Similar to noninflamed animals, estrogen modulated the magnitude of response in females. Ovariectomy reduced the visceromotor response to intracolonic mustard oil which was restored by E2 replacement (Ji et al., 2005; Lu et al., 2007). Intracolonic 5HTP, a serotonin precursor, induced colonic hypersensitivity without noticeable inflammation that was attenuated by ovariectomy and facilitated by E2 (Lu et al., 2009). OVx+E2 had a similar effect on colorectal pain in an inflammatory colitis model (Fan et al., 2009). In a model of bladder inflammation females developed behavioral responses faster than males, but the peak response was the same (Bon et al., 1997). In another model of bladder inflammation the visceromotor response to bladder distention increased during proestrus, when E2 was high, compared to diestrus, when E2

was low (Ball et al., 2010). These data suggest that visceral inflammation increases sensitivity, but does not alter the modulation by gonadal hormones.

3.1.3. Antinociceptive effects of E2 on visceral pain—In contrast to the pronociceptive effects of estrogen providing a possible explanation for sex differences to colon or bladder distention, estrogen appears antinociceptive in a model of ureteral stones. Although female rats spent a greater percentage of time in crises following ureteral stone implant compared to males (Affaitati et al., 2011), this was negatively correlated with estrogen levels. When E2 was high or decreasing during proestrus/estrus, there were significantly fewer ureteral crises than when E2 was low during met/di-estrus (Giamberardino et al., 1997). However, estrogen receptor or androgen receptor antagonists decreased the number of crises in females, with no effect in males (Affaitati et al., 2011). These paradoxical results might be due to the antagonists acting at subtypes of receptors for estrogens and androgens. This could unmask activity of other receptors. For example, ICI-182,780 antagonizes ERα and ERβ, but is an agonist at the G-protein coupled receptor, GPER (Prossnitz et al., 2008).

Increasing E2 to supraphysiological levels in intact female rats in the ureteral stone model decreased crises in females, but not males, while testosterone had no effect (Aloisi et al., 2010). Since these experiments were conducted in intact rats, the possibility of interactions between multiple hormones and receptors may have contributed to the fluctuations in the level of nociception/antinociception. Indeed, elevated levels of E2 and progesterone observed during pregnancy or pseudopregnancy are analgesic (Dawson-Basoa and Gintzler, 1996; Gintzler and Bohan, 1990). In addition, experimental conditions may have contributed to the observed outcome; ureteral crises were observed following stone implantation which required surgery and there was likely uncontrolled postoperative pain.

The role of estrogen on nociceptive sensitivity during hollow organ distention of female reproductive organs differs from bladder and colon. The percentage of escape responses to uterine distention with a balloon was greater during periods of lower circulating estradiol: metand diestrus compared to proestrus (Bradshaw et al., 1999). Correspondingly, ovariectomy increased escape responses to vaginal distention which was reversed by estrogen replacement (Bradshaw and Berkley, 2002). This hyperalgesia was likely peripherally mediated, resulting from an ovariectomy-induced decrease in vaginal wall thickness and/or increase in innervation density contributing to increased vaginal hypersensitivity and referred hyperalgesia (Berkley et al., 2007; Pessina et al., 2006; Ting et al., 2004). However, the distention threshold of primary afferents in the hypogastric nerve innervating the uterus and pelvic nerve innervating the vagina was lowest in proestrus (Robbins et al., 1992). Part of these contradictory results might be related to reproductive function vs. nociceptive sensitivity as afferent activity is not necessarily related to escape behaviors evoked by noxious stimuli.

Endometriosis is a condition in which uterine endometrium grows outside the uterine cavity. Clinically, this often results in pain. Endometriosis is modeled in rats by autotransplanting pieces of uterine horn to the abdominal mesentery. Vaginal hyperalgesia in endometriosis is estrogen modulated; greatest when E2 levels were highest during proestrus and least during estrus (Cason et al., 2003). Since the endometrial implants become innervated by sympathetic and sensory fibers from the thoracolumbar spinal cord and dorsal root ganglion (DRG) it is likely the vaginal hyperalgesia was centrally mediated (Berkley et al., 2004; McAllister et al., 2012). In contrast, in the absence of endometriosis, ovariectomy induced vaginal hyperalgesia which was reversed by estrogen replacement. However, ovariectomy had no effect on rats with endometrial hyperalgesia, but the hyperalgesia was attenuated by

E2, suggesting peripheral antinociceptive effects and central pronociceptive effects partially offset (Berkley et al., 2007).

In mice, long term ovariectomy increased mechanosensitivity in the area of referred pain from pelvic organs and visceral pain behavior was increased following administration of capsaicin into the colon. There was a corresponding increase in pERK in the spinal cord. Treatment that decreased pERK decreased hypersensitivity and vice versa. Furthermore, the referred pain only occurred at the level of the lumbosacral spinal cord (Klinger et al., 2011; Sanoja and Cervero, 2005; Sanoja and Cervero, 2008), suggesting the ovariectomy-induced hypersensitivity was peripherally mediated, perhaps a consequence of vaginal atrophy. Alternatively, the long duration loss of E2 may have led to osteoporosis-like symptoms. Similarly, rats ovariectomized for 4 weeks had greater mechanical and thermal sensitivity of the hindpaw and tail compared to ovariectomized rats with chronic E2 replacement (Sarajari and Oblinger, 2010). However, there was no change in the magnitude of the visceromotor response to colorectal distention in rats measured weekly for 8 weeks following ovariectomy (Traub et al., 2013).

3.1.4. Summary—The literature focusing on sex differences and hormonal modulation of acute and inflammatory visceral pain is complex. Overall, the majority of studies suggest E2 is pronociceptive and we contend it could partially account for the sex difference in visceral sensitivity. The majority of sex difference studies listed in table 1 (not all studies from the same lab drawing the same conclusion are listed) support greater nociceptive sensitivity in females. The remaining studies reported no sex difference or greater sensitivity in males. However, these latter studies measured different endpoints. The studies focusing on hormones indicate elevated levels of circulating E2 or withdrawal from the elevated levels likely contributed to the greater female visceral sensitivity; approximately 80% of the studies concluded that nociceptive responses were greater in cycling rats in proestrus compared to other cycle phases or that ovariectomy decreased responses and E2 replacement restored the response. In these cases, nociceptive sensitivity was determined hours to days following E2 replacement, when E2 levels were declining from peak values. Although several studies concluded E2 is antinociceptive these studies might have had a confounding peripheral component such as recent surgery or vaginal atrophy. The remaining studies used models with multiple conditions where the test stimulus and pathological condition were separate or focused on female-specific organs.

3.2. Sex differences and gonadal hormone modulation of deep somatic pain (Table 2)

Sex differences and hormonal modulation of deep somatic pain has been most extensively studied in the orofacial region examining muscles of mastication and the temporomandibular joint. While models of hindpaw inflammation can be considered superficial or subcutaneous and not necessarily deep tissue, we have included the formalin model due to the extensive literature on sex differences.

3.2.1. Orofacial pain—Temporomandibular disorders are more prevalent in women and inflammation of the masseter muscles or temporomandibular joint (TMJ) is used to model aspects of human temporomandibular disorder. In rats, masseter muscle or TMJ inflammation increased mechanosensitivity of the orofacial region and Fos expression in the medullary dorsal horn, but there was no sex difference (Bereiter et al., 2005b; Niu et al., 2012). However, following TMJ inflammation there was greater excitatory amino acid release in the trigeminal nucleus caudalis/upper cervical cord (Vc/C2) in males compared to females (Bereiter and Benetti, 2006). In the periphery, glutamate injected into the TMJ evoked greater jaw muscle activity in female rats compared to males. Subsequent mustard oil injected into the TMJ further increased jaw muscle activity but without a sex difference

(Cairns et al., 2002), suggesting a sex difference in glutamate receptor activity but not TRPA1. This is supported by greater NMDA receptor expression in the spinal cord of females (section 3.1.2) and greater NMDA receptor activity in female DRG neurons (section 3.4.1).

Underlying these sex differences could be sexually dimorphic effects of gonadal hormones. Masseter muscle inflammation-induced mechanical hyperalgesia was more robust following E2 replacement in ovariectomized rats (Liverman et al., 2009b; Traub et al., 2013). Jaw muscle activity evoked by glutamate injected into the TMJ was attenuated by ovariectomy and increased by E2 replacement. However, orchiectomy and E2 replacement had no effect (Cairns et al., 2002). Following TMJ inflammation by CFA, E2 dose-dependently upregulated NF-κB and downstream inflammatory cytokines in the synovial fluid and increased nociceptive behaviors as measured by a decrease in food intake (Kou et al., 2011).

In contrast, E2-evoked antinociception in TMJ inflammatory models has also been reported. TMJ inflammation induced by formalin produced a similar number of nociceptive behaviors in males and proestrous females, albeit lower than diestrous females and E2 reduced nociceptive behaviors in ovariectomized rats (Fischer et al., 2008). Similarly, TMJ inflammation induced by CFA evoked greater nociception in OVx+saline rats as measured by a different feeding behavior compared to OVx+E2 rats (Kramer and Bellinger, 2009).

It has been suggested that E2 antinociception in somatic inflammatory pain models is peripherally mediated via nongenomic signaling mechanisms (Favaro-Moreira et al., 2009) and several models of TMJ inflammation support an anti-inflammatory effect of E2. E2 attenuated plasma extravasation and neutrophil migration in the TMJ following TMJ inflammation in proestrus rats and OVx+E2 rats (Flake et al., 2006; Torres-Chavez et al., 2012). E2 and testosterone also attenuated these inflammatory measures in orchiectomized rats (Torres-Chavez et al., 2012). However, while E2 decreased CFA-induced TMJ inflammation (plasma extravasation) it increased TMJ afferent activity (Flake et al., 2005).

3.2.2. Hindpaw inflammatory pain—A number of studies have examined sex differences and the effects of gonadal hormones in models of hindpaw inflammation, most notably the formalin model. Formalin injected into the hindpaw produces an immediate 5–10 minute period of licking or shaking the injected limb (phase I; nociceptor activation) followed by a 5–10 minute lower activity interphase (thought due to an increase in descending inhibition) followed by a 30–40 minute phase II increase in excitatory activity (inflammation and central sensitization). Female rats displayed more licking/shaking in all 3 phases of the formalin response compared to males suggesting greater sensitivity (Gaumond et al., 2002). Formalin also evoked a more robust response in dorsal horn neurons during phase II in female rats and conditioning electrical stimulation more effectively attenuated the formalin response in males (You et al., 2006) similar to greater CPM in men. How this is modulated by gonadal hormones is unclear. In male rats, formalin-induced nociceptive responses were decreased by spinal administration of an ERα/β antagonist, GPER antagonist or aromatase inhibitor, suggesting pronociceptive activity at multiple membrane estrogen receptors at the spinal level (Zhang et al., 2012a). In female rats, ovariectomy decreased the nociceptive behavior during the interphase. This was blocked by replacing E2 and progesterone (E2/P) (Ceccarelli et al., 2006; Gaumond et al., 2002; Gaumond et al., 2005). Since the interphase is thought due to an increase in inhibitory processing, the positive correlation between E2/P and nociceptive responses suggest E2/P cause a deficit in inhibitory processing. Studies in female, but not male mice, further suggest this anti-inhibitory effect was due to activation of ERβ (Coulombe et al., 2011; Spooner et al., 2007).

This pronociceptive or anti-inhibitory effect of estrogen in the formalin test is contradicted by another series of reports that ovariectomy increased the response during phase II (excitatory response) of the formalin test compared to intact rats in proestrus or ovariectomized rats with steady state replacement of E2 (Hunter et al., 2011; Kuba et al., 2005; Mannino et al., 2007). Since the increase in nociceptive behavior in ovariectomized rats was only apparent following a high concentration of formalin (5%), it was possible that E2 had a peripheral anti-inflammatory effect reducing nociceptive behaviors in phase II. In agreement, ERα and ERβ selective agonists attenuated late phase 2 responses in mice (Coulombe et al., 2011).

Following longterm ovariectomy (6 months) licking behavior to 5% formalin was greater than in intact females. However, spinal reflexes, flinching and flexing, were not different. In addition, the flexion reflex (paw withdrawal from noxious heat) was unaffected by longterm ovariectomy suggesting that gonadal hormones modulated supraspinal rather than spinal processing of noxious stimuli (Ceccarelli et al., 2003a).

Conclusions drawn from the formalin model are further complicated by another study that reported that the biphasic response of formalin, this time injected into the lip, was increased by ovariectomy, but not orchiectomy, and accompanied by an increase in ERα in the trigeminal subnucleus caudalis and C1-C2 dorsal horn. This same study reported no effect of ovariectomy on responses to intraplantar injection of formalin compared to intact rats (1.5% formalin was used in the lip and hindpaw suggesting greater modulation in the orofacial region). Furthermore, there was no sex difference to formalin at either test site (Pajot et al., 2003).

3.2.3. Cancer Pain—There is no compelling evidence for a sex difference in cancer pain in humans (Edrington et al., 2004; Fillingim et al., 2009) and limited data regarding sex differences in pain resulting from cancer treatment. In rats mechanosensitivity in chemotherapy induced peripheral neuropathy was greater in females compared to males. Ovariectomy decreased and E2 replacement restored the hypersensitivity. A PKCε inhibitor decreased the neuropathy in males and ovariectomized females, but not intact or E2 treated females (Joseph and Levine, 2003).

3.2.4. Summary—Of the studies that looked at somatic deep tissue pain in males and females most found females more sensitive than males and two found no sex difference. This is consistent with the overall impression that females are more sensitive to pain than males. However, in contrast to visceral pain models, most studies focusing on the effects of hormones indicated E2 was antinociceptive, comparing intact females with the effects of ovariectomy and OVx+E2. It is possible the antinociceptive effects of E2 may have resulted from peripheral anti-inflammatory effects as antinociception mostly observed in inflammatory pain models.

3.3. The role of gonadal hormone receptor subtypes

3.3.1. Estrogen receptors—Behavioral and cellular studies support both genomic and non-genomic or rapid estrogen signaling in pain and nociception. ERα and ERβ are the classical estrogen receptors that function as ligand bound transcription factors. In addition, these classical receptors and GPER are also localized to plasma and intracellular membranes. E2 binding these receptors activates second messenger signaling pathways to modulate ion channels and receptors affecting neuronal activity and activating transcription factors within seconds to minutes (see (Gintzler and Liu, 2012; McEwen, 2001; Prossnitz and Barton, 2011; Srivastava et al., 2011; Vasudevan and Pfaff, 2008) for review).

Little is known about the role of different estrogen receptors in deep tissue pain. Our lab has reported opposing effects of selective activation of the classical estrogen receptors ERα and ERβ in ovariectomized rats (Cao et al., 2012; Ji et al., 2011)(FIGURE 2). Selective activation of ERα either systemically or at the level of the spinal cord increased the magnitude of the visceromotor response to colorectal distention four hours later, mimicking the effect of E2. A shorter duration response was noted for ERα, but not ERβ. Activation of ERα with a selective agonist facilitated the visceromotor response to colorectal distention within 15 minutes, suggesting the facilitation was via a nongenomic mechanism (Ji et al., 2011). In contrast, selective activation of ERβ decreased the magnitude of the visceromotor response to colorectal distention. The inhibition was not observed for 3–4 hrs after agonist administration leaving open the possibility this was a genomic process (Cao et al., 2012). When the ERβ agonist was administered to intact female rats overstimulating the ERβ system, the visceromotor response to colorectal distention was attenuated, suggesting the facilitatory effect of ERα activation masked the inhibitory effect of ERβ activation in intact rats.

The antinociceptive effect of ERβ activation supports a series of reports, in which an ERβ agonist inhibited hyperalgesia in several models of chemical, acute or persistent inflammatory pain and neuropathic pain, leading the authors to speculate ERβ mediated anti-inflammatory effects of estrogen (Gardell et al., 2008; Leventhal et al., 2006; Piu et al., 2008). In a different model of endometriosis than described above, in which human endometrial tissue is transplanted into athymic nude mice, an ERβ agonist decreased the size of the cysts in the abdominal cavity further supporting anti-inflammatory activity, but no effects on nociceptive processing were reported (Harris et al., 2005). In rat models of spontaneously developing inflammatory bowel disease or adjuvant-induced arthritis, an ERβ agonist attenuated diarrhea and reduced inflammatory scores in both models leading the authors to suggest ERβ modulates the immune system (Harris et al., 2003).

GPER is a G-protein coupled receptor that binds E2 activating several different intracellular signaling pathways (Prossnitz et al., 2007). Selective activation of GPER mediated visceral hypersensitivity induced by the 5-HT precursor 5HTP (Lu et al., 2009) and selective activation of GPER increased superficial pain related behaviors in mice (Deliu et al., 2012). GPER is expressed in primary afferents. While no sex difference in expression was reported, expression in females was decreased by ovariectomy (Takanami et al., 2010). In another study E2 binding GPER rather than ERα/β evoked mechanical hyperalgesia by increasing PKCε translocation in DRG cells. This obscured subsequent activation of β2-adrenergic receptor-induced hyperalgesia (Hucho et al., 2006; Khasar et al., 2005; Kuhn et al., 2008). Activation of ERα and GPER, but not ERβ, activated ERK in trigeminal ganglion neurons and contributed to masseter muscle pain (Liverman et al., 2009a; Liverman et al., 2009b) and G-1, a GPER agonist, rapidly increased Ca2+ in trigeminal ganglion neurons from female rats (Fehrenbacher et al., 2009).

In the central nervous system testosterone is converted into neurosteroidal E2 by aromatase, providing a mechanism for local and rapid modulation of nociceptive processing by E2 binding ERα, ERβ or GPER. Intrathecal administration of aromatase inhibitors attenuated nociceptive processing to noxious cutaneous stimuli in Japanese quail within minutes supporting rapid signaling in addition to slower evolving genomic modulation (Evrard, 2006; Evrard and Balthazart, 2004). In rats, spinal aromatase inhibitors shifted morphine antinociception from kappa opioid receptor dependent to independent. This was likely via rapid signaling as it occurred within 15 minutes (Liu et al., 2011). Clinically, aromatase inhibitors are used to treat pain from endometriosis (see (Ferrero et al., 2011; Nothnick, 2011; Pavone and Bulun, 2012) for review).

3.3.2. Androgen receptors—A general conclusion from many studies is that testosterone, presumably via androgen receptors, is protective or antinociceptive and the greater testosterone plasma concentration in males supports this hypothesis. Testosterone replacement in gonadectomized male rats was analgesic in the tailflick test (Frye and Seliga, 2001). Likewise, orchiectomy in males increased phase I and II responses to 2% formalin in rats which was reversed by testosterone replacement (Gaumond et al., 2005). There was no difference in nociceptive behaviors to a single injection of 5% formalin comparing intact and gonadectomized male rats. However, following repeated formalin injections in males, nociceptive behaviors decreased in intact, but not gonadectomized rats (Aloisi et al., 2003; Ceccarelli et al., 2003b). In intact rats, daily testosterone injections attenuated formalin-induced licking behavior in females, but not males (Aloisi et al., 2004), possibly a ceiling effect of testosterone in males. In males, orchiectomy increased nociceptive responses to formalin injected in the TMJ which was reversed by testosterone, but not E2 (Fischer et al., 2007). In a mouse model of muscle fatigue males showed less fatigue than females. In ovariectomized mice testosterone reduced fatigue, similar to males (Burnes et al., 2008). In rats, there was no sex difference in mechanical hyperalgesia following masseter muscle inflammation, but peripheral antinociceptive mechanisms were sexually dimorphic. A cannabinoid 1 receptor (CB1R) agonist produced greater antihyperalgesia in males than females which correlated with CB1R expression in the trigeminal ganglion. Orchiectomy reduced the CB1R mRNA in males with no effect of ovariectomy in females. Testosterone reversed the decrease in CB1R expression in males, but had no effect in females (Niu et al., 2012).

3.3.3. Summary—While not as extensively studied as estradiol, the majority of studies support an antinociceptive or protective role for testosterone. Although testosterone is aromatized into E2 in the brain and spinal cord, only a few investigators have addressed this aspect of testosterone function in deep tissue pain and nociception. More is known about estrogen receptors, both the classical genomic receptors (ERα and ERβ) and the more recently recognized GPER. Studies support both genomic and nongenomic roles of ERα and ERβ but specific contributions to nociceptive processing remain unclear. E2 acting at GPER activates second messenger pathways that either modulate neuronal excitability or modulate transcription.

3.4. Where do sex differences originate?

Sex differences in the response to noxious stimuli in conscious animals can occur at the level of primary afferents, the dorsal horn or at supraspinal levels. Sex differences in the response of primary afferents were reported in several models. Similarly, differences were reported at spinal and supraspinal levels contributing to differences in perception and descending modulation. While there are possible alternate explanations, data support sex differences in primary afferent activity. In contrast, sex differences in dorsal horn neuron activity could be intrinsic to the spinal cord or could result from sex differences in supraspinal processing and descending modulation.

3.4.1. Primary afferents—Estrogen receptors are expressed in primary afferents and estrogen modulates primary afferent activity. Estimates of estrogen receptor expression in DRG and trigeminal ganglion (TG) neurons range from 20% to “most cells” (Bereiter et al., 2005a; Papka and Storey-Workley, 2002; Taleghany et al., 1999). Generally, expression is greatest in the L6-S2 DRG and ERβ is expressed in more cells than ERα. Estrogen receptors are expressed in 20–70% of bladder afferents (Bennett et al., 2003; Ji et al., 2011) and in uterine-cervix afferents (Papka et al., 2001; Papka and Storey-Workley, 2002), but there is a lack of estrogen receptor alpha expression in rat lumbosacral colonic afferents (Ji et al., 2011).

There are only a handful of studies examining sex differences in the response to noxious stimuli of primary afferents innervating deep tissue. There was no sex difference in the evoked response of colonic afferents to colorectal distention (Ji et al., 2012b). One reason for this might be the observation that ovariectomy did not change the response of colonic afferents to colorectal distention compared to intact female rats, suggesting a lack of hormonal modulation of colonic afferents (Ji et al., 2011). However, males had greater spontaneous activity in lumbosacral (pelvic nerve) and thoracolumbar (splanchnic nerves) colonic afferent fibers in the dorsal roots (Ji et al., 2012b). Since females were more sensitive to colorectal distention than males (Figure 1), these observations suggest central processing rather than differences in primary afferent input accounts for the observed differences in behavior. This could reflect sex differences in transmitter release or receptor expression/function or hormonal influences within the CNS, either at the level of the spinal cord or at supraspinal sites. A similar conclusion was reported for muscle afferents; female rats had greater behavioral mechanosensitivity/lower thresholds than males, but muscle afferent fibers from males had lower mechanical thresholds than those from females (Hendrich et al., 2012).

Modulation of primary afferents by gonadal hormones has been studied in primary afferents that innervate sex-specific organs and common organs. Threshold for uterine and vaginal afferents innervated by the hypogastric and pelvic nerves, respectively, was lowest during proestrus and highest during diestrus. As previously mentioned this contradicts the behavioral studies from this group, but is thought to be related to reproductive function rather than nociception (Robbins et al., 1992).

Sex differences in excitability of primary afferents innervating tissues common to males and females may be due to genomic mechanisms or estrogen modulation by several different nongenomic signaling mechanisms. NMDA or glutamate injected into muscles of mastication evoked greater primary afferent discharges in female rats compared to males. Ovariectomy decreased the NMDA-evoked response which was reversed by E2 replacement (Cairns et al., 2001; Cairns et al., 2002; Dong et al., 2007). There was a greater percentage of GluN2B expressing masseter afferents in DRG from female rats with elevated E2 levels (OVx+E2, intact) compared to males, supporting other studies that E2 increases NMDA receptor activity (section 3.1.2.). Likewise, glutamate evoked greater activity in female rat facial cutaneous mechanoreceptors compared to males that was attenuated by an NMDA receptor antagonist (Gazerani et al., 2010). In unidentified DRG cells, NMDA evoked current was greater in cells from female rats compared to males and was E2 dependent (McRoberts et al., 2007). In cultured TG neurons bradykinin increased E2-dependent PLC signaling in female, but not male rats. Correspondingly, in ovariectomized rats, locally injected E2 increased bradykinin evoked hypersensitivity (Rowan et al., 2010). In support, E2 increased excitability of afferents innervating the TMJ which was further increased by inflammation (Flake et al., 2005). E2 increased pCREB in cultured DRG neurons, but not in autonomic pelvic ganglion neurons, via activation of pERK (Purves-Tyson and Keast, 2004). However, the specific estrogen receptors involved are unknown.

In contrast to the facilitatory effect of E2 in primary afferents described above, ovariectomy but not orchiectomy, increased locally injected ATP evoked mechanosensitivity of the hindpaw that was reversed by E2 (Ma et al., 2011). Consistent with this, E2 decreased ATP-induced calcium currents and P2X3 receptor mRNA in intact rat DRG cells (Ma et al., 2005). Similarly in mice, E2 attenuated ATP-induced Ca2+ currents by blocking L-type voltage gated calcium channels which was dependent on ERα activation (Chaban et al., 2011; Chaban et al., 2003; Cho and Chaban, 2012). This apparently involved estrogen receptor interaction with mGlu2/3 receptors (Chaban et al., 2011).

Of note, the inhibitory effect of E2 on ATP induced calcium currents was reported in physiologically unidentified DRG cells. Since visceral afferents constitute approximately 10% of DRG neurons, it is likely the aforementioned neurons innervated somatic tissue. However, in an animal model of colitis, E2 had an excitatory interaction with P2X receptors. Ovariectomy decreased visceral sensitivity and P2X3 receptor expression in DRG neurons which was reversed by E2 replacement, and E2 increased the magnitude of ATP-induced current in DRG cells (Fan et al., 2009). One possibility is the direction of modulation by E2 on P2X function changes during chronic inflammation. Indeed, colonic afferents from noninflamed rats do not express ERα and estrogen does not modulate colonic afferent activity (Ji et al., 2011). Following inflammation there may be an upregulation of ERα or a facilitatory effect mediated by ERβ or GPER may be unmasked. E2 might also have different effects on DRG cells that innervate viscera compared to somatic tissue.

3.4.2. Dorsal horn neurons—Following intrathecal administration, ER agonists/antagonists can modulate sensory and/or motor systems to affect responses. Recording from dorsal horn neurons provides evidence for second order sensory processing. Windup is a gain in the response of dorsal horn neurons to repetitive primary afferent stimulation. It is centrally mediated, NMDA receptor dependent and constitutes one of the most basic forms of dorsal horn neuronal plasticity. Electrical stimulation in the periphery evoked more robust windup in female rat dorsal horn neurons compared to males (Figure 3). Furthermore, only 37% of dorsal horn neurons in male rats showed windup vs. 82% in females (Chi-Square, $p<0.001$, unpublished observation). Windup is thought to be the mechanism underlying temporal summation in humans and the greater windup in female rats is consistent with greater temporal summation in women (see section 2.1.).

Several studies examining medullary or spinal dorsal horn neuronal responses to noxious deep tissue stimuli are similar to the behavioral outcomes providing a site for sex differences and hormonal modulation. The response of dorsal horn neurons to colorectal distention was greater in female rats compared to males (Ji et al., 2012b). Ovariectomy decreased the response of dorsal horn neurons to colorectal distention and E2 replacement recovered the response (Ji et al., 2003). Similarly, the response of medullary dorsal horn neurons responsive to temporomandibular joint stimulation/inflammation was more robust in proestrous rats compared to diestrous rats and ovariectomy decreased responses which were increased by E2 replacement (Okamoto et al., 2003; Tashiro et al., 2007). Surprisingly, there was no sex difference in the response of spinal dorsal horn neurons to cardiac stimulation with algogenic substances (Little et al., 2011). However, colonic inflammation induced a sexually dimorphic response of dorsal horn neurons. Compared to neurons that responded to colorectal distention in noninflamed rats, one phenotype of dorsal horn neuron was facilitated in females with no change in males. A second phenotype was facilitated in males and inhibited in females (Ji et al., 2012b). These data suggest sex differences in spinal processing of deep tissue stimuli contribute to behavioral differences in pain perception.

Most of these previous studies used estrogen replacement over days or bolus injections and examined responses hours to days later, suggesting the possibility of transcriptional effects underlying differences in neuronal responses. E2 also acts at membrane bound receptors initiating a rapid signaling cascade to alter cellular excitability. In ovariectomized rats E2 applied to the surface of the brainstem attenuated the response of medullary dorsal horn neurons to TMJ stimulation within minutes. An ERα agonist mimicked the inhibitory effect of E2, but an ERβ agonist facilitated neuronal responses, indicating receptor subtype selectivity in nociceptive processing (Tashiro et al., 2012). In a slice preparation, E2 potentiated synaptic signaling in the spinal cord via ERβ with no effect of ERα stimulation (Zhang et al., 2012b). Two points should be made here. First, the Tashiro paper supports membrane receptor initiated rapid signaling following selective activation of ERα and ERβ,

but a nonselective agonist such as E2 evoked an overall inhibitory response. In contrast, the same group reported E2 replacement over several days in ovariectomized rats facilitated the response of dorsal horn neurons in the same TMJ model (Tashiro et al., 2007). This suggests that genomic and nongenomic signaling might result in different outcomes in neuronal activity and overall behavioral response. Second, it is unclear what the phenotype of the medullary dorsal horn neuron was and whether it expressed ER. ERα is co-expressed with enkephalin providing a mechanism for antinociceptive activity of E2 in the spinal cord and presumably medullary dorsal horn (Amandusson et al., 1996).

3.4.3. Supraspinal processing—Supraspinal processing of nociceptive stimuli occurs at many sites throughout the brain. While there is significant literature addressing supraspinal processing of deep tissue pain, most experiments were done in males or in females without consideration of estrous status. Only recently have studies begun to examine sex differences in supraspinal processing or modulation by gonadal hormones.

Imaging studies in humans suggest chronic pain activates limbic pathways to a greater extent in women and cortical structures to a greater extent in men. Imaging of deep tissue pain in animals reported similar findings. Colorectal distention in female rats evoked greater changes in the ventromedial prefrontal cortex and broader limbic/paralimbic areas that are involved in the affective aspect of visceral pain. In contrast, male rats had greater cortical activation that lead to greater cortical inhibitory effects on limbic structures (Wang et al., 2009).

In ovariectomized rats TMJ inflammation induced E2-dependent hyperalgesia and upregulation of NGF and TRPV1 in the hippocampus, but not in the amygdala, prefrontal cortex or thalamus. Inhibition of hippocampal NGF or TRPV1 attenuated this hyperalgesia (Wu et al., 2012; Wu et al., 2010). However, E2 does modulate amygdala activity and nociceptive sensitivity. Site specific administration of E2 pellets to the amygdala increased the visceromotor response to colorectal distention, but had no effect on somatic sensation (Myers et al., 2010). However, activation of the amygdala by corticosterone reduced the estrous cycle fluctuation in the visceromotor response to colorectal distention, but cutaneous mechanosensitivity was greater during proestrus/estrus (Gustafsson and Greenwood-Van Meerveld, 2011). In contrast, E2 or E2 + P injected into the amygdala decreased nociceptive behavior on the hotplate and increased the tailflick latency (Frye and Walf, 2004; Walf and Frye, 2003).

Injection of nitroglycerin to model migraine induced sexually dimorphic Fos expression in multiple brain nuclei. Male rats had greater Fos expression in the paraventricular nucleus (PVH), supraoptic nucleus (SON) and nucleus trigeminalis caudalis (SPVC). In females, ovariectomy decreased nitroglycerin-induced Fos expression in the PVH, SON, central nucleus of the amygdala, nucleus tractus solitarius, area postrema and SPVC. Orchiectomy reduced Fos expression only the SPVC in males (Greco et al., 2012).

In contrast, there was no sex difference in Fos expression in second order neurons in the spinal or medullary dorsal horn following colorectal distention or TMJ inflammation (Bereiter et al., 2005b; Ji et al., 2012b; Murphy et al., 2009). However, there was significantly greater colorectal distention-induced Fos expression in retrogradely labeled spinal neurons that projected to the parabrachial nucleus (PBn) in male rats although there was no sex difference in the total number of spino-PBn neurons (Murphy et al., 2009). The PBn is a vital link in visceral nociceptive circuitry between the spinal cord and supraspinal sites including the amygdala and PAG. Sexually dimorphic activation of the PBn could contribute to sex differences in the affective component of pain and descending modulation. Indeed, a sex difference was observed in the functional projection of PAG neurons to the

RVM following hindpaw inflammation. While no sex difference was noted in Fos expression in the PAG by persistent inflammatory pain, significantly more PAG-RVM cells were activated in males in comparison with females even though females had a greater anatomical projection from the PAG to the RVM (Loyd and Murphy, 2006). Since the PAG-RVM pathway is well known for its role in descending pain modulation, the sex difference in spino-PBn and PAG-RVM circuit activation may contribute to the sexually dimorphic prevalence of some deep tissue pain conditions.

3.4.3.1. Stress and deep tissue pain: Women are usually more susceptible to stress-related disorders than men. Chronic stress is one of the many triggers of IBS, TMD and other functional pain syndromes. In many cases stress triggers the onset of or worsens the symptoms. Stress induces visceral hypersensitivity, but there are few papers examining sex differences or the role of gonadal hormones. Acute or mild chronic stress activates the emotional arousal circuit. Hypothalamic release of corticotropin releasing factor (CRF) and related peptides stimulate the hypothalamic-pituitary-adrenal (HPA) axis increasing circulating corticosterone (cortisol in humans) as well as activating the sympathetic nervous system. This increases visceral nociceptive processing at several levels of the nervous system (see (Dalla et al., 2011; Larauche et al., 2012b) for review).

In female rats, stress induced visceral hypersensitivity is estrogen dependent. Partial restraint stress in adult females induced visceral hypersensitivity that was decreased by ovariectomy and reversed by E2 replacement (Bradesi et al., 2002; Bradesi et al., 2003). In a new preparation modeling comorbidity of TMD, stress and IBS, visceral hypersensitivity persisted much longer in ovariectomized rats regularly injected with E2 compared to vehicle (Traub et al., 2013). In a mouse model of gastritis the inflammatory response was greater in females while the circulating levels of corticosterone decreased by more than half in females and not at all in males. Similarly, measures of anxiety increased in females, but not in males. However, there was no modulation of any responses in females across the estrous cycle (Painsipp et al., 2007).

Early life adversity has detrimental effects in adults that are sexually dimorphic. Maternal separation of all neonatal pups in a litter for 2 hours per day for 2 weeks induced visceral hypersensitivity to colorectal distention in male and female adults. When only half the litter was separated there was visceral hypersensitivity in adult females, but not males. Partial restraint stress alone in adults or following maternal separation increased the visceral hypersensitivity in females, with no effect in males (Rosztoczy et al., 2003). In contrast, a different strain of male rats was hypersensitive to colorectal distention following maternal separation and water avoidance stress as adults (Coutinho et al., 2002). In a different model, exposing neonatal rats to an unpredictable pairing of an odor and shock resulted in visceral hypersensitivity to colorectal distention in adult females, but not males. Furthermore, estrogen modulated the sensitivity in the adult females; ovariectomy decreased the visceral hypersensitivity which was reversed by E2 replacement (Chaloner and Greenwood-Van Meerveld, 2013).

Neonatal bladder inflammation also induced colorectal hypersensitivity in adult females. Following neonatal insult colorectal hypersensitivity in estrous/diestrous rats was normalized to the level observed in proestrous rats (Miranda et al., 2011). This may reflect the greater visceral hypersensitivity in proestrous rats without neontatal insult. This model also suggests neonatal insult in one viscus can modulate sensitivity in other viscera in adults contributing to increased comorbid pain conditions.

3.4.4. Summary—Sex differences in deep tissue pain can occur at multiple levels of the neuraxis. The majority of animal studies show that female gonadal hormones exert an

excitatory effect on primary afferents, spinal and supraspinal neurons. In primary afferents the interaction of E2/ERs and additional specific receptors appears to increase or decrease afferent excitability. E2 attenuates P2X receptor responses to ATP. This could be interpreted as another example of the peripheral anti-nociceptive/anti-inflammatory effect of E2. On the other hand, E2 increases NMDA receptor activity which could occur in the absence of inflammation or perhaps the activity in DRG cells *in vitro* reflects what happens in the central terminal of primary afferents; increased NMDA receptor activity increasing transmitter release.

Sex differences at the level of the spinal cord are present, and apparently are independent of primary afferent differences. However, whether this represents the source of sex differences in deep tissue pain is unknown at present. In addition, the supraspinal studies suggest sex differences could arise in several sites in the brain. Stress results in sexually dimorphic visceral hypersensitivity that is E2 dependent.

4. Conclusion

That there exists a sex difference in deep tissue pain is well supported. The majority of studies concluded the prevalence and severity of chronic pain conditions is disproportionately greater in women, and the majority of animal studies, especially in rodents, support greater nociceptive processing in females. Much evidence suggests gonadal hormones contribute to sex differences, but underlying mechanisms are unclear. Hormones can modulate nociceptive processing at many levels of the neuraxis or in the periphery. Often the same stimulus and hormonal complement has different effects depending on the site of action or underlying conditions. Possible peripheral anti-inflammatory/anti-nociceptive effects of E2 could oppose pro-nociceptive activity within CNS circuitry. In addition, it is still not clear if the absolute or changing concentrations of hormones are more important in determining pro- or anti-nociceptive direction. Many studies in women suggest deep tissue pain symptoms are exaggerated in the perimenstrual period or during menses. This can be interpreted as evidence that gonadal hormones are antinociceptive since pain is greater when hormone levels are low. However, it can also be interpreted to suggest that withdrawal of hormone facilitates transcriptional regulation affecting nociceptive processing that takes several days to fully manifest. Unfortunately, the rapidly changing hormonal levels during the rodent estrous cycle make clarification of this question difficult. On the other hand, animal experimentation allows us to address questions about underlying mechanisms that cannot be addressed in humans. The role of membrane receptor initiated rapid signaling, perhaps by locally synthesized hormones, neurosteroids, is just beginning to be carefully examined in pain modulation. While evidence supports a role in nociceptive processing, the relative importance of rapid signaling vs. classical genomic mechanisms is unknown. Future studies will hopefully address some of these issues in determining the mechanisms underlying sex differences in pain.

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Highlights

- Humans and animal studies support greater deep tissue pain in females
- The sex difference is partially dependent on gonadal hormones
- Gonadal hormones are pro- or anti-nociceptive depending on organ/condition

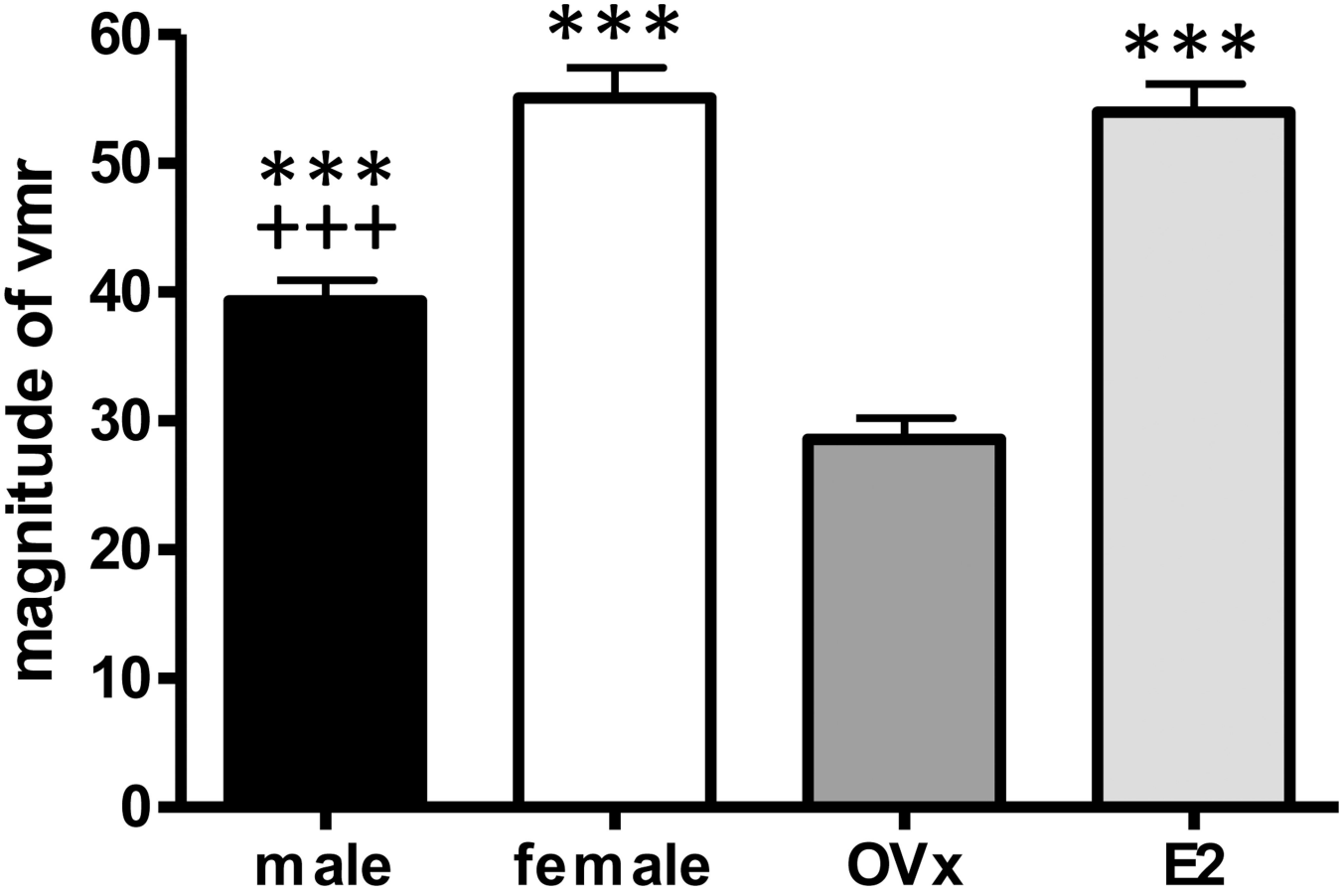

Figure 1.

Sex difference and effect of hormone modulation on the magnitude of the visceromotor response (vmr) to colorectal distention. The vmr was significantly greater in female rats compared to males. Ovariectomy significantly decreased the vmr compared to intact females and replacement E2 restored the vmr to a magnitude similar to intact rats. One way ANOVA $p<0.0001$, n=55–61/group. *** $p<0.001$ vs. OVx; +++ $p<0.001$ vs. female.

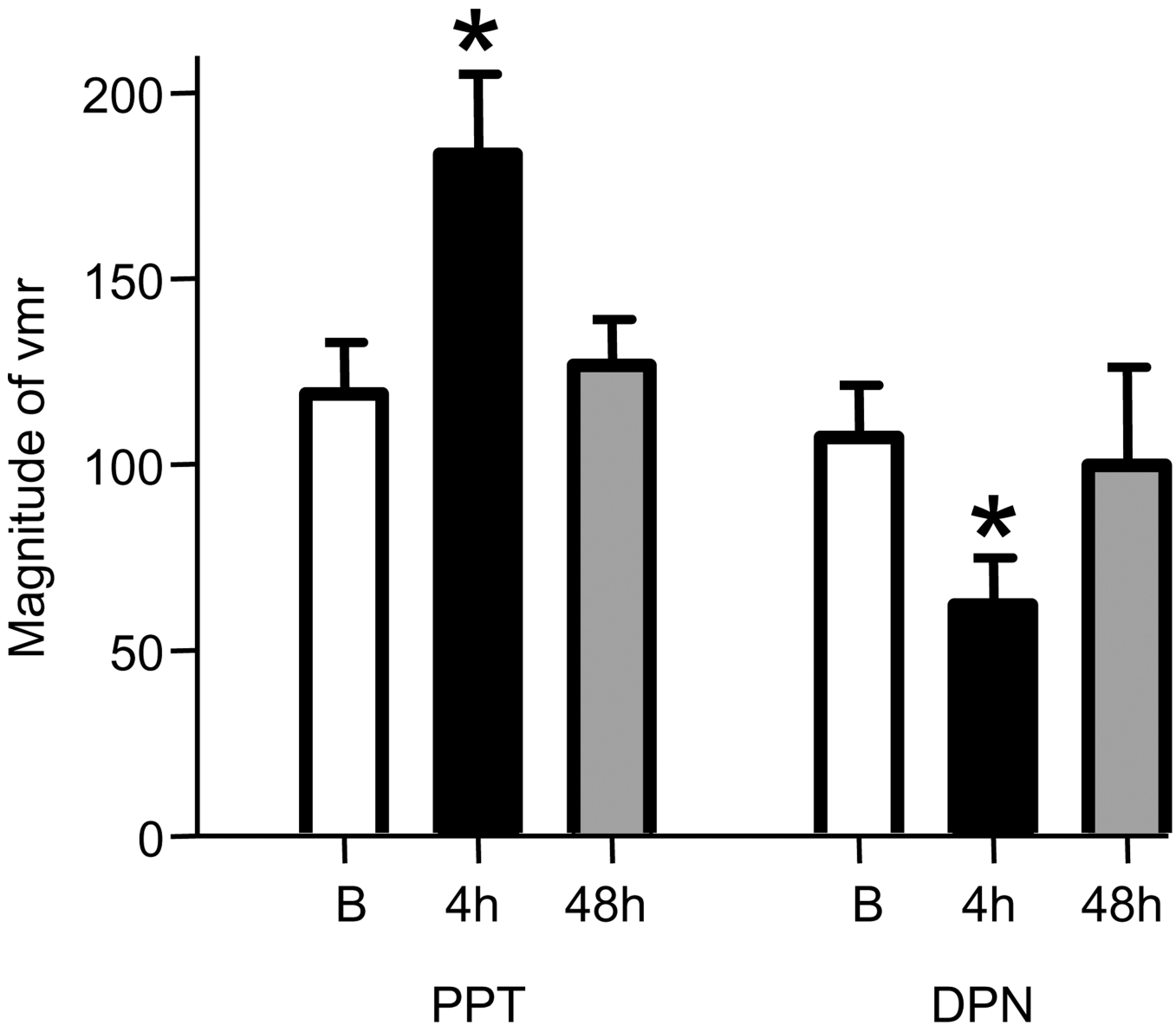

Figure 2.

The effect of s.c. administration of the ERα agonist PPT and the ERβ agonist DPN on the visceromotor response. The effect of each agonist was significantly greater than baseline four hours after injection and returned to baseline by 48 hours (1 way RM ANOVA). * $p<0.05$ vs. baseline (B) prior to agonist administration. Figures are derived from data originally published in (Ji et al., 2011) and (Cao et al., 2012) with permission.

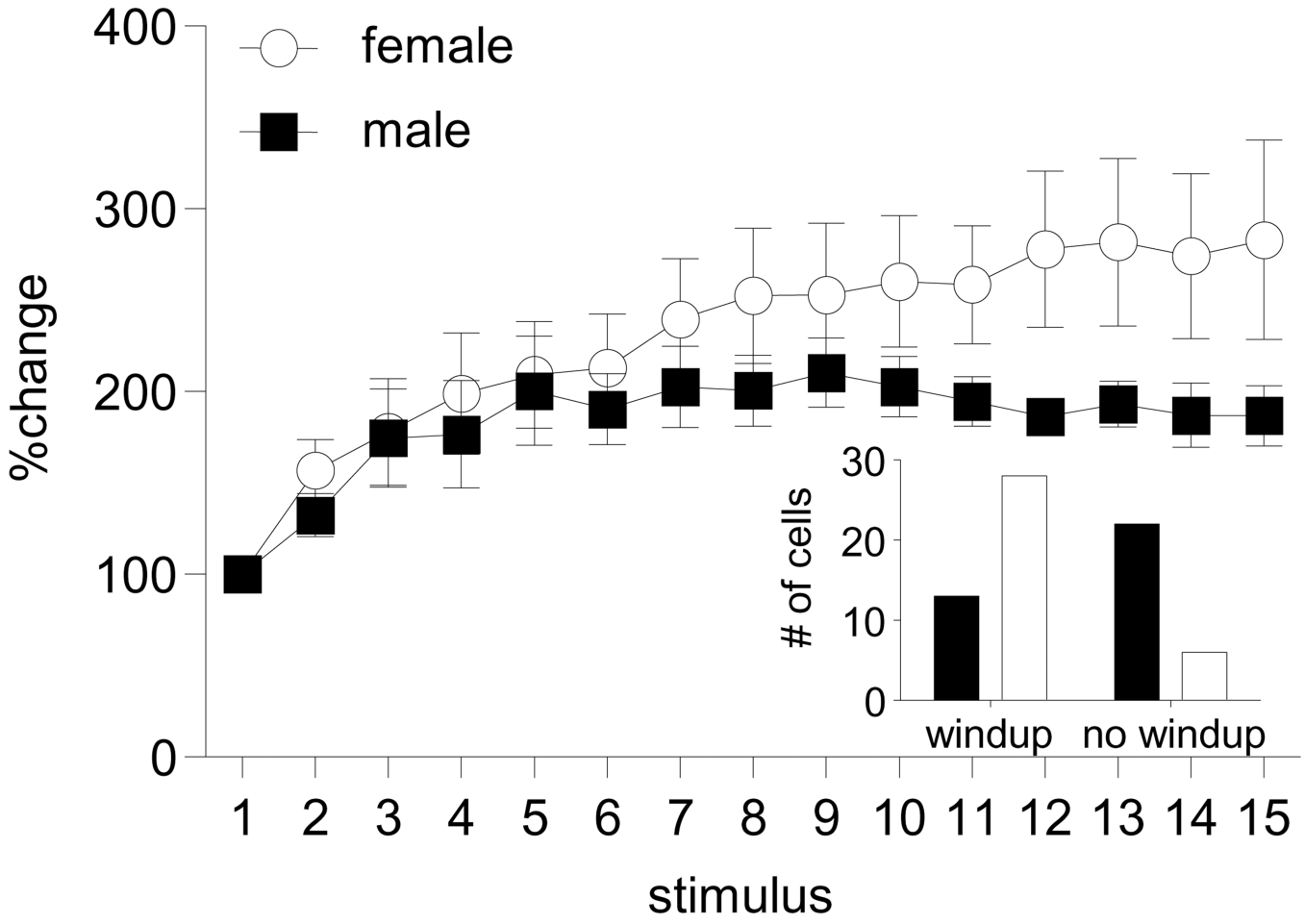

Figure 3.

Sex difference in magnitude and incidence of windup. Response to electrical stimulation at 1 Hz normalized to response to the first stimulus. Data are mean ± sem. There is a time × sex interaction, $p<0.005$, n=13 male, 28 female. Inset: Significantly more cells showed windup in female rats compared to males (Chi-square: $p<0.001$). This parallels greater temporal summation in women.

Table 1

Sex differences and hormonal modulation of visceral stimuli [a]

Ref	Species/prep	Test (stim→response)	Result	Additional results and Notes
Sex difference				
Holdcroft (Holdcroft et al., 2000)	Rat/ M,F	CRD→VMR	F>M;	Greater female sensitivity to CRD; increased during high E2.
Ji (Ji et al., 2012)	Rat/ M,F	CRD→VMR; normal, inflamed colon	F>M. inflame F>M	Greater female sensitivity to CRD ± inflammation. NMDA antagonist more potent in males, greater NMDA receptor activity, expression in membrane in females.
Bourdu (Bourdu et al., 2005)	Rat/ M,F	CRD→VMR; colitis	F>M	Greater female sensitivity to CRD ± inflammation.
Wang (Wang et al., 2009)	Rat/ M,F	CRD→ VMR, imaging, pain behavior	VMR: M=F Pain scores F>M	No SD in the VMR, but females had higher pain scores. There was a SD in brain activity to CRD.
Rosztoczy (Rosztoczy et al., 2003)	Rat/ M,F	CRD→VMR	basal M=F; mat.sep F>M; mat.sep+ stress F>M	No SD in basal visceral sensitivity. Maternal separation ± adult stress increased visceral sensitivity that was greater in females.
Kamp (Kamp et al., 2003)	Mice/different strains/ M,F	CRD→VMR	F>M	Greater female sensitivity to CRD in some strains.
Larauche (Larauche et al., 2012)	Rat/ M,F	CRD→intracolonic pressure	basal M=F; acute stress ↓M,F; repeated stress ↑F.	No SD in basal visceral sensitivity, greater effect of chronic stress in females. Intracolonic pressure differs from abdominal muscle VMR.
Ness (Ness et al., 2001)	Rat/ M,F	UBD→VMR	F,p>M	Greater female sensitivity to UBD
Affaitati (Affaitati et al., 2011)	Rat/ M,F	Ureteral stone→ crises;	F>M	Greater ureteral stone pain in females; ER, AR antagonists ↓ pain in females only.
Aloisi (Aloisi et al., 2010)	Rat/ M,F; supraphys E2 or T	Ureteral stone→ crises	F>M	Greater ureteral stone pain in females; Supraphys E2 was antinociceptive in females only (similar to pregnancy?); testosterone had no effect.
Larsson (Larsson et al., 2003)	Mice/ M,F	CRD→ VMR	F=M	Duration of VMR was half duration of distention. Unusual response.
Hormone modulation				
Ji (Ji et al., 2008)	Rat/ F, cycle	CRD→VMR	proestrus>met/diestrus	E2 is pronociceptive.
Ji (Ji et al., 2003)	Rat/ OVx,+E2	CRD→VMR	E2>OVx	E2 is pronociceptive.
Ji (Ji et al., 2005)	Rat/ F,OVx,+E2,+prog	CRD→VMR; normal, inflamed colon	E2=F>OVx; E2>E2+P. inflame: E2>OVx>F.	E2 is pronociceptive, progesterone blunted effect of E2.
Tang (Tang et al., 2008)	Rat/ OVx,+E2	CRD→VMR	E2>OVx. +APV: E2>OVx.	E2 is pronociceptive. E2 increased GluN1, pGluN1 expression, E2 decreased potency of NMDAr antagonist.

Ref	Species/prep	Test (stim→response)	Result	Additional results and Notes
Ji (Ji et al., 2011)	Rat/ OVx,+E2,+PPT	CRD→VMR	E2=PPT>OVx	ERα activation is pronociceptive, mediated effect of E2 on VMR. A MEK inhibitor attenuated the effect of PPT.
Fan (Fan et al., 2009)	Rat/ F,OVx,+E2,+prog	CRD→VMR; colitis	E2>OVx=E2+prog	E2 is pronociceptive in colitis model, progesterone blunted effect of E2.
Lu (Lu et al., 2007)	Rat/ OVx,+E2	CRD→VMR; normal, inflamed colon	E2>OVx	E2 is pronociceptive. E2 increased pCREB in spinal cord.
Lu (Lu et al., 2009)	Rat/ F,OVx,+E2; ER antagonists	CRD→VMR; normal, inflamed colon	E2,E2+prog,E2+ICI>OVx	E2 is pronociceptive via GPER, not ERα/β.
Bradesi (Bradesi et al., 2003)	Rat/ F,OVx,+E2	CRD→VMR	E2>OVx stress: E2>OVx.	E2 is pronociceptive in stress model. NK-1 antagonist was antinociceptive and E2 dependent.
Ball (Ball et al., 2010)	Rat/ F, cycle	UBD→VMR; normal, inflamed bladder	Inflam ↑VMR, met,p>di; nal ↑VMR, e>met>di>p	E2 is pronociceptive; influences opioid receptor in normal and inflamed bladder.
Robbins (Robbins et al., 2010) Peng (Peng et al., 2008)	Rat/ OVx,+E2	UBD→VMR;	E2>OVx*	E2 withdrawal (changing concentration) is pronociceptive. Constant E2 had no effect.
	Rat/ F, cycle	Uterine capsaicin evokes PnUR	p>met	E2 is pronociceptive; increases cross organ sensitization. NMDA receptor dependent.
Peng (Peng et al., 2010)	Rat/ OVx,+E2	colonic mustard oil evokes PnUR	E2>OVx	Cross organ sensitization is facilitated by E2. E2 ↑pAKT→↑pGluN2B→↑cross organ sensitization
Lin (Lin et al., 2006)	Rat/F,OVx,+E2	Repetitive Stimulation to potentiate PnUR	E2>OVx	E2 potentiates PnUR, is NMDA dependent.
Cason (Cason et al., 2003)	Rat/ F, cycle	VD→ escapes (Vaginal Hyperalgesia); endometriosis	Endometriosis↑, proestrus>estrus	Endometriosis increased vaginal hyperalgesia when E2 was high in cycling rats (proestrus). No estrous cycle effect in normal rats.
Berkley (Berkley et al., 2007)	Rat/ OVx, estropause	VD→ escapes (Vaginal Hyperalgersia); Endometriosis	Intact: Endo ↑VH. OVx: ↑VH, E2↓VH; OVx + Endo: VH, ↓ by E2	Endometriosis hyperalgesia is centrally mediated, OVx hyperalgesia is peripherally mediated; OVx + Endo had opposing effects.
Bradshaw (Bradshaw and Berkley, 2002)	Rat/ F,OVx,+E2	VD→escapes (Vaginal Hyperalgesia)	OVx>E2	E2 is antinociceptive. Ovariectomy induced vaginal hyperalgesia, relieved by E2 replacement.
Giamberardino (Giamberardino et al., 1997)	Rats/ F, cycle	Ureteral stone→ crises	met/di>p/e	E2 is antinociceptive
Cao (Cao et al., 2012)	Rat/ OVx,ERβ agonist	CRD→VMR	ERβ agonist ↓VMR	ERβ activation is antinociceptive, opposes ERα.

[a]Behavior: only those aspects of paper relevant to sex differences or hormonal modulation of behavioral responses are listed.

Abbreviations: VMR: visceromotor response; CRD: colorectal distention; UBD: urinary bladder distention; VD: vaginal distention; PnUR: pelvic nerve to urethra reflex; M,F,p,e,met,di: intact male, intact female, proestrous, estrous, metestrous, diestrous; OVx: ovariectomized; +E2: OVx with E2 replacement; GDx: gonadectomy in males; SD: sex difference; ↑/↓: increase/decrease. PPT: ERα agonist; Pn: pelvic nerve

Table 2

Sex differences and hormonal modulation of deep somatic stimuli [a]

Ref	Species/prep	Test (stim→response)	Result	Additional results and Notes
Sex difference				
(Burnes et al., 2008)	Mouse/ M,F,OVx, +testosterone	Muscle fatigue; ASIC3 wildtype and KO	Wt: F>M=OVx+T. KO: Fwt=MKO>Mwt.	Greater muscle fatigue (pain) in females. Testosterone was protective against muscle pain (low pH) but dependent on ASIC3.
Niu (Niu et al., 2012)	Rat/ M,F	masseter CFA→ mechanosensitivity	M=F. CB1R agonist: antihyperalgsia M>F	No SD in muscle hyperalgesia, but SD in peripheral modulation of muscle pain by CB1R agonist. Speculate testosterone is antinociceptive/protective.
Cairns (Cairns et al., 2002)	Rat/ M,F,OVx,GDx, +E2	Glu, m.oil in TMJ → jaw muscle activity	F>M (Glu); OVx↓,+E2↑; GDx,+E2 no change. m.oil: ↑activity but no SD.	Glutamate sensitized TMJ to further inflammation w/o SD. E2 in OVx was pronociceptive to glutamate.
Gaumond (Gaumond et al., 2002)	Rat/ M,F,GDx,OVx	Hindpaw formalin response	F>M: I, interphase, II; F>OVx: interphase; GDx>M: I, late II; OVx=GDx.	Formalin evoked greater responses in females, suggests E2 was antinociceptive by decreasing interphase inhibition; Testosterone was protective; GDx eliminated sex difference.
Coulombe (Coulombe et al., 2011)	Mice/ M,F, OVx+α/β agonist, ERαKO, ERβKO	Hindpaw formalin response	Interphase: ERβKO↓, β ag↑; Phase I: ERαKO↑, α ag↓. ↑	In females, ERα was antinociceptive, ERβ was pronociceptive (↓ inhibitory mechanisms). Minimal effect in males.
Zhang (Zhang et al., 2012)	Rat/ M,F,OVx,+E2, antags	Hindpaw Mech Threshold, Thermal Threshold; Formalin response	E2 ↓MTh, ↓TTh: M=F=OVx. Formalin males: ↓ by ICI, G-15, ATD	Spinal E2 was pronociceptive in M and F (no SD); mERs mediated effects of E2.
Joseph (Joseph and Levine, 2003)	Rat/ M,F,OVx,+E2	Chemotherapy-Induced Peripheral Neuropathy	F>M; OVx↓SD; E2 ↑SD; GDx: no effect. Inhibit PKCε: ↓hyp in M=OVx>>E2,F	CIPN F>M; SD was E2 dependent, E2 was pronociceptive. PKCε modulated pain but did not explain SD or E2 effect.
Spooner (Spooner et al., 2007)	Mice/ M,F, ERβKO	Hindpaw formalin response; Hot plate	Wt>KO; female effect only; Fos: wt>KO; HP:F=FβKO>M=MβKO	E2 was pronociceptive via ERβ dampening endogenous pain inhibitory mechanism→ ↑ spinal nociceptive activity. Females more sensitive to HP, ERβ independent.
Gaumond (Gaumond et al., 2005)	Rat/ M,F,GDx,OVx +E2, +T for 3wks	Hindpaw formalin response	GDx ↑phase I, II, T↓ I,II; OVx ↓ interphase; E +Prog restored interphase; E2, Prog had no effect.	GDx increased formalin response, reversed by testosterone (antinociceptive). OVx increased interphase inhibition, reversed by E2+prog (anti-inhibitory).
Pajot (Pajot et al., 2003)	Rat/ M,F,GDx,OVx	Formalin response to lip, foot	Lip: OVx>F; GDx=M. Foot: OVx=F, GDx=M.	E2 was antinociceptive to lip formalin, not hindpaw. Orofacial region more sensitive than hindpaw.
Ma (Ma et al., 2011)	Rat/ M,F,OVx,+E2, GDx	OVx induced Mech hyperalgesia, ATP antag in hindpaw	Mhyp: OVx>E2; GDx:no effect. ATPantag: reversed OVx Mhyp; GDx:no effect.	E2 was antinociceptive. E2 in females modulated P2X3 signal transduction, pain. Genomic mechanism. OVx↑, E2↓ P2X mRNA, protein in DRG.
Fischer (Fischer et al., 2008)	Rat/M,F,OVx,+E2, GDx	Glutamate/formalin in TMJ	di>p,M; OVx=di>F	E2 was antinociceptive to intra TMJ glutamate or formalin. No effect of estrous cycle phases during formalin test.
Hormone modulation				

Ref	Species/prep	Test (stim→response)	Result	Additional results and Notes
Sex difference				
Aloisi (Aloisi et al., 2003)	Rat/ M, GDx	Hindpaw formalin response, repeated injections	GDx increased formalin response over time	Testosterone was protective
Ceccarelli (Ceccarelli et al., 2006)	Rat/ F, OVx	Hindpaw formalin response, repeated injections	F>OVx	Cycling hormones were pronociceptive vs. OVx. Females had greater Fos expression in the arcuate nucleus.
Ceccarelli (Ceccarelli et al., 2003)	Rat/ F, longterm OVx (6 months)	Hindpaw formalin; thermal pain	Formalin licking: OVx>F; flinching, flexing OVx=F;	Replacement E2 antinociceptive to supraspinal (licking), but not spinal (flick, flex) responses to formalin.
Kuba (Kuba et al., 2005)	Rat/ OVx, +E2	Hindpaw formalin response	OVx > E2 (phase II) E2 capsules ↓ late phase but only high concentration of formalin	E2 was antinociceptive. Chronic E2 from capsules potentially anti-inflammatory in periphery.
Mannino (Mannino et al., 2007)	Rat/ F cycling, OVx, +E2 capsules	Hindpaw formalin response	OVx> p=E2	E2 was antinociceptive
Sanoja (Sanoja and Cervero, 2005; Sanoja and Cervero, 2008)	Mice/F,OVx,+E2	Mech hyp; Therm hyp; colonic capsaicin	Mhyp: OVx>E2. Thyp: no effect. Cap behavior: OVx>E2. No effect of estrous cycle.	E2 was antinociceptive. OVx increased hyperalgesia which was reversed by E2.
Fischer (Fischer et al., 2007)	Rat/M,F,GDx,OVx,+T	TMJ formalin response	GDx>M; OVx=OVx+T; GDx=GDx+E2>GDx+T	Testosterone was protective decreasing the formalin response in males. E2 had no effect in GDx. Testosterone had no effect in GDx.

[a]Behavior: only those aspects of paper relevant to sex differences or hormonal modulation of behavioral responses are listed.

Abbreviations: M,F,p,e,met,di: intact male, intact female, proestrous, estrous, metestrous, diestrous; OVx: ovariectomized; +E2: OVx with E2 replacement; GDx: gonadectomy in males; +T: GDx + testosterone replacement; SD: sex difference; ↑/↓: increase/decrease. ag: agonist; antag: antagonist; Glu: glutamate; KO: knockout; mER: membrane bound estrogen receptor; Pn: pelvic nerve; PPT: ERα agonist; m.oil: mustard oil; wt: wildtype; ICI: ICI-182,780; G-15: GPER antagonist; ATD: aromatase inhibitor;