The influence of estrogen on sex-related differences in pain perception using dog as an animal model

L. Miguel Carreira1,2,3* and Pedro Azevedo1

1Faculty of Veterinary Medicine, Department of Clinic – Surgery, University of Lisbon (FMV/ULisboa), Av. da Universidade Técnica de Lisboa, Polo Universitário Alto da Ajuda, 1300-477 Lisbon, Portugal
2Centre for Interdisciplinary Research in Animal Health (CIISA), FMV/ULisboa, Av. da Universidade Técnica de Lisboa, Polo Universitário Alto da Ajuda, 1300-477 Lisbon, Portugal
3Anjos of Assis Veterinary Medicine Centre (CMVAA), Rua Dª. Francisca da Azambuja Nº 9 – 9A; 2830-077 Barreiro, Portugal

Abstract

Objective: Evaluate the effects of the steroidal sexual hormone estrogen upon sex-related differences in pain perception within a scenario of perioperative orthopedics surgery, using the dog as an animal model.

Materials and methods: A sample of 60 dogs (n=60) of both genders divided by three groups each with 20 individuals: neutered females (NF), non-neutered females (NNF), and males (ML) was used. These animals were regular hospital patients submitted to orthopaedic surgery (OTS), and were evaluated for their pain level using the Melbourne Pain Scale (MPS). The patients’ pain level was evaluated over 3 peri-operative time points: M0 (immediately before surgery), M1 (24 hours after surgery) and M2 (8 days after surgery).

Results: Patients experienced lower pain levels across the time points, and this reduction was statistically significant between M0M1, M1M2 and M0M2, for the groups NF, NNF and ML (p<0.001 for all). Statistically significant differences in the final pain scores assessed with the MPS were found only between pair-wise NF/ML at M0 (p=0.016) and M1 (p=0.042). Correlations between NNF and ML were strong at M0 (rho=0.81), M1 (rho=0.64), and M2 (rho=0.79).

Conclusions: The NF group can be representative of women with low estrogens concentration. The MPS used to rate pain levels was useful in identifying and grading the NF, NNF and ML groups’ pain in patients during the peri-operative period, with males always presenting lower values than females. It is possible to conclude that steroid hormones, such as estrogens, influence sex-related differences in pain perception between females and males, with statistically significant differences registered between the NF and ML groups. Therefore, analgesic protocols developed by clinicians should always consider the gender and gonadal status of the patients.

Introduction

Since 2001, pain has been considered the fifth vital sign, which has led to research in different fields attempting to clarify pain’s associated pathophysiological mechanisms [1-3]. Pain is a dynamic phenomenon influenced by several factors, including excitatory and inhibitory regulatory mechanisms, which have important consequences on individuals’ responses to analgesics [4-6]. Several clinical studies carried out in different species such humans, dogs, and rodents have shown that gender influences pain stimulus modulation and pain perception, with substantial differences in pain perception between males and females [6-9]. In humans, women have lower pain thresholds than men, and women experience higher pain perception than men. These differences can be explained by various biological factors, such as the steroidal action of sexual hormones, which forms one of the main mechanisms that modulates variations in pain perception between genders [6-9]. In the nervous system, steroid hormones, such as estrogen, induce many effects including modulation of neurotransmitters in the brain, spinal cord and peripheral nerves, alteration of the excitability of specific regions of the brain, and influence available receptors for themselves and other ligands, such as opiates and serotonin, which are expressed in various parts of the nociceptive pathway [15-19]. These effects could impact pain perception. An individual’s inability to communicate pain does not mean that an individual cannot experience pain, thus requiring pain therapy [14]. Pain is a transversal issue that requires study under a zooubicuity scenario in both animal and human patients, since the inability to verbally express pain is not exclusive to animals but also applies to human infants, nonverbal, comatose, and cognitively impaired patients [20-23]. In veterinary medicine, patients express pain in different ways. The difficulty of assessing and quantifying pain is an important topic associated with species, age and breed-related differences, and the subjectivity and variability related to pain scales may result in the failure or success of determining patients’ therapeutic levels [24-27]. The development of multidimensional, or composite, pain scales considering physiological and behaviour characteristics and variations, such as the Melbourne Pain Scale (MPS), was significant, due to its ability to evaluate dogs’ pain in a multi-dimensional fashion [28,29]. The Melbourne Pain Scale (MPS) delivers one example. MPS is based on the assessment of six categories, which includes objective physiological measurements, such as heart rate, respirations, activity levels, etc.

Correspondence to: L. Miguel Carreira, Department of Clinics - Surgery, Faculty of Veterinary Medicine, University of Lisbon (FMV/ULisboa), Av. da Universidade Técnica de Lisboa, Polo Universitário Alto da Ajuda, 1300-477 Lisbon, Portugal, Tel: +351213652893; E-mail: miguelscarreira@fmv.ulisboa.pt

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as heart and respiratory rates, response to intervened tissues palpation, activity, posture, mental status and vocalization [30,31]. By translating a subjective assessment of pain into an objective score with recognized clinical relevance, it is possible to obtain useful, reliable, nonbiased, and repeatable data, reducing the variability between clinicians [32]. This study evaluated the effects of the steroidal sexual hormone estrogen upon sex-related differences in pain perception within a scenario of perioperative orthopedics surgery, using the dog as an animal model.

**Materials and methods**

The study used a sample of 60 dogs (n=60) of both genders divided by three groups each with 20 individuals: neutered females (NF), non-neutered females (NNF), and males (ML). These animals were regular hospital patients, that underwent orthopaedic surgery (OTS) to correct the presence of fractures, and at no time were these animals used as experimental animals. The owners of the patients signed a consent form for participation. The patients’ pain level was evaluated using the Melbourne Pain Scale (MPS) over 3 peri-operative time points: M0 (immediately before surgery), M1 (24 hours after surgery) and M2 (8 days after surgery). MPS allow us to evaluating physiological and behavioural parameters for each patient; scoring from 0 to 27. Patients’ pain score were always evaluated by the same two researchers, and in order to not influence the results, the observers were blind to information regarding the gender and gonadal status of the animal and the type of osteosynthesis each patient undergone. At the end, the mean of obtained values for final pain score of each patient was used to the statistical analysis. By assuming that individuals vary in their experience and expression of pain, and considering that different breeds typically present different responses to pain—where small breeds are generally more reactive than large breeds [33], and some breeds tend to be more stoic than others (for example, Poodle versus Rottweiler) [32,34]. We evenly distributed the different breeds between the females and males, to achieve more uniform results when we looked at pain level versus stoic than others (for example, Poodle versus Rottweiler) [32,34]. We

**Discussion**

The aim of the study was to evaluate the influence of the steroidal hormone estrogen in modulating sex-related differences in pain perception within a scenario of perioperative orthopedics surgery, using the dog as an animal model. Individuals of both genders (females and males) were similar regarding age, body weight, breed and surgery parameters. Surgery type and duration presented a very similar distribution between both genders, without statistically significant differences between females and males (p=0.857).

Identical nocicepti stimuli, applied under similar environmental conditions, promoted a highly-variable pain response across individuals, since pain is more than an objectively quantifiable physiological response: it is an experience [28]. Methods for assessing pain—perception or intensity—in animals is challenging, and still remains subjective due to its observational nature; therefore, it presented some limitations [28,30,33,36,37]. Subjective evaluation through observation and animal variation behaviors are commonly used for pain assessment, making difficult to obtain reliable, repeatable, useful, and nonbiased data [36-41]. Ideally, a subjective scoring scale correlates to objective measurements with a well-established clinical relevance [32]. In an effort to provide reliable pain evaluation scales, several pain scales have been developed, integrating behavioral and physiological observations that can be scored. Differences between pre- and post-operative physiological values were presumed to be indicators of patient pain intensity [42]. However, variations in these physiological data have not yet been established as true pain severity indicators in animals [43-47]. Pre-emptive pain surgical rating is important before conducting surgical procedures, to allow surgeons to develop analgesic protocols suitable to each patient’s clinical condition and to ensure comfort for the patient by controlling the expected pain level resulting from the surgery [29].

Another factor to consider is an individual’s response to opioid analgesics, which appears to vary with the patient’s gender. Some studies concluded that different responses to opioid analgesics between genders are modulated mainly by G-protein [7,48]. The endogenous μ-opioid neurotransmitters are related to stress and pain suppression. In animals, opioids tend to demonstrate more effective action in males than in females [49-51]. This is in line with the results of this study, where although all patients underwent the same post-operative,
multimodal analgesic protocol that proved to be suitable for nearly all the patients submitted to surgery, the overall response in males was better than in females. Therefore, females provided a lower score over the post-surgery time points considered in the study. In addition, the number of females (7) submitted to the rescue analgesic protocol with buprenorphine, to achieve a comfortable state, was higher than the number of males (4). Females represented 63% of the group where the rescue analgesic protocol was used. Among the female patients, five were NF and only two were NNF. Previous studies on the nociceptive pain and inflammatory model assessed differences in the perception of pain between genders [12]. In humans, studies suggest that women have lower pain thresholds than men: therefore women have higher perceptions of pain than males, which can be explained by the steroidal action of sexual hormones, such as estrogen [6,10-13,24].

Many of the central regions involved in pain and analgesia, specifically the periaqueductal grey, bone marrow, and dorsal root ganglia, contain receptors for estrogens, and can synthesise them locally, particularly in the hippocampus by using endogenous cholesterol [18,55-59]. By binding to specific receptors in tissues, sexual hormones initiate, terminate, or amplify a transcription signal, affecting the transcriptional events that influence the expression of various neurotransmitters and receptors, which are then translated into a functional clinical significance [52,53,60]. Sexual hormones act as neuroactive steroids, affecting the nervous system dynamics and brain functions via neurotransmission modulation [61,62], by controlling neuronal excitability through specific interactions with neurotransmitter receptors (genomic action) and ion channels (non-genomic action) [8,63,64]. Glutamate, γ-Aminobutyric acid (GABA), and acetylcholine (ACh) are first-generation neuromessengers, which are stored in pre-synaptic vesicles and can be quickly released. In contrast, the neuroactive steroids—produced in the mitochondrias and microsomes of neurons and glial cells—are slowly released via passive diffusion [56]. Changes in serum estrogen concentrations are accompanied by changes in a variety of other neurotransmitters, such as serotonin, ACh, dopamine, and β-endorphins. Their reduction accompanies decreases in serum estrogen concentrations [54,65]. Since estrogen has significant anti-nociceptive actions, lower estrogen levels are associated with increased pain and with impairment of descending

### Table 1. Characterisation of the sample according to age, body weight, breed and type of surgical procedure. Also the final scores using the MPS at M0, M1 and M2.

| Parameter                        | N  | Mean (SD) | N  | Mean (SD) | N  | Mean (SD) |
|---------------------------------|----|-----------|----|-----------|----|-----------|
| **Age (years)**                  |    |           |    |           |    |           |
| Total                           | 60 | 5.12 (1.46)| 20 | 5.28 (1.47)| 20 | 5.20 (1.52)| 20 | 4.93 (1.49)|
| **Weight (Kg)**                  |    |           |    |           |    |           |
| Total                           | 60 | 12.54 (3.92)| 20 | 12.07 (4.11)| 20 | 12.48 (3.90)| 20 | 13.13 (3.75)|
| **Surgery duration (minutes)**   |    |           |    |           |    |           |
| Total                           | 60 | 63.24 (22.24)| 20 | 62.57 (21.94)| 20 | 64.02 (20.89)| 20 | 64.30 (23.23)|
| **Breed**                        |    |           |    |           |    |           |
| Crossbreed                      | 35 | 17 (18)   | 8  | 3 (5)     | 6  | 3 (3)     |
| Poodle                          | 3  | 2 (1)     | 4  | 1 (3)     | 4  | 2 (2)     |
| Boxer                           | 20 | 12.07 (4.11)| 20 | 12.48 (3.90)| 20 | 13.13 (3.75)|
| Rottweiller                     | 5  | 1 (1)     | 5  | 1 (1)     | 5  | 1 (1)     |
| Pit-bull                         | 4  | 1 (3)     | 4  | 1 (3)     | 4  | 2 (2)     |
| French Bulldogue                | 4  | 1 (3)     | 4  | 1 (3)     | 4  | 2 (2)     |
| **Surgical procedure**           |    |           |    |           |    |           |
| Diaphyseal fracture of the femur| 23 | 6 (4)     | 4  | 4 (13)    | 4  | 4 (13)    |
| Proximal epiphyseal fracture of the femur | 5 | 1 (3) | 5 | 1 (3) | 5 | 1 (3) |
| Distal epiphyseal fracture of the femur | 8 | 3 (2) | 8 | 3 (2) | 8 | 3 (2) |
| Diaphyseal fracture of the tibia | 9  | 2 (2)     | 9  | 2 (2)     | 9  | 2 (2)     |
| Proximal epiphyseal fracture of the tibia | 4 | 1 (2) | 4 | 1 (2) | 4 | 1 (2) |
| Distal epiphyseal fracture of the tibia | 5  | 1 (3) | 5  | 1 (3) | 5  | 1 (3) |
| Diaphyseal fracture of the humerus | 4  | 1 (2) | 4  | 1 (2) | 4  | 1 (2) |
| Proximal epiphyseal fracture of the humerus | 2  | 1 (1) | 2  | 1 (1) | 2  | 1 (1) |
| **MPS Score**                    |    |           |    |           |    |           |
| Neutered Females (NF)           |    |           |    |           |    |           |
| M0                              | 11.65 (4.06) | 1.41 | 9.51 (3.44) | 1.0 | 8.89 (2.93) | 0.89 |
| M1                              | 4.06 (2.10)  | 1.04 | 3.59 (1.52) | 0.75 | 3.56 (1.16) | 0.72 |
| M2                              | 5.0 (0.0)    | 0.0  | 0.0 (0.0)    | 0.0  | 0.0 (0.0)    | 0.0  |
| Non-Neutered Females (NNF)      |    |           |    |           |    |           |
| M0                              | 19.0 (9.0)   | 6.0  | 18.0 (7.0)   | 2.0  | 18.0 (5.0)   | 2.0  |
| M1                              | 9.0 (6.0)    | 2.0  | 7.0 (1.0)    | 1.0  | 7.0 (1.0)    | 1.0  |
| M2                              | 6.0 (3.0)    | 1.0  | 5.0 (2.0)    | 2.0  | 5.0 (2.0)    | 2.0  |
| Males (ML)                      |    |           |    |           |    |           |
| M0                              | 11.65 (4.06) | 1.41 | 9.51 (3.44) | 1.0 | 8.89 (2.93) | 0.89 |
| M1                              | 4.06 (2.10)  | 1.04 | 3.59 (1.52) | 0.75 | 3.56 (1.16) | 0.72 |
| M2                              | 5.0 (0.0)    | 0.0  | 0.0 (0.0)    | 0.0  | 0.0 (0.0)    | 0.0  |

Sample (N): Standart deviation (SD); Kilogram (Kg); Mean (x); Melbourne pain scale (MPS); Moment immediately after surgery (M0); Moment 24 hours after surgery (M1), Moment 8 days after surgery (M2); Orthopedics surgery OSTS
By using a NF group in our study, we can evaluate what is happening in women with low estrogens concentration, as occurring in hysterectomy or with the onset of menopause, and try to understand if estrogen levels played a major role in differences of pain perception, by comparing the final MPS scores in the NF and NNF groups with the ML group. NFs presented lower estrogens levels than NNFs; thus, we expected that differences in pain perception would be more marked between the NF/ML pair-group, than in the NNF/ML pair-group. According to the results, the evolution of pain experienced by patients, evaluated at the three times considered, showed the same trend for all three groups (NF, NNF, ML), suggesting that post-surgery pain levels decreased consistently over time. According to the results, females showed higher final pain scores using the MPS at all time points (M0, M1, M2), than did males. At the end of the study (M2), the average scores obtained were 1.41 for the NF group, 1.00 for the NNF group, and 0.89 for the ML group, which demonstrated overall good pain control, considering that the maximum MPS score is 27. No significant differences for the final pain score were registered between the NF and NNF groups (p=0.06). Nevertheless, this p-value is very close to a significant result of p=0.05, suggesting that a larger sample size might have achieved a significant difference between these groups. Statistically significant differences in pain level scores were registered between the NF and ML groups at M0 (p=0.016), and at M1 (p=0.042), with greatly improved patients’ comfort occurring within the first 24 hours after surgery. Comparisons between the NNF and ML groups presented no statistically significant differences at any time point (M0, M1, M2).

**Conclusion**

Human and animals pain perception is influenced by several biological variables. The MPS used to rate pain levels was useful in identifying and grading the NF, NNF, and ML groups’ pain in patients during the peri-operative period, with males always presenting lower values than females. It is possible to conclude that steroid hormones, such as estrogens, influence sex-related differences in pain perception between females and males, with statistically significant differences registered between the NF and ML groups. Therefore, analgesic protocols developed by clinicians should always consider the gender and gonadal status of the patients.

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**References**

1. Hellyer P, Rodan I, Brunt J, Downing R, Hagedorn J, Robertson SA (2007) AHA/AAFP pain management guidelines for dogs and cats. *J Feline Med Surg* 9: 466-480.
2. Rollin BE (2008) The ethics of pain management. In: Handbook of veterinary pain management. Gaynor JS, Mair WW (eds). St. Louis, Mosby 2-11.
3. Dyson DH (2008) Perioperative pain management in veterinary patients. * Vet Clin North Am Small Anim Pract* 38: 1309-1327. [Crossref]
4. Mira F, Costa A, Mendes E, Azevedo P, Carreira LM (2015) A pilot study exploring the effects of musical genres on the depth of general anesthesia assessed by haemodynamic responses. *J Feline Med Surg pii*: 1098612X15588968. [Crossref]
5. Mira F, Costa A, Mendes E, Azevedo P, Carreira LM (2015) Influence of music and its genres on respiratory rate and pupil diameter variations in cats under general anesthesia: contribution to promoting patient safety. *J Feline Med Surg* 17: 643-648. [Crossref]
6. Palmeira CC, Ashmawi HA, Pouso I (2011) Sex and pain perception and analgesia. *Rev Bras Anestesiol* 61: 814-828. [Crossref]
7. Wiesenfeld-Hallin Z (2005) Sex differences in pain perception. *Gend Med* 2: 137-145. [Crossref]
8. Aloisi AM, Ceccarelli I, Herlegen T (2000) Gonadectomy and persistent pain differently affect hippocampal c-Fos expression in male and female rats. *Neurosci Lett* 281: 29-32. [Crossref]
9. Hurley RW, Adams MC (2008) Sex, gender, and pain: an overview of a complex field. *Anesth Analg* 107: 309-317. [Crossref]
10. Berkley KJ (1997) Sex differences in pain. *Behav Brain Sci* 20: 371-380. [Crossref]
11. Fillingim RB, Gear RW (2004) Sex differences in opioid analgesia: clinical and experimental findings. *Eur J Pain* 8: 413-425. [Crossref]
12. Gaumond I, Arsenault P, Marchand S (2005) Specificity of female and male sex hormones on excitatory and inhibitory phases of formalin-induced nociceptive responses. *Brain Res* 1052: 105-111. [Crossref]
13. Thompson AD, Angelotti T, Nag S, Mokha SS (2008) Sex-specific modulation of spinal nociception by alpha-adrenoreceptors: differential regulation by estrogen and testosterone. *Neurosci Lett* 1268-1277. [Crossref]
14. Benedetto-Castellote S (1995) Fisiologia del Nervio. In: Fisiologia Veterinaria. Garcia-Sanchez A, Castelojo F, CruzPalomino LF, Gonzalez-Gallego J, Lopez de Silanes MD, Salido Ruiz G, (eds). Madrid: McGraw-Hill 11-40.
15. Craft RM (2007) Modulation of pain by estrogens. *Pain* 132 Suppl 1: S3-S12. [Crossref]
16. Aloisi AM, Bonifazi M (2006) Sex hormones, central nervous system and pain. *Horm Behav* 50: 1-7. [Crossref]
17. Smith YR, Stohler CS, Nichols TE, Bueller JA, Koeppa RA, Zubieta JK (2006) Pronociceptive and antinociceptive effects of estradiol through endogenous opioid receptors. *Eur J Pain* 10: 100-106. [Crossref]
neurotransmission in women. J Neurosci 26: 5777–5785. [Crossref]
18. Vincent K, Tracey I (2008) Hormones and their interaction with the pain experience. Rev Pain 2: 20-24. [Crossref]
19. Sarajian S, Oblinger MM (2010) Estrogen effects on pain sensitivity and neuropathic expression in rat sensory neurons. Exp Neurol 224: 163-169. [Crossref]
20. Chang PC, Yeh CH (2005) Agreement between child self-report and parent proxy-report to evaluate quality of life in children with cancer. Psychoneuroendocrinology 14: 125-134. [Crossref]
21. Loewenstein DA, Arguelles S, Bravo M, Freeman RQ, Arguelles T, Acevedo A, et al. (2001) Caregivers’ judgments of the functional abilities of the Alzheimer’s disease patient: a comparison of proxy reports and objective measures. J Gerontol B Psychol Sci Soc Sci 56: 78–84. [Crossref]
22. Schnakers C, Chatelle C, Vanhaudenhuyse A, Majerus S, Ledoux D, et al. (2010) The Noiceesion Coma Scale: a new tool to assess nociceision in disorders of consciousness. Pain 148: 215-219. [Crossref]
23. Fuchs-Lacelle S, Hadjistavropoulos T (2004) Development and preliminary validation of the pain assessment checklist for seniors with limited ability to communicate (PACSLAC). Pain Manage Nurs 5: 37-49. [Crossref]
24. Molony V, Kent JE (1997) Assessment of acute pain in farm animals using behavioral and physiological measurements. J Anim Sci 75: 266-272. [Crossref]
25. Schnitzler A, Plocher M (2000) Neurophysiology and functional neuroanatomy of pain neurotransmission. In: Handbook of veterinary pain management. Gaynor JS, Muir WW (eds). St Louis: Mosby 430-442. [Crossref]
26. VMA (2001) adopts position regarding animal pain. J Am Vet Med Assoc 218:1694.
27. American Animal Hospital Association; American Association of Feline Practitioners; American Animal Hospital Association; American Association of Feline Practitioners; American Animal Hospital Association; American Association of Feline Practitioners. (2001) AAHA/AAFP Pain Management Guidelines Task Force Members, Helffier P, Rodan I, Brun I, et al. (2007) AAHA/AAPF pain management guidelines for dogs and cats. J Amer Anim Hosp Assoc. 43: 235-248. [Crossref]
28. Hansen BD (2003) Assessment of pain in dogs: veterinary clinical studies. ILAR J 44: 197-205. [Crossref]
29. Mich PM, Helffier PW (2008) Objective, Categoric Methods for Assessing Pain and Analgesia. In: Handbook of veterinary pain management. Gaynor JS, Muir WW (eds). St Louis: Mosby:78-107
30. Molony V, Kent JE (1997) Assessment of acute pain in farm animals using behavioral and physiological measurements. J Anim Sci 75: 266-272. [Crossref]
31. Schnitzler A, Plocher M (2000) Neurophysiology and functional neuroanatomy of pain neurotransmission. In: Handbook of veterinary pain management. Gaynor JS, Muir WW (eds). St Louis: Mosby 430-442. [Crossref]
32. Sharron M (2013) The challenges of assessing osteoarthritis and postoperative pain in elderly patients: a comparison of proxy reports and objective measures. J Gerontol B Psychol Sci Soc Sci 56: 78–84. [Crossref]
33. National Academies Press (US) (2009) NRC - National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals. Recognition and Alleviation of Pain in Laboratory Animals. Washington DC: Recognition and Assessment of Pain. [Crossref]
34. Dobromylskij P, Flecknell PA, Lascelles BD, et al. (2000) Pain assessment. In: Flecknell PA, Waterman-Pearson A, et al., editors. Pain management in animals. London: Saunders 53–79. [Crossref]
35. Plumb DC (2015) Plumb’s Veterinary Drug Handbook, 8th ed. Wiley-Blackwell.
36. Rialland P, Authier S, Guillot M, Del Castillo JR, Veiller-Lermeux D, et al. (2012) Validation of orthopedic postoperative pain assessment methods for dogs: a prospective, blinded, randomized, placebo-controlled study. Gilestro GF, ed. PLoS ONE 7: e49480. [Crossref]
37. de Oliveira FA, Luna SP, do Amaral JB, Rodrigues KA, Sant’Anna AC, et al. (2014) Validation of the UNESP-Butucaru unidimensional composite pain scale for assessing postoperative pain in cattle. BMC Vet Res 10: 200. [Crossref]
38. Stallard P, Williams L, Vellman R, Lenton S, McGrath PJ, et al. (2002) The development and evaluation of the pain indicator for communicatively impaired children (PICIC). Pain 98: 145-149. [Crossref]
39. Wiseman ML, Nolan AM, Reid J, Welsh E (1998) Relationship between physiological factors and clinical pain in dogs scored using a numerical rating scale. J Small Anim Pract 39: 469–474. [Crossref]
40. Gellasch KL, Kruse-Elliot KT, Osmond CS, Shih AN, Bjorling DE (2002) Comparison of transferal administration of fentanyl versus intramuscular administration of buprenorphine for analgesia after onychectomy in cats. J Am Vet Med Assoc 220: 1020–1024. [Crossref]
41. Drendel AL, Kelly BT, Ali S (2011) Pain assessment for children: overcoming challenges and optimizing care. Pediatr Emerg Care 27: 773-781. [Crossref]
42. Marco CA, Plewa MC, Buderer N, Hymel G, Cooper J (2006) Self-reported pain scores in the emergency department: lack association with vital signs. Acad Emerg Med 13: 974–979. [Crossref]
43. Claiborne J, Nag S, Mokka SS (2006) Activation of opioid receptor like-1 receptor in the spinal cord produces sex-specific antinociception in the rat: estrogen attenuates antinociception in the female, whereas testosterone is required for the expression of antinociception in the male. J Neurosci 26: 13048-13053. [Crossref]
44. Zubieta JK, Smith YR, Bueltl JA, Xu Y, Kilbourn MR, et al. (2002) mu-opioid receptor-mediated antinociceptive responses differ in men and women. J Neurosci 22: 5100-5107. [Crossref]
45. Cook CD, Barret AC, Roach AL, Bowman JR, Packer MJ (2000) Sex-differences in the antinociceptive effects of opioids: importance of rat genotype, nociceptive stimulus intensity, and efficacy at the mu opioid receptor. Psychopharmacology 150: 430-442. [Crossref]
46. Dahlan A, Kest B, Wamaxan AR, Sarton E (2008) Sex-specific responses to opiates: animal and human studies. Anesth Analg 107: 83-95. [Crossref]
47. Brody S, Carlstrom K, Lagrelius A, Lundell NO, Mollerstrom G, Poussette A (1987) Serum sex hormone binding globulin (SHBG), testosterone/SHBG index, endometrial pathology and bone mineral density in postmenopausal women. Acta Obstet Gynec Scand 66: 357-360. [Crossref]
48. Beyenburg S, Stoffel-Wagner B, Bauer J, Watzka M, Blumcke I, et al. (2001) Neuroactive steroids and seizure susceptibility. Epilepsy Res 44: 141-153. [Crossref]
49. Mellon SH (1994) Neurosteroids: biochemistry, modes of action, and clinical relevance. J Clin Endocrinol Metab 78: 1003-1008. [Crossref]
50. Shibuya K, Takata N, Hojo Y, Furukawa A, Yasumatsu N, et al. (2003) Hippocampal cystochrome P450s synthesize brain neurosteroids which are paracrine neumodulators of synaptic signal transduction. Biochem Biophys Acta 1619: 301-316. [Crossref]
51. Sinchak K, Mills RH, Tao L, LuPolt P, Lu JK, et al. (2003) Estrogen induces de novo progesterone synthesis in astrocytes. Dev Neurosci 25: 343-348. [Crossref]
52. Amatue SK, Alt JJ, Stamps CL, McCarthy MM (2004) Brain estradiol content in newborn rats: sex differences, regional heterogeneity, and possible de novo synthesis by the female telencephalon. Endocrinology 145: 2906-2917. [Crossref]
53. Evvard HC (2006) Estrogen synthesis in the spinal dorsal horn: a new central mechanism for the hormonal regulation of pain. Am Physiol Regul Integr Comp Physiol 291: R291-R299. [Crossref]
54. Greenspan JD, Craft RM, LaResche L, Arendt-Nielsen L, Berkley KJ, et al. (2007) Studying sex and gender differences in pain and analgesia: a consensus report. Pain 132 Suppl 1: S26-45. [Crossref]
55. Murrell JC, Paatha EP, Scott EM, Reid J, Hellebrekers LJ (2008) Application of a modified form of the Glasgow pain scale in a veterinary teaching centre in the Netherlands. Vet Rec 162: 403-408. [Crossref]
56. Simpson ER (2003) Sources of estrogen and their importance. J Steroid Biochem Mol Biol 86: 225-230. [Crossref]
57. Robichaud M, Debonnel G (2005) Oestrogen and testosterone modulate the firing activity of dorsal raphe nucleus serotoninergic neurons in both male and female rats. J Neuroendocrinol 17: 179-185. [Crossref]

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Glob Anesth Perioper Med, 2016 doi: 10.15761/GAPM.1000152 Volume 2(3): 194-199
63. Rupprecht R, Holsboer F (1999) Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci* 22: 410-416. [Crossref]

64. Teyler TJ, Vardaris RM, Lewis D, Rawitch AB (1980) Gonadal steroids: effects on excitability of hippocampal pyramidal cells. *Science* 209: 1017-1018. [Crossref]

65. Rudick CN, Woolley CS (2000) Estradiol induces a phasic Fos response in the hippocampal CA1 and CA3 regions of adult female rats. *Hippocampus* 10: 274-283. [Crossref]