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Dependency on sex and stimulus quality of nociceptive behavior in a conscious visceral pain rat model

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ABSTRACT

Visceral pain may be influenced by many factors. The aim of this study was to analyze the impact of sex and quality of intracolonic mechanical stimulus on the behavioral manifestations of visceral pain in a preclinical model.

Male and female young adult Wistar rats were sedated, and a 5 cm long latex balloon was inserted into the colon. Sedation was reverted and behavior was recorded. The pressure of the intracolonic balloon was gradually increased using a sphygmomanometer. Visceral sensitivity was measured as abdominal contractions in response to mechanical intracolonic stimulation. Two different types of stimulation were used: tonic and phasic. Phasic stimulation consisted of repeating several times (3x) the same short stimulus (20 s) within a 5 min interval allowing a 1 min break between individual stimuli. For tonic stimulation the stimulus was maintained throughout the whole 5 min interval. Both phasic and tonic stimulation produced a pressure-dependent increase of abdominal contractions. The abdominal response was more intense under phasic than under tonic stimulation, but with differences depending on the sex of the animals: females exhibited more contractions than males and of similar duration at all pressures, whereas duration of contractions pressure-dependently increased in males. The duration of tonically stimulated contractions was lower and not sex- or pressure-dependent. In the rat, responses to colonic distension depend on the quality of the stimulus, which also produces sex-dependent differences that must be taken into account in the development of models of pathology and visceral pain treatments.

1. Introduction

Pain is a protective mechanism allowing perception of damage. Visceral pain is defined as pain that arises from internal organs. In hollow organs, it may occur due to excessive contraction, stretching or ischemia [1]. Visceral pain is also the defining characteristic of various gastrointestinal (GI) disorders, including irritable bowel syndrome (IBS) where, in the absence of specific markers, is a factor that allows diagnosis [2].

To assess visceral pain, one of the most widely used methods is colorectal distention (CRD). In these studies, an inflatable balloon is inserted into the distal colon to mechanically stimulate it. This methodology has been applied in animal models [3–6] and also in some studies of rectal sensitivity in volunteer patients [7–9]. In animals, pain responses can be evaluated by different methods like abdominal withdrawal reflex or by electromyography [2]. A more simple and effective analysis method consists of visual recording of abdominal contractions [3–6,10].

Some visceral pain studies use phasic stimulation, which consists of repeating the same short stimulus several times within a certain period of time, with a few seconds of rest between individual stimuli [7,11–13]. Other studies use tonic stimulation, in which the same stimulus is maintained throughout a defined period of time [3,4,8,10,14]. Although the type of stimulus chosen may lead to important differences, the reports comparing results obtained with both methods are scarce [15,16].

In addition, there is growing interest in investigating differences between genders and how sexual hormones influence visceral pain and related pathologies [3,4,11,13]. The sensitivity thresholds seem lower in

Abbreviations: CRD, colorectal distension; GI, gastrointestinal; IBS, irritable bowel syndrome; OVX, ovariectomized.

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women when close to menstruation [17]. In animal studies, somatic and visceral sensitivity also varies with the phase of the estrous cycle. In rats, these phases are proestrus, estrus, metestrus, and diestrus (Table S1, Supplementary Material). Proestrus corresponds to the follicular state in humans and is related to an increase in the concentration of circulating estradiol. Estrus corresponds to the decline in estradiol. Ovulation occurs at the end of this phase. Metestrus and diestrus display high levels of progesterone and correlate, respectively, with the early and late secretory phases of the human female cycle [18]. High levels of estradiol in proestrus and estrus are associated with high visceral sensitivity [3,4].

Due to the lack of studies combining both factors, our aim was to analyze, in a preclinical model, the impact of sex and quality of intracolonic mechanical stimulation on the behavioral manifestations of visceral pain.

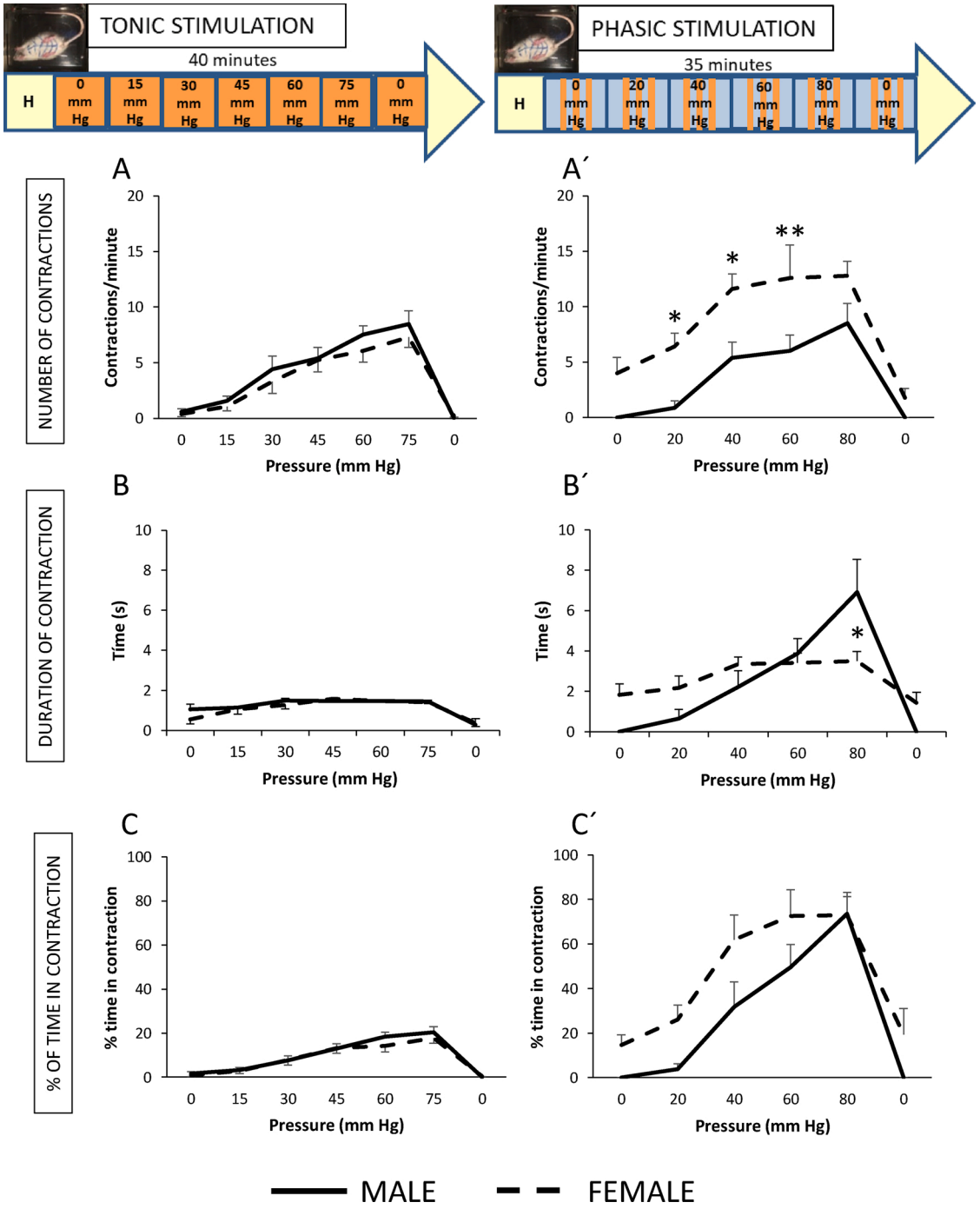

Fig. 1. Colonic sensitivity to tonic or phasic mechanical stimulation in male and female rats. After 5 min of habituation (H), rats were subjected to tonic or phasic mechanical intracolonic stimulation (see Methods for details). For tonic stimulation, pressure was increased from 0 to 75 mmHg, in steps of 15 mmHg every 5 min, to finally return to 0 mmHg again; for each pressure value, a single stimulus was applied and maintained for 5 min (orange rectangles). For phasic stimulation, pressure was increased from 0 to 80 mmHg, in steps of 20 mmHg every 5 min, to finally return to 0 mmHg again; for each pressure value, 3 stimuli of 20 s (orange vertical lines) were applied, 1 min apart. For tonic (A, B, C) or phasic stimulation (A´, B´, C´) number of contractions per minute (A, A´), duration of contractions (B, B´) and % of time in contraction (C, C´) were measured. Experimental groups were males (tonic stimulation n = 7, phasic stimulation n = 8) and females (tonic stimulation n = 8, phasic stimulation n = 5). Data represent the mean ± SEM. * $p < 0.05$, ** $p < 0.01$ vs male (two-way ANOVA followed by Bonferroni post-hoc test).

2. Materials and methods

The experiments were designed and performed according to the EU Directive for the Protection of Animals Used for Scientific Purpose (2010/63/EU) and Spanish regulations (Law 32/2007, RD 53/2013 and order ECC/566/2015) and approved by the Ethical Committee at Universidad Rey Juan Carlos (URJC) and Comunidad Autónoma de Madrid (PROEX 063/18, PROEX 023/19). The number of animals used, and their suffering were minimized.

Male (N = 7–8/group) and female (N = 5–8/group) young adult (2–4 months old) Wistar rats were obtained from the Veterinary Unit of URJC and randomly housed (2–4/cage) in standard transparent cages (60 × 40 × 20 cm) in a temperature (20 °C) and humidity (60 %) controlled room, with a 12 h light/12 h dark cycle (lights off between 20:00 and 08.00 h). Animals had free access to standard laboratory rat chow (Harlan Laboratories Inc.) and tap water.

Body weight was recorded, and the female estrous cycle phase was analyzed by vaginal cytology [3,4], using conventional methods (Table S1, Supplementary material). Afterwards, rats were prepared for colorectal sensitivity experiments as previously described [6]. Briefly, after sedation with Sedator® (medetomidine hydrochloride, 1 mg/kg, ip), a 10 cm longitudinal line was drawn over the *linea alba* of the abdomen. Transverse lines were drawn every 2 cm to better visualize the contractions during the recordings. Then, fecal material was gently removed from the rectum and a 5 cm long latex balloon lubricated with vaseline was inserted through the anus into the colon so that the tip of the balloon was 7 cm inside the colorectum. The catheter to which the balloon was connected was fixed to the tail of the rat with Parafilm®, to avoid its expulsion.

Sedation was reverted with Revertor® (atipamezole hydrochloride, 0.66 mg/kg, ip). After waking up (normally in <5 min), the rat behavior was recorded using a video camera (iPad; Apple, Madrid, Spain) located 30 cm below the recording cage floor. The first 5 min were only used to confirm the normal behavior of the rat after recovery from sedation and were discarded; thereafter, the pressure of the intracolonic balloon was gradually increased using a sphygmomanometer. Two different protocols of stimulation were applied (Fig. 1): tonic stimulation, pressure was increased from 0 to 75 mmHg, in steps of 15 mmHg every 5 min, and finally returned to 0 mmHg again (for each pressure value, a single stimulus was applied and maintained for 5 min); phasic stimulation, pressure was increased from 0 to 80 mmHg, in steps of 20 mmHg every 5 min, and finally returned to 0 mmHg again (for each pressure value, 3 stimuli of 20 s were applied, 1 min apart).

The videos were exported as a series of frames (1 s-1), using Quick Time Player Pro for Windows (v.7.7.4; Apple Inc.). Visceral sensitivity was measured as abdominal contractions in response to mechanical intracolonic stimulation. An abdominal contraction was considered as a depression of the abdomen where transverse lines approached one another. Thus, each frame was analyzed to determine if the rat abdomen was contracted or relaxed. This information was used to determine, for each condition (stimulus quality/sex), the average number and duration of contractions, as well as the average percentage of time spent by the rat contracting the abdomen during each 5 min or 20 s period, depending on the kind of stimulation applied (tonic or phasic, respectively). The average number of contractions is represented per time unit (min).

Statistical analyses were performed using Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA). Results are expressed as mean ± SEM. One or two-way ANOVA followed by Bonferroni's post-hoc test was used for analyses of colonic sensitivity. Student's *t*-test was used to compare body weight means between males and females. Fisher's exact test was used to compare proportions of females in each phase of the cycle. Differences were considered significant when $p < 0.05$.

3. Results

As expected for young adult age, the body weight of female rats was significantly lower than that of male animals ($p < 0.001$). No difference was found between weights of males used for tonic (389 ± 12 g) and phasic (389 ± 9 g) stimulation experiments. However, females used in phasic stimulation experiments displayed a significantly lower body weight (206 ± 4 g) than those used in tonic stimulation assays (258 ± 4 g) ($p < 0.0001$).

Female rats were only found in proestrus and estrus phases, and in similar proportions (proestrus: 50 %, tonic vs 40 %, phasic; estrus: 50 %, tonic vs 60 %, phasic; $p > 0.05$).

Before pressure application, males used in the tonic stimulation experiment presented 0.6 ± 0.28 contractions/min. Then, the number of contractions progressively increased in response to increasing intracolonic pressure, indicating sensitiveness to colonic mechanical stimulation (Fig. 1A). At the highest pressure applied (75 mmHg), 8.48 ± 1.16 contractions/min were detected. During stimulation (15−75 mmHg), the average duration of contractions remained within a narrow range of values (between 1 and 1.5 s), except at the end of the experiment when pressure application ceased. At that point, the mean number of contractions dropped dramatically (0.02 ± 0.03), and their mean duration was practically zero (Fig. 1B). The percentage of time that animals remained in contraction (Fig. 1C) showed a progressive pressure-dependent increase and reached maximum values of 20 ± 2.6 % at the highest pressure (75 mmHg). The pressure-dependency statistical analysis of the different parameters is shown in Table S2 (Supplementary material).

Females under tonic stimulation showed a similar behavior to males and the graphs obtained for both sexes closely overlapped for the three parameters considered, without statistically significant differences (Fig. 1A–C; Table S2, Table S3, Supplementary material).

Under phasic stimulation, the number of contractions/min obtained in males (Fig. 1A) also progressively increased with pressure (Table S2), reaching maximum values of 8.5 ± 1.8 at the highest pressure (80 mmHg). No contractions were detected when pressure returned to zero. At 60 mmHg (used in both protocols), contractions/min for tonic (7.5 ± 0.8) and phasic (6 ± 1.8) stimulation were not significantly different ($p > 0.05$) (Figure S1A, Supplementary Material).

Interestingly, the mean contraction duration obtained with phasic stimulation in males pressure-dependently increased (Table S2) with a maximum of 6.9 ± 1.6 s at 80 mmHg (Fig. 1B′). Moreover, at 60 mmHg, contraction durations under phasic (3.8 ± 0.7 s) and tonic (1.4 ± 0.04 s) stimulation showed statistically significant differences ($p < 0.01$) (Figure S1B).

The percentage of time that the abdomen remained contracted in males during phasic stimulation was higher than in tonic stimulation, with statistically significant differences at 60 mmHg (49.5 ± 10.1 %, phasic vs 18.4 ± 1.8 %, tonic, $p < 0.05$; Figs. 1C,1C′, S1C, Table S3).

Under phasic stimulation, females showed a greater number of contractions/min than males at all pressures, with statistically significant differences at 20, 40, and 60 mmHg (Fig. 1A). The mean duration of contractions in females showed little variation upon stimulation (Table S2), with values between 1.8 and 3.5 s (Fig. 1B′). Up to 40 mmHg, females presented longer contractions than males, without reaching statistically significant values (Table S3), but with higher pressures (60 and 80 mmHg), males gradually increased contraction duration with values significantly higher than those of females at 80 mmHg. In general, the percentage of time in contraction tended to be higher also in females than males at all pressures, except for 80 mmHg, where graphs overlapped (Fig. 1C′). Indeed, the two-way ANOVA demonstrated statistically significant differences between the curves (Table S3: $F(1.67) = 15.27$; $p \leq 0.001$). In females, all parameters showed higher values under phasic than under tonic stimulation at 60 mmHg (Figure S1).

4. Discussion

In this study, performed in rats, phasic intracolonic mechanical stimulation elicited more (females) or longer lasting (males) behavioral

responses than tonic (sustained) stimuli, which did not induce significant sex-dependent differences.

As in our previous reports [5,6], in males under tonic stimulation, the number of contractions and % of time in contraction increased in a pressure-dependent manner, while contraction duration did not. Whereas other authors obtained qualitatively similar results [3,4,10,12], quantitative differences could be due to the different rat strains used [19], different tonic stimulation protocols (some authors stimulate for longer periods but allow resting periods between stimuli [3,4,12,14]) or different recording and evaluation methods [19].

To our surprise, when tonic stimulation was applied to females (which were all found in proestrus/estrus phases), no differences with males were observed. In previous studies, females were more sensitive than males [3,4,12]. Furthermore, rodent females in the proestrus/estrus phase were more sensitive to painful stimuli than those in metestrus/diestrus [4]. The effect of sex hormones may explain sex-dependent differences in visceral pain because in ovariectomized rats (OVX), the number of contractions was similar for females and males under tonic stimulation [14]. Moreover, visceral hypersensitivity produced by early life adversity in female rats was completely reversed by OVX and restored with estradiol replacement [12]. However, the mentioned studies were carried out using stress paradigms or IBS models, where hypersensitivity development is favored. Differences in the duration of mechanical stimulus or application of rest periods between stimuli may also contribute to explain discrepancies between reports. In contrast with those previous preclinical findings, our results are more in agreement with rectal sensitivity studies, in which tonic stimulation did not reveal differences between sexes in the pain threshold in healthy patients [9], nor in discomfort pressures in patients with IBS [8].

Even though not exactly the same pressures were used for tonic and phasic stimulation, the biggest difference between both sets of experiments was the quality of each pressure stimulation. For direct comparisons, 60 mmHg, applied in both protocols, was used. Compared with tonic stimulation, application of the phasic protocol produced a higher % of time in contraction in both females and males, with a non-significant trend of females presenting higher values than males in this parameter (Fig. 1C'). The increased % of time in contraction found in males was probably due to a pressure-dependent increase in the mean contraction duration, whereas in females it was higher because of an increased number of contractions/min (in females, duration was higher than in tonic stimulation protocol, but not pressure-dependent). Since females in phasic experiments were smaller compared with those used in tonic experiments, the more intense responses found in these smaller animals could be due to mechanical issues associated with a smaller diameter of the anus and colorectum. However, in a non-invasive radiographic study performed for gastrointestinal motility evaluation (see [5] for detailed description of this technique) just one week before the present experiments, the diameters of the fecal pellets found in the last 5 cm of the female distal colon (the very same region occupied by the balloon during visceral pain experiments) were not significantly different (5.39 ± 0.08 mm, tonic vs 5.82 ± 0.41 mm, phasic; $p > 0.05$), suggesting that the procedure of insertion and inflation of the balloon at similar pressure values did not necessarily cause more stimulation in females used for the phasic protocol.

In agreement with previous reports in both rats [15,16] and patients [8], phasic stimulation seems to be more powerful than tonic stimulation in triggering abdominal contractions and producing sex-dependent differences, possibly due to different abdominal muscle fiber composition [20,21]. Thus, in *abdomini rectum*, type II, fast fibers predominate over type I, slow fibers in both males and females, which could explain the more intense responses in phasic *vs* tonic stimulation. In addition, compared with females, males have a higher proportion of IIA fibers (which perform rapid and repetitive movements of little intensity) and fewer IIB fibers (which are used in fast and intense movements), which could explain the more intense reaction of the females during the experiments with phasic stimulation. An increased recruitment of type IIA fibers might explain the pressure-dependent contraction duration found in males.

Our differences in the response to tonic and phasic stimulation could also be, at least partly, related with sex hormones levels. In our experiments, females were in proestrus/estrus, associated with the highest levels of estrogens [3]. Estrogens can have pronociceptive but also antinociceptive effects, preventing pain via vagal activation. In OVX rats treated with estrogen supplements, systemic injection increased the response to pain, while luminal application reduced it by vagal activation upon release of serotonin by mast cells [22]. Alternatively, tonic stimulation may activate the endogenous opioid system, which produces analgesic effects and is also influenced by estrogen levels [23]. There are estrogen receptors in the central and peripheral nervous system and production of estrogens can be both peripheral (in ovaries) and central (in the central nervous system). At the spinal level, there are MOR and KOR opioid receptors and estrogens act as molecular transducers of spinal antinociception in females. Abundance of MOR and KOR receptors has been found in females during proestrus, but not during diestrus, or in males, showing an influence of estrogens on their expression [23].

In conclusion, both stimulation type and sex strongly influence visceral pain behavior in rats, possibly associated with abdominal musculature composition features. The prolonged colonic mechanical stimulation during the tonic protocol could activate different pathways to those triggered during phasic stimulation, and this different activity could be further modulated by estrogen activity. These differences should be taken into account when designing visceral pain experiments.

Author contributions

RA designed the study. General parameters were obtained by YLT. LLG and YLT performed the visceral pain experiments. LLG performed colonic sensitivity and vaginal cytology analysis. LLG and RA wrote the manuscript. All authors approved the final version of the manuscript.

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CRediT authorship contribution statement

L. López-Gómez: Investigation, Formal analysis, Writing - original draft, Visualization, Writing - review & editing. **Y. López-Tofiño:** Investigation, Writing - review & editing. **R. Abalo:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neulet.2021.135667.

References

[1] B. Feng, T. Guo, Visceral pain from colon and rectum: the mechanotransduction and biomechanics, J. Neural Transm. 127 (2020) 415–429, https://doi.org/10.1007/s00702-019-02088-8.

[2] B. Greenwood-Van Meerveld, D.K. Prusator, A.C. Johnson, Animal models of gastrointestinal and liver diseases. Animal models of visceral pain: pathophysiology, translational relevance, and challenges, Am. J. Physiol. Gastrointest. Liver Physiol. 308 (11) (2015) G885–G903, https://doi.org/10.1152/ajpgi.00463.2014.

[3] D.K. Prusator, B. Greenwood-Van Meerveld, Gender specific effects of neonatal limited nesting on viscerosomatic sensitivity and anxiety-like behavior in adult rats, Neurogastroenterol. Motil. 27 (1) (2015) 72–81, https://doi.org/10.1111/nmo.12472.

[4] D.K. Prusator, B. Greenwood-Van Meerveld, Sex-related differences in pain behaviors following three early life stress paradigms, Biol. Sex Differ. 7 (1) (2016) 29, https://doi.org/10.1186/s13293-016-0082-x.

[5] P. Mosińska, M. Martín-Ruiz, A. González, V. López-Miranda, E. Herradón, J. A. Uranga, G. Vera, A. Sanchez-Yanez, M.I. Martín-Fontelles, J. Fichna, R. Abalo, Changes in the diet composition of fatty acids and fiber affect the lower gastrointestinal motility but have no impact on cardiovascular parameters: in vivo and in vitro studies, Neurogastroenterol. Motil. 31 (9) (2019), e13651, https://doi.org/10.1111/nmo.13651.

[6] M. Martín-Ruíz, J.A. Uranga, P. Mosinska, J. Fichna, K. Nurgali, M.I. Martín, Fontelles R. Abalo, Alterations of colonic sensitivity and gastric dysmotility after acute cisplatin and granisetron, Neurogastroenterol. Motil. 31 (3) (2019), e13499, https://doi.org/10.1111/nmo.13499.

[7] C. Ng, M. Danta, G. Prott, C.A. Badcock, J. Kellow, A. Malcolm, Modulatory influences on antegrade and retrograde tonic reflexes in the colon and rectum, Am. J. Physiol. Gastrointest. Liver Physiol. 287 (5) (2004) G962–G966, https://doi.org/10.1152/ajpgi.00460.2003.

[8] E.A. Mayer, S. Berman, L. Chang, B.D. Naliboff, Sex-based differences in gastrointestinal pain, Eur. J. Pain 8 (5) (2004) 451–463, https://doi.org/10.1016/j.ejpain.2004.01.006.

[9] A. Icenhour, F. Labrenz, T. Roderigo, C. Siebert, S. Elsenbruch, S. Benson, Are there sex differences in visceral sensitivity in young healthy men and women? Neurogastroenterol. Motil. 31 (9) (2019), e13664 https://doi.org/10.1111/nmo.13664.

[10] J.P. Russell, E. Mohammadi, C.O. Ligon, A.C. Johnson, M.D. Gershon, M. Rao, Y. Shen, Chi-Chung Chan, H.S. Eidam, M.P. DeMartino, M. Cheung, A.I. Oliff, S. Kumar, B. Greenwood-Van Meerveld, Exploring the potential of RET kinase inhibition for irritable bowel syndrome: a preclinical investigation in rodent models of colonic hypersensitivity, J. Pharmacol. Exp. Ther. 368 (2) (2019) 299–307, https://doi.org/10.1124/jpet.118.252973.

[11] A.Z. Murphy, S.K. Suckow, M. Johns, R.J. Traub, Sex differences in the activation of the spinoparabrachial circuit by visceral pain, Physiol. Behav. 97 (2) (2009) 205–212, https://doi.org/10.1016/j.physbeh.2009.02.037.

[12] A. Chaloner, B. Grennwood-van Meerveld, Sexually dimorphic effects of unpredictable early life adversity on visceral pain behavior in a rodent model, J. Pain 14 (3) (2013) 270–280, https://doi.org/10.1016/j.jpain.2012.11.008.

[13] C. Knuesel, M. Oulevey-Meier, B. Flogerzi, M. Krayer, I. Gschossmann, J. Miller, L. Tovar, S. Janko, J.M. Gschossmann, Effect of estrogen on visceral sensory function in a non-inflammatory colonic hypersensitivity rat model, Neurogastroenterol. Motil. 28 (10) (2016) 1570–1579, https://doi.org/10.1111/nmo.12857.

[14] B. Myers, J. Schulkin, B. Greenwood-Van Meerveld, Sex steroids localized to the amygdala increase pain responses to visceral stimulation in rats, J. Pain 12 (4) (2011) 486–494, https://doi.org/10.1016/j.jpain.2010.10.007.

[15] S.V. Coutinho, P.M. Plotsky, M. Sablad, J.C. Miller, H. Zhou, A.I. Bayati, J. A. McRoberts, E.A. Mayer, Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat, Am. J. Physiol. Gastrointest. Liver Physiol. 282 (2) (2002) G307–G316, https://doi.org/10.1152/ajpgi.00240.2001.

[16] V. Martínez, M. Ryttinger, M. Kjerling, M. Astin-Nielsen, Characterisation of colonic accommodation in Wistar Kyoto rats with impaired gastric accommodation, Naunyn Schmiedebergs Arch. Pharmacol. 376 (3) (2007) 205–216, https://doi.org/10.1007/s00210-007-0195-1.

[17] L.A. Houghton, R. Lea, N. Jackson, P.J. Whorwell, The menstrual cycle affects rectal sensitivity in patients with irritable bowel syndrome but not healthy volunteers, Gut 50 (4) (2002) 471–474, https://doi.org/10.1136/gut.50.4.471.

[18] A.F. Ajayi, R.E. Akhigbe, Staging of the estrous cycle and induction of estrus in experimental rodents: an update, Fertil. Res. Pract. 6 (1) (2020) 1–15, https://doi.org/10.1186/s40738-020-00074-3.

[19] D. O'Malley, M. Julio-Pieper, S.M. O'Mahony, T.G. Dinan, J.F. Cryan, Differential visceral pain sensitivity and colonic morphology in four common laboratory rat strains, Exp. Physiol. 99 (2) (2014) 359–367, https://doi.org/10.1113/expphysiol.2013.076109.

[20] G.L. Costerbosa, A.M. Barazzoni, M.L. Lucchi, R. Bortolami, Histochemical types and sizes of fibers in the rectus abdominis muscle of guinea pig: adaptive response to pregnancy, Anat. Rec. 217 (1) (1987) 23–29, https://doi.org/10.1002/ar.1092170105.

[21] G. Vesentini, G. Marini, F. Piculo, D.C. Damasceno, S.M.M. Matheus, S.L. Felisbino, I.M.P. Calderon, A. Hijaz, A.M.P. Barbosa, M.V.C. Rudge, Morphological changes in rat rectus abdominis muscle induced by diabetes and pregnancy, Braz. J. Med. Biol. Res. 51 (4) (2018) e7035, https://doi.org/10.1590/1414-431x20177035.

[22] X.J. Yan, C.C. Feng, Q. Liu, L.Y. Zhang, X. Dong, Z.L. Liu, Z.J. Cao, J.Z. Mo, Y. Li, J. Y. Fang, S.L. Chen, Vagal afferents mediate antinociception of estrogen in a rat model of visceral pain: the involvement of intestinal mucosal mast cells and 5-hydroxytryptamine 3 signaling, J. Pain 15 (2) (2014) 204–217, https://doi.org/10.1016/j.jpain.2013.10.012.

[23] A.R. Gintzler, E.M. Storman, N.J. Liu, Estrogens as arbiters of sex-specific and reproductive cycle-dependent opioid analgesic mechanisms, Vitam. Horm. 111 (2019) 227–246, https://doi.org/10.1016/bs.vh.2019.06.002.